Substance P Regulates Somatostatin Expression in Inflammation

Arthur M. Blum, David E. Elliott, Ahmed Metwali, Jie Li, Khurram Qadir and Joel V. Weinstock

_J Immunol_ 1998; 161:6316-6322; 
http://www.jimmunol.org/content/161/11/6316

References
This article cites 34 articles, 19 of which you can access for free at: http://www.jimmunol.org/content/161/11/6316.full#ref-list-1

Subscription
Information about subscribing to _The Journal of Immunology_ is online at: http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Substance P Regulates Somatostatin Expression in Inflammation

Arthur M. Blum, David E. Elliott, Ahmed Metwali, Jie Li, Khurram Qadir, and Joel V. Weinstock

Substance P (SP) and somatostatin (SOM) are made at mucosal surfaces and sites of inflammation. There is a SP/SOM immunoregulatory circuit that modulates the IFN-γ response in murine schistosomiasis. SP enhances, while SOM decreases, IFN-γ secretion. Various inflammatory mediators induce macrophages to make SOM, but no known factor limits this expression. It was discovered that SP regulates SOM synthesis. Splenocytes from normal, uninfected mice cultured with LPS, IFN-γ, or IL-10 for 4 h strongly expressed SOM mRNA, but failed to do so in the presence of SP. The inhibition with 10⁻⁹ M SP was >85% shown by quantitative PCR. Also, splenocyte SOM content decreased from 10⁴₈ pg to <10 pg/4 x 10⁶ cells following SP exposure. Immunohistochemistry identified SOM solely within splenic macrophages following cytokine stimulation. Mice infected with Schistosoma mansoni form granulomas in the liver and intestines resulting from deposition of parasite eggs in these organs. The granulomas contain macrophages that make SOM constitutively. SP at 10⁻⁸ M decreased SOM mRNA expression >90% in dispersed granuloma cells cultured for 4 h or longer. Specific SP receptor antagonists blocked SP suppression of SOM expression in splenocytes and dispersed granuloma cells, showing that an authentic SP receptor mediated the regulation. Additional studies revealed that IL-4 antagonized the SP effect in the spleen. It is concluded that in granulomas and splenocytes from mice with schistosomiasis and in splenocytes from uninfected animals that 1) SP inhibits macrophage SOM induction and ongoing expression at the mRNA and protein levels acting through the SP receptor, and 2) IL-4 can antagonize this SP effect. The Journal of Immunology, 1998, 161: 6316–6322.

Interferon-γ is an important Th1 cytokine that enhances the cellular immune response to intracytoplasmic pathogens. Substance P (SP) and somatostatin (SOM) are short polypeptides present at mucosal surfaces and at other sites of chronic inflammation. In murine schistosomiasis, ova induce granulomas in the liver and intestines. Both SP and SOM are involved in the modulation of T cell IFN-γ secretion in murine schistosomiasis. SP enhances, while somatostatin decreases IFN-γ responses (1, 2). This regulation is significant, because they also modulated IgG2a production, which is IFN-γ-dependent (3). Also, specific SP (NK1) and SOM (SSTR2) receptors, which are expressed on T cells (4, 5), mediate the effects of SP and SOM on IFN-γ secretion (6). It is likely that other granuloma inflammatory cell types also express these receptors. Thus, there is a SP/SOM immunoregulatory circuit in schistosomiasis that may help modulate the balance between the Th1 and Th2 response.

We recently showed that various lymphoid organs express preprosomatostatin (ppSOM) mRNA and that macrophages, induced by various inflammatory mediators such as IFN-γ, IL-10, TNF-α, PGE2, cAMP analogues, and LPS, make large amounts of SOM (7). The goal of this study was to determine whether there are regulatory factors that can limit macrophage SOM expression. Because SP and SOM have opposing immunoregulatory functions, it was ascertained if SP could alter ppSOM mRNA expression in immunocytes.

