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Somatostatin receptors on human lymphocytes and leukaemia cells

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SUMMARY

Receptors for somatostatin were identified on mitogen-activated human peripheral blood lymphocytes (PBL) and human leukaemic cells in 87·5% of lymphoblastic leukaemia and in 12·5% of non-lymphocytic leukaemia, using a somatostatin radiobinding assay. The specific binding of ^{125}I-somatostatin of these cells increased linearly with the cell numbers and was suppressed by non-iodinated somatostatin. We investigated the distribution of fluorescent somatostatin to mitogen-activated PBL by using a fluorescence-activated cell sorter (FACS). Over 95% of the cell populations bound fluorescent somatostatin and no distinct predilection was found among certain lymphocyte subpopulations and somatostatin receptor-positive cells. Scatchard analysis showed a single class (low affinity) of binding site on mitogen-activated PBL and two classes (high and low affinity) of specific binding sites on lymphoblastic leukaemia cells.

INTRODUCTION

Somatostatin, a tetradecapeptide isolated from the hypothalamic tissue, inhibits the release of growth hormone (GH), TSH, ACTH, glucagon, insulin, secretin, gastrin and cholecystokinin (Krulich, Dhariwal & McCann, 1968). This peptide is present in the hypothalamus, cortex, midbrain, brain stem, spinal cord, sensory ganglia, delta cells of islets of Langerhans' and epithelium of stomach and intestine (Arimura, 1981). Such widespread distributions of somatostatin suggest a diversity of physical functions.

Evidence is accumulating that immune and neuroendocrine systems communicate via factors common to both systems. We reported that certain human lymphoid cell lines bear receptors for somatostatin (Nakamura *et al.*, 1987). We have now obtained evidence that mitogen-activated peripheral blood lymphocytes (PBL) and lymphocytic leukaemia cells express receptors for somatostatin. Scatchard analysis indicated that the mitogen-activated PBL bear comparatively low-affinity receptors for somatostatin, and that lymphocytic leukaemia cells have at least two classes of binding sites; low-affinity receptors with similar characteristics on mitogen-activated PBL, and high-affinity receptors uniquely expressed on malignant lymphoid cells.

The role of somatostatin receptors in immunoregulatory functions and the relation between expression of high-affinity receptors and lymphoid malignancy are discussed.

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MATERIALS AND METHODS

Sample preparations

Leukaemic cells were obtained from 16 Japanese patients in the Second Department of Internal Medicine, Chiba University Hospital, Japan. Heparinized samples of peripheral blood were obtained and mononuclear cells were isolated by a standard centrifugation step with Ficoll–Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ). Surface antigen analyses of leukaemic cells were performed using a fluorescent-activated cell sorter (FACStar; Becton-Dickinson FACS Systems, Mountain View, CA) by indirect immunofluorescence using monoclonal antibodies (mAb) to human lymphocytes and fluorescent-conjugated F(ab′)$_2$ fragments of goat anti-mouse immunoglobulin serum (Cappel Laboratories, West Chester, PA). Acute undifferentiated leukaemia (AUL) was diagnosed, based on the following criteria: lack of morphologic signs for myeloid differentiations; negative staining for periodic acid–Schiff (PAS), non-specific esterase, myeloperoxidase (MPO) and platelet peroxidase (PPO); lack of reactivity with anti-T-cell antibodies and an antibody to common ALL antigen; negativity for expression of cytoplasmic immunoglobulins. Cells from three acute lymphoblastic leukaemia (ALL), one adult T-cell leukaemia (ATL), four chronic lymphocytic leukaemia (CLL), five acute non-lymphocytic leukaemia (ANLL) and three chronic myelocytic leukaemia (CML) were used for subsequent experiments.

For preparation of mitogen-activated PBL, cells were obtained from heparinized blood of normal individuals and were purified by centrifugation on a Ficoll–Hypaque gradient. The cells were then further incubated for 48 hr at 37° in RPMI-1640 medium supplemented with L-glutamine, 10% foetal

bovine serum and 10 μg/ml of phytohaemagglutinin-P (PHA-P; Sigma Chemical Co., St Louis, MO).

Binding studies

Measurement of somatostatin binding to peripheral blood lymphocytes and leukaemia cells was carried out by radio-binding assay, using ^{125}I-labelled somatostatin, as described elsewhere (Nakamura *et al.*, 1987). To measure somatostatin binding, ^{125}I-somatostatin and 0·5–2 × 10^6 cells were incubated in 0·2 ml phosphate-buffered saline (PBS; pH 7·4) containing 10 mM MgCl, 1% bovine serum albumin and 50 μg/ml Thimerosal as an antiseptic (SOM-buffer). After incubation for 120 min at 22°, the bound radioactivity was separated from the free tracer by centrifugation. The cells were washed twice with SOM-buffer and the radioactivity counted in a scintillation counter (LKB Industries Inc., Rockville, MD). Specific binding of ^{125}I-somatostatin was calculated by subtracting the non-specific binding (in the presence of 10^{-4} M unlabelled somatostatin) from the total binding.

Determination of specific binding of radioligand

The specificity of ^{125}I-somatostatin binding was determined by competitive inhibition, under the same conditions described above. A mixture of 100 μl of the competitors (somatostatin, insulin and glucagon), at varying dilutions, and ^{125}I-somatostatin was incubated with 2 × 10^6 cells for 120 min at 22°. After washing, the bound radioactivity was counted, and the specific binding of somatostatin to the cell surface was determined by subtracting the binding in the presence of the competitor from the total binding. The dissociation constant (K_d) and number of binding sites (B_{max}) for each leukaemia cell were determined by Scatchard plots.

Preparation of fluorescent somatostatin

Fluorescent somatostatin was prepared by a method similar to that used to prepare fluorescent substance P as described by Payan *et al.* (1984a). 5-([4,6-dichlorotriazinyl] amino)-fluorescein hydrochloride (DTAF; Sigma) and 0·25 mg of somatostatin (Sigma) were reacted in 0·15 M NaCl, buffered with 1 M sodium carbonate (pH 9·3), for 3 hr at 22°. The mixture was applied to one end of a 250-μm tick DC-Fertigplatter Kiesel gel 60F254 (Merck, Darmstadt, FRG) that was developed in a sealed chamber with chloroform:methanol:glacial acetic acid (15:5:1). Somatostatin conjugated to DTAF (SOM-DTAF) migrated as a single spot with a Rf of 0·13.

The SOM-DTAF-containing silica gel was scraped from the plate and eluted with six portions of methanol. The eluate was vacuum dried and resuspended in 0·3 ml of PBS.

Cell-surface analysis by a fluorescence-activated cell sorter (FACS)

Cells were prepared in SOM-buffer at 2 × 10^6-ml and 0·1-ml aliquots were used for binding studies.

Mouse monoclonal antibodies to human lymphocytes (anti-CD3, CD4, CD8, CD20 and CD25 antibodies) and phycoerythrin (PE)-conjugated monoclonal rat anti-mouse kappa chain antibody were purchased from Becton-Dickinson Immunodiagnostics (Mountain View, CA).

For dual-colour FACS analysis, each monoclonal mouse anti-human lymphocyte antibody was added (10 μl/2 × 10^6 cells) to the cell suspension and the mixtures were incubated for 45 min at 4°. Cells were washed twice in SOM-buffer and 0·1 ml of PE-conjugated monoclonal rat anti-mouse kappa chain antibody was added to each sample. After further incubation for 45 min, cells were washed twice and were resuspended in 0·1 ml of SOM-buffer, then 10 μl of SOM-DTAF were added and the preparations were incubated for 90 min at 22°. After this procedure, 1 ml of SOM-buffer was added to the samples and the cells were analysed by FACS.

RESULTS

^{125}I-somatostatin binding to human PBL, granulocytes, red blood cells and mitogen-activated lymphocytes

Figure 1 shows the representative binding profile of ^{125}I-somatostatin to resting PBL, granulocytes, red blood cells and PHA-activated lymphocytes from normal individuals. The specific binding of ^{125}I-somatostatin to mitogen-activated lymphocytes increased linearly with the number of mitogen-activated lymphocytes, but binding sites were not evident when granulocytes, red blood cells and resting PBL were used.