It was shown that SP, at physiologic concentrations and operating through the authentic SP receptor (NK1), inhibits macrophage SOM mRNA and peptide expression both in normal splenocytes from uninfected mice and in granuloma cells from schistosome-infected animals. Also, IL-4 can block this SP effect. These data further suggest that SP and SOM are important opposing immunoregulatory molecules that help differentiate Th1 and Th2 responses.

Materials and Methods

Mice and schistosome infection

This study used female CBA mice bought from the National Cancer Institute (Washington, DC). Also used were C57BL/6 IL-4⁻/⁻ and littermate controls (IL-4⁺/+), and C57/B129 Ray-I T and B cell-deficient mice (8). Breeding colonies for the various C57BL/6 and C57/B129 Ray-I mice were maintained at the University of Iowa. At 7–8 wk of age, some mice were infected s.c. with 50 cercariae of the Puerto Rican strain of Schistosoma mansoni (9, 10).

Dispersal of granuloma cells and splenocytes and cell culture

Livers of mice sacrificed during the 8 wk of infection were homogenized for 30 s at low speed in a Waring blender. Granulomas were collected by 1 × g sedimentation and washed three times in RPMI 1640 medium (hereafter referred to as RPMI). To prepare a single cell suspension from these granulomas, the intact granulomas were incubated in a shaking water bath at 37°C for 30 min in RPMI containing 0.5% collagenase (type I from Clostridium histolyticum; Sigma, St. Louis, MO). The softened granulomas were disrupted further by repeated suction and expulsion through a 1-ml syringe. The dispersed granuloma cell suspensions were passed through a sterile gauze to exclude nondispersed fragments. The cells were collected...
by centrifugation, washed three times in RPMI, and counted. Cell viability was determined by eosin Y exclusion.

Single-cell suspensions of splenocytes were prepared from individual spleens from 8-wk-infected or uninfected mice by gentle teasing in RPMI. The cells were briefly resuspended in distilled water to lyse RBC. The splenocytes then were washed three times in a large volume of RPMI.

Cells usually were cultured for 4 h in tissue culture flasks (Corning, Cambridge, MA) with 25 ml of medium (about 4 x 10^7 cells/flask) at 37°C. Some cells were cultured for 18 h to collect supernatant for IL-4 measurement by ELISA. The culture medium was RPMI containing 10% FCS, 10 mM HEPES buffer, 2 mM L-glutamine, 100 U/ml penicillin, 5 µg/ml streptomycin, and 100 µg/ml streptomycin (all from Sigma). The cells were cultured alone or in the presence of rIL-10 (30 ng/ml) (R&D Systems, Minneapolis, MN), LPS (30 µg/ml) (Sigma), IFN-γ (200 U/ml) (Sigma), and/or SP antagonists (10 mM). The SP antagonists used were CP69,345 (Pfizer, Groton, CT) and SR 140333 (a gift from Dr. X. Emonds-Alt, Sanofi Recherche, France).

**SOM extraction**

Splenocytes were exposed to the appropriate stimulants for 4 h. SOM then was extracted from splenocytes before chromatography (11). Splenocytes (about 1.5 x 10^7) were added to 1 ml of 1 M acetic acid solution, which was heated in boiling water for 2 min and then cooled on ice. The cell remnants then were sonicated for six 5-s bursts on ice. Next, the acetylated and boiled cell extracts were centrifuged for three 10-min intervals at 630 x g to remove particulate matter. The extracts then were dried in a vacuum concentrator and reconstituted in appropriate solutions for HPLC. Extracts from each of three separate experiments were pooled for each HPLC fractionation and RIA measurement.

**HPLC**

Extracts first were passed through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) that was prewetted with 100% acetonitrile followed by 0.05% trifluoroacetic acid (TFA) (Pierce, Rockford, IL) in water. The extracts then were loaded onto the cartridge, washed with 0.05% TFA (20 ml), and eluted with 50% acetonitrile in 0.05% TFA (3 ml). The eluates were dried in a vacuum concentrator.