To confirm the specificity of ^{125}I-somatostatin binding to mitogen-activated PBL, competitive inhibition studies were carried out using as inhibitors non-labelled somatostatin and other unrelated peptides. Only somatostatin inhibited the ^{125}I-somatostatin binding to activated lymphocytes (data not shown).

Scatchard analysis shown in Fig. 2 suggested the presence of a single class of binding sites with 1·25 × 10^{-11} M–2·5 × 10^{-5} M of

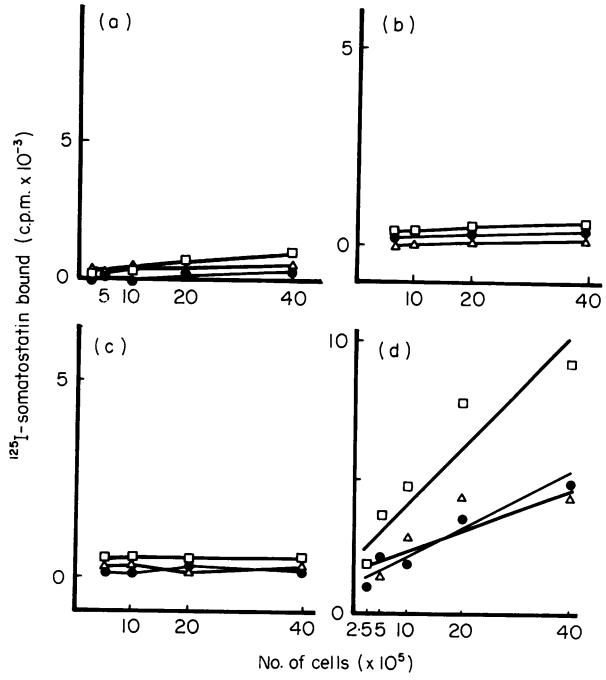

Figure 1. ^{125}I-somatostatin binding to human peripheral blood lymphocytes (a), granulocytes (b), red blood cells (c) and PHA-activated lymphocytes (d). ^{125}I-somatostatin and 0·5–2 × 10^6 cells were incubated for 120 min at 22°. Specific binding of ^{125}I-somatostatin (●) was calculated by subtracting the non-specific binding (in the presence of 10^{-4} M unlabelled somatostatin; △) from the total binding (□).

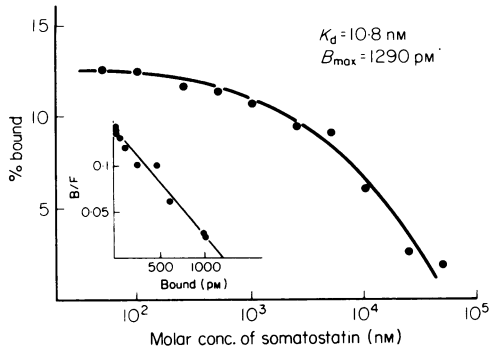

Figure 2. Inhibition of the specific binding of ^{125}I-somatostatin to PHA-activated human peripheral blood lymphocytes in the presence of increasing concentrations of unlabelled somatostatin. Each point indicates the mean of triplicate representative experiments. The dissociation constant (K_d) and number of binding sites (B_{max}) for cells were determined by Scatchard plots (insert).

somatostatin. The K_d of 10·8 nM and B_{max} of 1290 pM were estimated. This B_{max} corresponds to $7{\cdot}0 \times 10^5$ binding sites per cell.

Fluorescent somatostatin binding to mitogen-activated lymphocytes

To examine the lymphocyte subpopulation which may uniquely express the somatostatin receptor, we performed dual parameter FACS analysis using fluorescent-labelled somatostatin (SOM-DTAF) and PE-conjugated monoclonal anti-human lymphocyte antibodies (anti-CD3, CD4, CD8, CD20 and CD25 antibodies) and representative results are given in Fig. 3. SOM-DTAF bound to over 95% of the mitogen-activated lymphocytes. There was no distinct predilection among certain lymphocyte subpopulations and somatostatin receptor-positive cells.

^{125}I-somatostatin binding to human leukaemia cells

To clarify the causative relationship between neoplastic change of lymphoid cells and the expression of somatostatin receptors on the cell surface, we performed ^{125}I-somatostatin binding studies, using leukaemia cells of lymphoid or non-lymphoid origin. Figure 4 shows the representative binding profile of ^{125}I-somatostatin to leukaemia cells from four different patients (CLL, ATL, CML and AUL). It is apparent that the specific binding increased linearly with the cell number, in the case of CLL and ATL, but no specific binding of ^{125}I-somatostatin was observed with cells from CML and AUL patients. The same analytical studies of somatostatin receptors on leukaemia cells from 16 cases were done using ^{125}I-somatostatin and competitor. As is evident in Table 1, on all three ALL cells (two pre-B-cell and one T-cell origin), one ATL and on two of three CLL cells from B-cell origin, there were binding sites for somatostatin. In contrast, of the five leukaemia cells from non-lymphoid origin and three CML cells tested, only one was found to possess receptors for somatostatin.

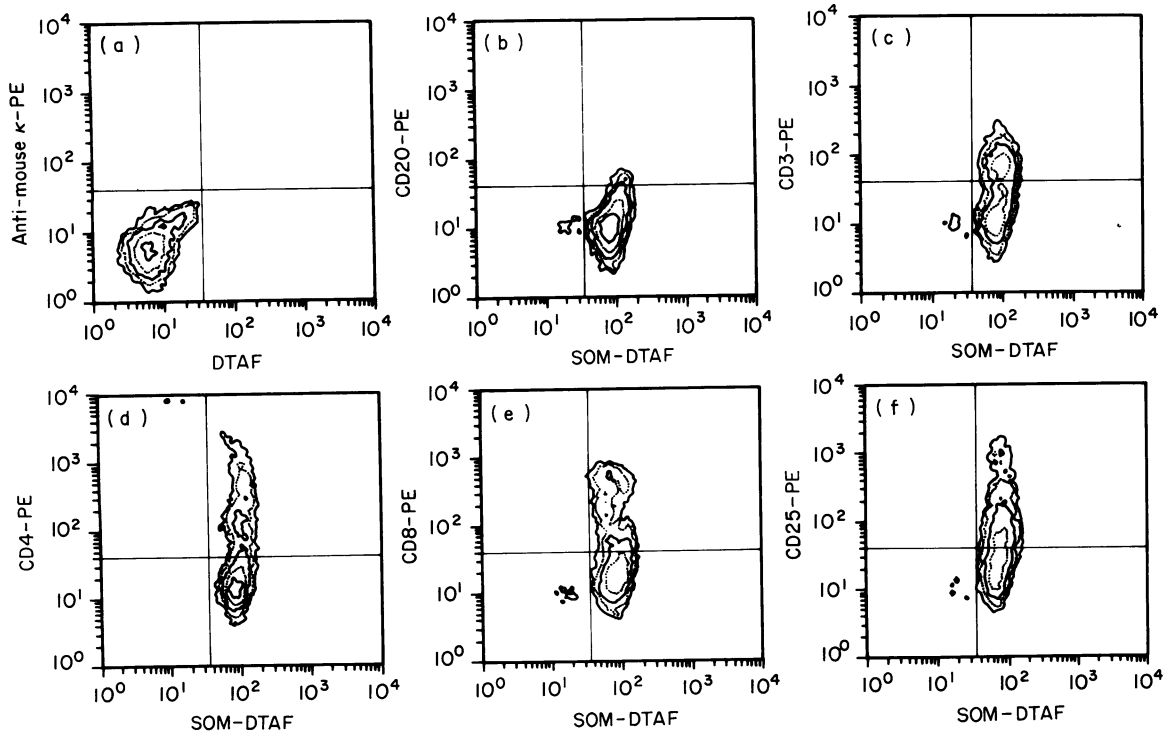

Figure 3. Dual parameter FACS analysis of fluorescein-somatostatin (SOM-DTAF) to PHA-activated normal human peripheral blood lymphocytes (PBL). PBL cultured with PHA for 48 hr were stained with monoclonal mouse anti-human lymphocyte antibodies anti-CD20 (b), anti-CD3 (c), anti-CD4 (d), anti-CD8 (e) and anti-CD25 (f) antibodies and phycoerythrin (PE)-conjugated monoclonal rat anti-mouse kappa chain antibody, followed by SOM-DTAF. Horizontal axes indicate fluorescence intensity and vertical axes indicate PE intensity, respectively. (a) Shows cells stained with DTAF and PE-conjugated anti-mouse kappa chain antibody.