SOM was identified by elution profile using a HPLC system (Waters Associates). Peptide fractionation was performed on a C18 reverse-phase column (Waters Associates). Peptides were eluted over 45 min at a flow rate of 1 ml in a linear gradient of 20–45% acetonitrile in 0.05% TFA (Pierce). Samples were dissolved in 20% acetonitrile containing 0.05% TFA. To determine the elution time of the immunoreactive SOM, 1-ml fractions of eluate were collected serially, dried, reconstituted in buffer, and assayed for SOM by RIA. The elution time of authentic SOM-14 was determined by injecting synthetic SOM 1–14 (Sigma) into the C18 column and monitoring its elution using a model 410 multiwave detector (Waters Associates) set at 214 nm. Blank control samples were run through the HPLC column before injecting each experimental sample and assayed for SOM by RIA to assure that there was no carryover of SOM between runs.

**RIA**

Extracts were dissolved in 0.5 ml of 0.1 M Tris-acetate buffer, pH 7.4, containing 0.2% BSA (Sigma). The RIA was performed as previously reported (11). 3H-[3,5]-somatostatin (New England Nuclear, Boston, MA) and rat anti-SOM mAb (S607; a generous gift from Dr. X. Emonds-Alt, University of California, Los Angeles, CA) were diluted in the Tris-acetate buffer containing 0.2% BSA. Synthetic SOM 1–14 (SOM) was purchased from Sigma.

**Immunohistochemistry**

Murine splenocytes (5 x 10^7/well) were incubated at 37°C for 4 h in four-chamber tissue culture slides (Lab-Tek, Nunc, Naperville, IL) with rIL-10 (30 ng/ml). Adherent cell and cytospin preparations (derived from nonadherent cell population) were used for immunohistochemical detection of intracellular SOM. Cytospin preparations were prepared by centrifuging 4 drops of a suspension of cells (1.2 x 10^6/ml), for 10 min at 32.2 x g onto slides that were precleaned with acid-alcohol (Cytospin II, Shandon Southern Instruments, Sewickley, PA).

Cells preparations were fixed for 5 min in 95% ethanol with 5% glacial acetic acid (v/v) and washed three times for 5 min each in Trizma buffer solution (TBS). Slides were submerged for 15 min at room temperature in TBS containing 0.1% (v/v) Triton X-100 (Sigma), followed by 5 min in TBS, 2 min in methanol, and 30 min in 3% H2O2, (v/v) in methanol. Slides subsequently were washed in methanol for 2 min, TBS for 5 min, and three times in TBS containing 0.1% (v/v) BSA for 3 min. After treatment with hyaluronidase (1 mg/ml in 100 mM sodium acetate, 0.85% (v/v) NaCl) (Type IV-S; Sigma) for 30 min at 37°C, splenocyte preparations were blocked for 30 min at room temperature with 1% rabbit serum in TBS containing 0.5% BSA. This was repeated using a blocking kit to block nonspecific avidin–biotin binding (SP-2001, Vector Laboratories, Burlingame, CA). Slides were washed three times in TBS, incubated with anti-FcγRII/III mAb (2.4G2, HB-197; American Type Culture Collection, Manassas, VA) for 30 min at room temperature and washed three times more in TBS with 0.1% Triton X-100. Splenocyte preparations were incubated overnight at 4°C in a humidity chamber with 5 µg/ml biotinylated anti-SOM mAb (CURE.S607) or preabsorbed S607 as control diluted in TBS containing 0.1% Triton X-100, 1% rabbit serum, and 0.1% BSA. CURE.S607 was biotinylated using a biotin reagent (BHNS; Zymed Laboratories, South San Francisco, CA) according to the instructions from the manufacturer. Control mAb was biotinylated S607 mAb preabsorbed for 30 min at 37°C with 30 M excess of synthetic SOM 1–14 (Sigma). Cell preparations were washed three times in TBS with 0.1% BSA. Then, they were exposed to avidin–horseradish peroxidase (1:1000 dilution; Zymed) followed by 0.05% diaminobenzidine (Sigma) containing 0.1% H2O2. Cells were counterstained using Gils No. III hematoxylin (Sigma).

**RNA extraction and competitive PCR assay for SOM cDNA**

Each experiment used RNA from splenocytes or granuloma cells pooled from three to four separate mice. Total cellular RNA was extracted from cell suspensions by homogenization in guanidinium/acid-phenol as previously described (12). Cellular RNA (5 µg) was reverse-transcribed with Moloney-monkey leukemia virus (400 U) using an 18-mer of oligo-dT (0.5 µg) as primer. The first strand cDNA was diluted to 250 µl, and 15 µl (0.3 µg RNA) was added to PCR buffer containing 2 U Taq DNA polymerase, 1.4 mM MgCl2, 50 mM KCl, and 100 mM Tris, pH 8.3, in a total volume of 50 µl. The sense primer to amplify ppSOM was 5'-ATGCTGTCTT GCCGCTCCTCCAGT-3', and the anti-sense primer was 5'-ACAGAGATG GAATGCTTCCAG-3'. The PCR consisted of 35 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min. Products of RT-PCR amplification was analyzed by agarose gel electrophoresis using 1.7% Nusieve GTG agarose (FMC Bioproducts, Rockland, ME) in 0.5× TBE buffer. The ppSOM competitive mimic plasmid was made as previously described (7). The ppSOM mimic plasmid was 2.219 x 10^6 g/M. Known quantities of mimic plasmid DNA containing double-stranded abbreviated ppSOM cDNA were added to PCR reactions containing cDNA from mRNA reverse-transcribed with oligo-dT.

Total RNA preparations contained equivalent 18S and 28S RNA bands. RNA extracts were quantified spectrophotometrically. In most experiments, samples were compared for content of actin to further confirm equivalent mRNA content and reverse transcription.

**FIGURE 1.** SP inhibits ppSOM mRNA expression in splenocytes. Splenocytes (4 x 10^6/flask) from uninfected, normal CBA mice were cultured in vitro in RPMI for 4 h alone (cells only) or with LPS (30 µg/ml), rIFN-γ (200 U/ml), or rIL-10 (30 ng/ml). Some cultures also received SP (10 µM). After the incubation, splenocyte RNA was extracted, reverse-transcribed, and amplified by PCR for ppSOM cDNA. Data are representative of two to three separate experiments.
Table I. SP inhibits IL-10-, LPS-, and IFN-γ-induced ppSOM expression in splenocytes from uninfected CBA mice

<table>
<thead>
<tr>
<th>Cells</th>
<th>mRNA ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells only</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Cells + IL-10 (30 ng/ml)</td>
<td>4.75 ± 1.0</td>
</tr>
<tr>
<td>Cells + IL-10 + SP</td>
<td>0.55 ± 0.1*</td>
</tr>
<tr>
<td>Cells + LPS (30 μg/ml)</td>
<td>5.50 ± 2.1</td>
</tr>
<tr>
<td>Cells + LPS + SP</td>
<td>0.88 ± 0.2*</td>
</tr>
<tr>
<td>Cells + IFN-γ (200 U/ml)</td>
<td>5.00 ± 1.4</td>
</tr>
<tr>
<td>Cells + IFN + SP</td>
<td>0.60 ± 0.5*</td>
</tr>
</tbody>
</table>

* Cells were cultured 4 h as described in Fig. 1 with indicated factors. All data are mean fentogram ppSOM mRNA/0.4 μg total cellular mRNA ± SD from each of two separate experiments.

IL-10, LPS, or IFN vs IL-10, LPS, or IFN + SP; p < 0.01.

Results

SP inhibits ppSOM mRNA induction in splenocytes

It previously was shown that LPS, IFN-γ, and IL-10 induce ppSOM mRNA expression in dispersed splenocytes from normal CBA, B129 or C57BL/6 mice cultured in vitro (7). SOM and SP work antagonistically to modulate IFN-γ secretion from T cells (1, 2). Because SOM and SP function in a common IFN-γ immunoregulatory circuit, it was determined if SP could affect ppSOM mRNA expression.