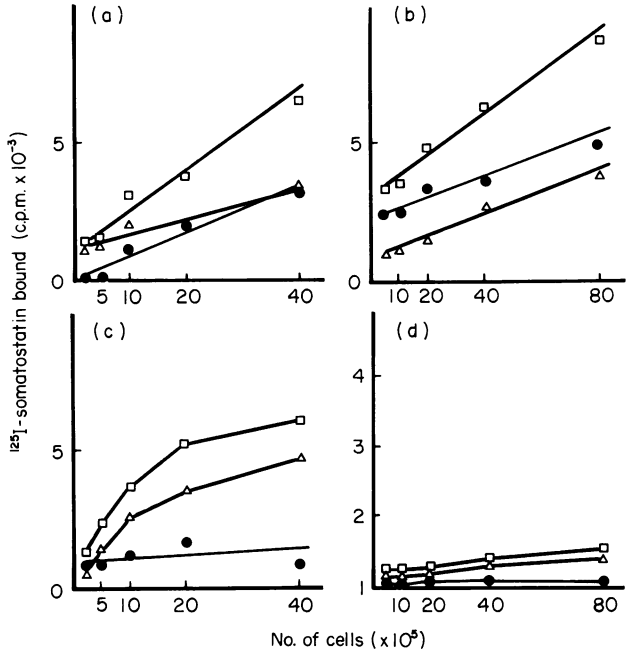

Figure 4. ^{125}I-somatostatin binding to four representative human leukaemia cells. Leukaemia cells from patients with (a) CLL (chronic lymphocytic leukaemia), (b) ATL (adult T-cell leukaemia), (c) CML (chronic myelocytic leukaemia) and (d) AUL (acute undifferentiated leukaemia) and ^{125}I-somatostatin were incubated for 120 min at 22°. Specific binding of ^{125}I-somatostatin (●) was calculated by subtracting the non-specific binding (in the presence of 10^{-4} M unlabelled somatostatin; △) from the total binding (□).

Table 1. Somatostatin receptors on human leukaemia cells

Leukaemias	Cases	Somatostatin receptor (+)	Somatostatin receptor (−)
ALL*	3	3	0
ATL	1	1	0
CLL	4	3	1
ANLL	5	1	4
CML	3	0	3

* ALL; acute lymphoblastic leukaemia, ATL; adult T-cell leukaemia, CLL; chronic lymphocytic leukaemia, ANLL; acute non-lymphocytic leukaemia, CML; chronic myelocytic leukaemia.

When the data were plotted using Scatchard analysis (Fig. 5) two classes of binding sites had a K_d of 1·59 nM (high-affinity receptors) and 16·6 nM (low-affinity receptors), respectively. The B_{max} of high-affinity receptors was calculated to be 270 pM, a value corresponding to $1{\cdot}6 \times 10^5$ binding sites per cell, and that of low-affinity receptors was 1410 pM ($8{\cdot}5 \times 10^5$ sites/cell),

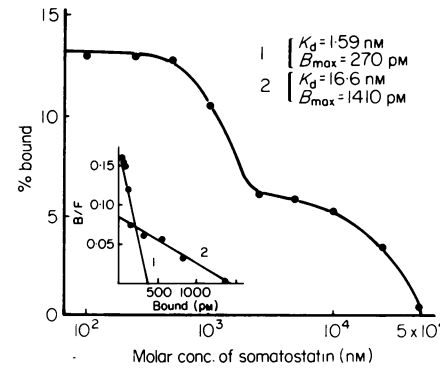

Figure 5. A representative inhibition of the specific binding of ^{125}I-somatostatin to leukaemic cells from a patient with chronic lymphocytic leukaemia in the presence of increasing concentrations of unlabelled somatostatin. Each point indicates the mean of triplicate experiments. The dissociation constant (K_d) and number of binding sites (B_{max}) for cells were determined by Scatchard plots (insert).