Splenocytes from normal CBA mice did not express ppSOM mRNA when cultured in vitro with SP for up to 18 h. As expected, cells cultured with LPS, rIFN-γ, or rIL-10 for as little as 4 h expressed ppSOM mRNA strongly. However, SP at 10⁻⁹ M or higher strikingly antagonized LPS, rIFN-γ, or rIL-10 induction of ppSOM mRNA (Fig. 1). The inhibition with 10⁻⁸ M SP was always >85% as demonstrated by quantitative PCR (Table I). Also, this inhibition was sustained when cells were cultured in vitro for up to 48 h (data not shown). Higher concentrations of SP (i.e., 10⁻⁷ M) frequently resulted in no detectable ppSOM cDNA. Experiments using LPS or rIFN-γ gave similar results (Fig. 1 and Table I).

SP modulates ongoing ppSOM expression in granulomas

Animals infected with S. mansoni form Th2-type granulomas in the liver and intestines resulting from deposition of parasite eggs in these organs. The granulomas contain macrophages that make SOM (13). It was determined if SP could modulate ongoing SOM expression.

Dispersed granuloma cells cultured in vitro for up to 18 h expressed ppSOM mRNA constitutively. Exposure to LPS, rIFN-γ, or rIL-10 did not alter endogenous ppSOM mRNA levels. However, cells cultured with SP for as little as 4 h had substantially less ppSOM mRNA. The decrease in ppSOM mRNA expression was

>75% or >90% when SP was used at 10⁻⁹ or 10⁻⁸ M, respectively (Fig. 2), as measured by quantitative PCR (Table II). The SP suppression of ppSOM mRNA expression was sustained in 18-h cultures (data not shown).

The SP effect is neurokinine 1 (NK1) receptor-mediated

SP belongs to a family of molecules called tachykinins. It functions through the NK1 receptor. The brain, spleen, and granulomas of mice infected with schistosomiasis express this receptor (4). There are several potent and highly selective nonpeptide NK1 receptor antagonists like SR140333 and CP96345. SR140333 is a NK1 receptor antagonist particularly potent in mice (14). Experiments employed SR140333 and CP96345 to determine whether they could block SP modulation of ppSOM mRNA expression.

Splenocytes from uninfected, normal CBA mice were cultured in vitro alone or in the presence of rIL-10 with or without SP. Some wells also contained either of the two SP receptor antagonists. As seen in Fig. 3, SR140333 totally prevented SP from inhibiting ppSOM induction. CP96345 afforded similar results (data not shown). Neither inhibitor used alone induced ppSOM expression.

Fig. 3 also shows that SP receptor antagonists block SP modulation of ongoing ppSOM expression within dispersed granuloma cells cultured in vitro.

SP can also govern SOM protein production

It next was determined if SP modulation of splenocyte ppSOM mRNA expression resulted in a change in splenocyte SOM content. Splenocytes from uninfected mice were cultured for 4 h in vitro in the presence or absence of rIL-10 and/or SP (10⁻⁶ M) in RPMI. Following the incubation, the cells were boiled and sonicated in acetic acid solution to extract SOM. The extracts then

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** SP inhibits ongoing ppSOM mRNA expression in granuloma cells. Dispersed granuloma cells from CBA mice infected with S. mansoni for 8 wk were cultured in vitro, as described in Fig. 1, in the presence or absence of SP at the indicated concentration. SP at 10⁻⁹ and 10⁻⁸ M suppressed ppSOM mRNA expression by >75 and >90%, respectively. Data are representative of two separate experiments.

![FIGURE 3](http://www.jimmunol.org/)

**FIGURE 3.** SP receptor antagonist blocks SP regulation of ppSOM mRNA expression. Splenocytes from uninfected CBA mice or dispersed granuloma cells from 8-wk-infected animals were cultured as described in Fig. 1. Some cultures contained rIL-10 (30 ng/ml), SP (10⁻⁶ M) and/or SP receptor antagonist SR140333 (10⁻⁷ M) as indicated in the figure. Data are representative of two separate experiments.
were eluted through an HPLC column. The column eluant was collected in 1-ml fractions that subsequently were assayed from SOM content using an RIA.

Immunoreactive SOM only was evident in the elution fraction corresponding to the elution time of synthetic SOM 1–14. Splenocytes cultured with rIL-10 for 4 h contained large amounts of authentic SOM. However, cells exposed to medium containing SP had no detectable SOM (Table III). The RIA was sensitive down to 5 pg/ml.

**Splenic macrophages store immunoreactive SOM**

We previously showed that granuloma macrophages contain SOM. We used immunohistochemistry, employing an anti-SOM mAb, to locate the storage site of SOM within the mixed spleen cell population. Immunoreactive SOM localized solely to mononuclear cells with bean-shaped nuclei and other morphologic features of macrophages (Fig. 4).

**Neither T cells nor B cells are required for SP regulation of ppSOM mRNA induction**

It is likely that macrophages are the major source of SOM in the granuloma (13) and spleen. The above experiments suggest that SP interacts with unknown cellular elements of the spleen and granuloma to govern ppSOM expression. Rag-1 mutant mice do not produce mature B or T lymphocytes because of mutation in their recombination activation gene (8). Their spleens contain predominantly NK cells and monocytes. We used splenocytes from uninfected Rag-1 mice to determine whether SP could still regulate ppSOM mRNA expression in the absence of mature T or B cells.

Splenocytes from Rag-1 mice can express ppSOM mRNA. Fig. 5 shows that in the absence of T and B cells, SP still modulates ppSOM mRNA expression. Thus, SP can alter SOM production without the aid of either T or B cell interactions. Also, the NK1 receptor antagonist, SR140333, blocks SP modulation of ppSOM expression in Rag-1 mice.

**IL-4 is a natural antagonist of the SP effect**

Splenocytes from schistosome-infected mice of either the CBA or C57BL/6 strain constitutively express ppSOM mRNA (7). Unlike normal spleen cells from uninfected mice of either strain or granuloma cells, this ppSOM mRNA expression was not subject to SP regulation (data not shown).

Splenocytes from mice infected with schistosomiasis produce IL-4 constitutively, which is not the case for spleen cells from uninfected animals (15). This prompted us to determine whether IL-4 naturally antagonized SP regulation of ppSOM mRNA expression. The first series of experiments studied ppSOM expression and SP regulation in IL-4 mutant mice unable to make IL-4.

C57BL/6 IL-4−/− mice infected with schistosomiasis expressed ppSOM mRNA in the spleen at levels similar to that of wild-type controls. However, unlike the IL-4+/+ mice, SP could abrogate ppSOM mRNA expression in splenocytes from IL-4−/− animals (Fig. 6) (Table IV). To explore the importance of IL-4 in regulating the SP effect, splenocytes from IL-4−/− mice were cultured for 4 h in the presence or absence of SP and/or rIL-4 before ppSOM mRNA quantification. Fig. 7 and Table IV show that rIL-4 antagonized SP regulation of ppSOM mRNA expression in IL-4−/− mice. Recombinant IL-4 use alone did not affect ppSOM mRNA levels.

Next, splenocytes from uninfected, normal CBA mice were cultured for 4 h with rIL-10 to induce ppSOM mRNA expression.

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/Content/6319.png)

**FIGURE 4.** Immunohistochemistry showing SOM (black) in splenic macrophages from CBA mice. Cells were counterstained using Gills No. III hematoxylin. (Magnification, ×400).

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/Content/6320.png)

**FIGURE 5.** SP suppresses ppSOM mRNA expression in splenocytes from C57/B129 Rag−/− mutant mice. Splenocytes were cultured, as describe in Fig. 1, with rIL-10 (30 ng/ml) or rIL-10 plus SP (10−6 M). Data are representative of two separate experiments. MW, molecular weight standards.

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/Content/6321.png)

**FIGURE 6.** SP inhibits ppSOM mRNA expression in splenocytes from IL-4−/− mutant mice, but not littermate controls infected with schistosomiasis. Dispersed splenocytes from IL-4+/+ (control) or IL-4−/− (IL-4 KO) C57BL/6 mice infected with S. mansoni for 8 wk were cultured in vitro, as described in Fig. 1, either alone (cells only) or with SP (10−6 M). Splenocytes from infected mice always constitutively express ppSOM mRNA. Data are representative of three separate experiments.
and/or SP (10^−6 M), described in Fig. 1, either alone (cells only) or with rIL-4 (200 U/ml) in separate experiments. ND, not done.