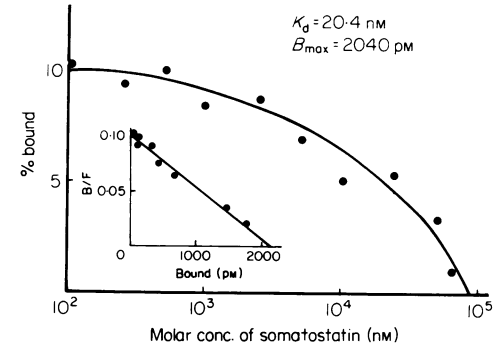

Figure 6. A representative inhibition of the specific binding of ^{125}I-somatostatin to leukaemic cells from a patient with acute non-lymphocytic leukaemia in the presence of increasing concentrations of unlabelled somatostatin. Each point indicates the mean of triplicate experiments. The dissociation constant (K_d) and number of binding sites (B_{max}) for cells were determined by Scatchard plots (insert).

respectively. Similar observations were made using different leukaemia cells of lymphoid origin (data not shown). In contrast, only one case of ANLL which expressed somatostatin receptors revealed a single comparatively low-affinity binding site with a K_d of 20·4 nM and B_{max} of 2040 pM ($12{\cdot}2 \times 10^5$ sites/cell) (Fig. 6).

DISCUSSION

Much attention has been directed to the regulatory loop between the immune and the neuroendocrine systems and attempts have been made to determine the bi-directional effects of neuropeptides on the immune regulation, from the viewpoint of the neuro-immuno-endocrine axis (Payan & Goetzl, 1985; O'Dorisio *et al.*, 1981; Stanisz, Befus & Bienenstock, 1986). Many neuroendocrine peptides of the central nervous system, including α- and β-endorphin, ACTH and met- and leu-

enkephalins inhibit or stimulate various functions of lymphocytes (Alvareg-Mon, Kehrl & Fauci, 1985; Johnson *et al.*, 1984).

We found in the present study that mitogen-activated PBL bear receptors for somatostatin, as determined by a radiobinding assay using ^{125}I-somatostatin. We also noted that human leukaemia cells possess the same receptors, in 87·5% of patients with lymphocytic leukaemia and in 12·5% of non-lymphocyte leukaemia patients, respectively. Scatchard analyses indicated that the activated PBL from normal individuals and AML cells from one patient bear a single class of low-affinity receptors for somatostatin with a K_d of approximately 10–20 nM. In contrast, most lymphocytic leukaemia cells were found to express two classes of binding sites, high- and low-affinity somatostatin receptors.

Somatostatin was originally characterized as an inhibitor of GH, ACTH, LH, FSH and prolactin (Arimura, 1981), and an inhibitory effect of somatostatin on adenylate cyclase activity was observed in various tissues (Reisine, 1985). The presence of receptors for somatostatin on pituitary membranes and other endocrine and nervous organs has also been characterized and the biological actions of somatostatin are apparently receptor-mediated (Higuchi *et al.*, 1985; Srikant & Patel, 1985; Reubi & Landolt, 1984). We previously reported that the human T-cell lymphotrophic/leukaemia virus (HTLV-1) producer adult T-cell leukaemia (ATL) cell line, MT-2, had receptors for somatostatin with a K_d of 0·64 nM (Nakamura *et al.*, 1987). Somatostatin receptors were first quantified in GH_4C_1 pituitary tumour cells using ^{125}I-somatostatin (Koch & Schonbrunn, 1984). In the central nervous system, the highest concentration of somatostatin receptors was found in the cerebral cortex, followed by thalamus, hypothalamus, striatum, amygdala and hippocampus (Higuchi *et al.*, 1985). Somatostatin receptors have also been characterized in the pituitary, adrenal cortex and pancreatic acinar cells (Natozaki *et al.*, 1986). In all these tissues, a single class of high-affinity binding sites (K_d; 0·3–1·0 nM) was identified. We noted high affinity receptors for somatostatin on MT-2 cells (Nakamura *et al.*, 1987) and on lymphoblastic leukaemia cells in the present work.