Operates through the authentic SP receptor (NK1). Altogether, the latter observation implies that the SP expression, and specific SP receptor antagonists completely inhibit inflammatory cells. As little as 10^−9 M SP strongly affects ppSOM mRNA expression within the inflammatory cells even when cultures received large quantities of supplemental rIL-4 (Fig. 9).

Discussion

The data currently presented show that SP inhibits LPS-, IFN-γ-, and IL-10-induced ppSOM mRNA expression in splenocytes from normal, uninfected mice. Moreover, SP down-modulates ongoing ppSOM mRNA expression in granulomas of schistosome-infected animals. Our HPLC data also suggest that this inhibition in mRNA production results in less SOM peptide synthesis within the inflammatory cells. As little as 10^−9 M SP strongly affects ppSOM expression, and specific SP receptor antagonists completely inhibited this regulation. This latter observation implies that the SP operates through the authentic SP receptor (NK1). Altogether, the above findings further support our contention that SP regulation of SOM expression is biologically relevant.

As true for SOM, authentic SP and its mRNA are readily detected in schistosome granulomas (6, 10). Schistosome granulomas are focal inflammatory responses rich in eosinophils and macrophages as well as other inflammatory cell types. Unlike SOM, which is made by granuloma macrophages (13), SP in schistosome granulomas probably derives from eosinophils (16). However, additional reports suggest that macrophages from the peritoneal cavity and other locations and the P388 macrophage cell line (17) are other sources of SP.

We showed here, using immunohistochemistry, that macrophages are the likely source of SOM in the spleen. Moreover, macrophage-like but not T or B cell lines contain ppSOM mRNA (7). Splenocytes from Rag-1 mice, which do not have mature T or B cells, expressed ppSOM mRNA, further supporting the above contention.

Inflammatory cells can display the NK1 SP receptor. Some human PBL (18), murine lymphocytes from Peyer’s patches and spleen (19), and several B (20, 21) and T cell lines bind SP. In murine schistosomiasis, splenocytes and dispersed granuloma inflammatory cells express a functional SP receptor (1) that can signal to enhance IFN-γ production (1, 4). Splenocytes, thymocytes, granuloma T cells, and several granuloma-derived, CD4^+ Th2 cell lines express SPr mRNA (4).

Yet, SP blocked ppSOM mRNA production in Rag-1 splenocytes, suggesting that neither T nor B cells are required for this SP receptor-dependent, regulatory process. Guinea pig (22), rat (23, 24), and mouse (25, 26) peritoneal macrophages also can bind SP. Although not yet fully demonstrated, our experiments using Rag-1 mice suggest that SP regulates macrophage SOM synthesis through direct interaction with SP receptors located on these cells.

Table IV. SP inhibits ppSOM mRNA expression in splenocytes from C57BL/6 IL-4^−/−, but not IL4^−/−, mice infected with S. mansoni and IL-4 prevents SP inhibition of ppSOM mRNA expression in IL-4^−/− animals

<table>
<thead>
<tr>
<th>Cells^b</th>
<th>IL-4^−/−</th>
<th>IL-4^−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells only</td>
<td>3.50 ± 0.7</td>
<td>3.50 ± 0.7</td>
</tr>
<tr>
<td>Cells + SP</td>
<td>3.80 ± 0.6</td>
<td>0.80 ± 0.13^c</td>
</tr>
<tr>
<td>Cells + SP + IL-4</td>
<td>ND</td>
<td>4.20 ± 0.6</td>
</tr>
<tr>
<td>Cells + IL-4</td>
<td>ND</td>
<td>4.00 ± 0.5</td>
</tr>
</tbody>
</table>

^a Cells were cultured 4 h as described in Fig. 1 with indicated factors. All data are mean femtogram ppSOM mRNA/0.4 μg total cellular mRNA ± SD from each of two separate experiments. ND, not done.

^b Cells were cultured with rIL-4 (200 U/ml) and/or SP (10^−6 M).

^c Cells vs cells + SP, p < 0.01.

Table V. Recombinant IL-4 prevents SP regulation of ppSOM mRNA expression in splenocytes from uninfected CBA mice

<table>
<thead>
<tr>
<th>Cells^b</th>
<th>mRNA ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells only</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cells + IL-10</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>Cells + IL-10 + SP</td>
<td>0.4 ± 0.2^c</td>
</tr>
<tr>
<td>Cells + IL-10 + SP + IL-4</td>
<td>6.0 ± 2.8</td>
</tr>
<tr>
<td>Cells + IL-10 + IL-4</td>
<td>5.0 ± 1.4</td>
</tr>
</tbody>
</table>

^a Cells were cultured 4 h as described in Fig. 1 with indicated factors. All data are mean femtogram ppSOM mRNA/0.4 μg total cellular mRNA ± SD from each of two separate experiments.

^b Cells were cultured with rIL-10 (30 ng/ml), rIL-4 (200 U/ml), and/or SP (10^−6 M).

^c IL-10 vs IL 10 + SP, p < 0.01.
SP can increase IFN-γ production and effect other aspects of immune responses. In murine schistosomiasis, SP substantially enhances IgG2a production through its effects on IFN-γ secretion (3). Also, SP may increase PWM-induced, duodenal IFN-γ synthesis in humans (27). Moreover, a SP antagonist given in vivo to mice impedes an appropriate IFN-γ response to intestinal salmonellosis (28). It is possible that SP affects the production of other regulatory cytokines (29–31) and Ig isotypes (21, 32–34).

Schistosome granulomas contain Th1 cells that are the major source of IFN-γ within these lesions (35). SOM inhibits IFN-γ secretion. The effect of SP on IFN-γ and SOM synthesis in schistosomiasis suggests that SP can promote IFN-γ production in Th2-type responses both through stimulating IFN-γ secreted from T cells and inhibiting SOM production.

While SP blocks leukocyte ppSOM mRNA expression, IL-4 can counter this SP regulation. This was demonstrated both in normal C57BL/6 and CBA mice and in C57BL/6 IL-4−/− mice. Even SP used at 10−6 M proved ineffective in the presence of IL-4. IL-4 is a Th2 cytokine that induces many of the phenotypic characteristics of a Th2 response (36). Early expression of IL-4 helps block development of IFN-γ-producing T cells, whereas, IFN-γ inhibits growth of IL-4 generating T cells. SP augments T cell IFN-γ release (1) and thereby promotes Th1 responses. SOM suppresses T cell IFN-γ release (2) to aid Th2 responses. SP inhibits SOM secretion, effectively preventing SOM-mediated inhibition of IFN-γ synthesis. In this context, prevention of the SP effect by IL-4 is important, because IL-4 allows continued SOM production that in turn further inhibits T cell IFN-γ secretion.

However, this IL-4 regulation of SP was not evident in the granuloma. Schistosome granulomas make large amounts of IL-4. Yet, SP was still a powerful inhibitor of SOM mRNA expression in dispersed granuloma cells. Granuloma cells cultured with supplemental rIL-4 also failed to express ppSOM mRNA after SP stimulation, suggesting that insufficient endogenous production of IL-4 was not the deciding factor. Thus, there are probably additional immunoregulatory factors that govern ppSOM mRNA expression at sites of inflammation.

Lymphocytes at mucosal surfaces and at other sites of inflammation are exposed to multiple Ags. The immune system is under constant stimulation and must be tightly regulated to avoid needless organ damage. At mucosal surfaces and other sites of inflammation, SP may prompt Th1 cells to secrete IFN-γ and, therefore, inhibit Th2 cell development. Somatostatin provides the opposite signal, suppressing IFN-γ release. Thus, these neuropeptides, which are part of the innate immune system, may help tune both the intensity and nature of the T cell inflammatory response. In this report, we showed that SP can inhibit SOM synthesis by inflammatory cells and that IL-4 can oppose this action. These new observations further suggest that SP and SOM are opposing components of an important immunoregulatory circuit.

References