Human and murine lymphocytes have also been noted to bear receptors for somatostatin. Bhathena *et al.* (1981) reported that human blood mononuclear leucocytes posses approximately 300–500 somatostatin receptors/cell with a K_d of 0·5 μM. Scicchitano *et al.* (1987) reported that murine Peyer's patch and splenic T and B cells from both organs showed specific binding of somatostatin. They also described that 80–84% of Peyer's patch Thy-1.2-positive, Ly-1-positive or L3T4-positive cells and 94% of Lyt-2-positive cells bound somatostatin, that over 80% of B cells from this organ bound somatostatin and that approximately 30% of Thy-1.2-positive, Ly-1-positive or L3T4-positive cells bound somatostatin. In human systems, we did not obtain evidence to support the proposal that resting PBL possess receptors for somatostatin; however, there is strong evidence for the presence of somatostatin receptors on mitogen-activated PBL and the majority of lymphoblastic leukaemia cells. Using fluorescent-labelled somatostatin, we found that somatostatin bound over 95% of the mitogen-activated lymphocytes, and no distinct predilection was present among lymphocyte subsets and somatostatin receptor-positive cells. This is in contrast to the findings of Organist *et al.* (1987). These workers prepared a monoclonal antibody against the IM-9 lymphoblast substance P receptor molecule and demonstrated, by two-colour FACS analysis with fluorescein-substance P and rhodamine-labelled anti-receptor antibody, that substance P receptors express a distinct population of human PBL.

Most recently, Scicchitano *et al.* (1988) reported that the murine IgA-secreting plasmacytoma, MOPC-315, expresses somatostatin receptors. Although the functional significance of receptors for somatostatin found on lymphocytes remains obscure, the findings do support the view that the presence of high-affinity recepters for somatostatin closely correlates with the neoplastic transformation of cells from lymphoid origin. The low-affinity receptors for somatostatin on the activated PBL may function as molecules linking the regulatory loop between the immune and the neuroendocrine systems.

Somatostatin was found to block VIP-stimulated cyclic AMP formation in human peripheral lymphocytes (O'Dorisio *et al.*, 1981) and to prevent forskolin-stimulated adenylate cyclase activity in S49 lymphoma cells, which lack the stimulatory guanine nucleotide site (NS) (Jacobs, Aktories & Schultz, 1983). This peptide also inhibits the proliferation of the lymphoid cell line, Molt-4, PHA-stimulated human T lymphocytes (Payan, Hess & Goetzl, 1984b) and concanavalin A-activated rat lymphocytes (Mascardo, Barton & Sherline, 1984). In contrast to these findings, Nordlind & Mutt (1985) stated that somatostatin enhanced the proliferative response of mouse spleen cells. Bi-directional effects of this peptide on the proliferative responses of the murine plasmacytoma MOPC-315 cells were also described (Scicchitano *et al.*, 1988), and controversial results may be reflected in the multimodal action of somatostatin on lymphoid cells. We have evidence to support the hypothesis that low-dose somatostatin enhances the mitogen response of human PBL, while a high-dose inhibits the same proliferative response *in vitro* (our unpublished observations).

It appears that mitogen-activated human PBL and most lymphoblastic leukaemia cells bear receptors for somatostatin. Evidence is accumulating that the immune and neuroendocrine systems communicate by virtue of signal molecules and receptors common to both systems. The role of somatostatin and its receptors on lymphoid cells in immune regulation or dysregulation remains obscure, hence further investigations are required.

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