The somatostatin immunoregulatory circuit present at sites of chronic inflammation

Joel V Weinstock and David Elliott

Division of Gastroenterology–Hepatology, Department of Medicine, University of Iowa, Iowa City, Iowa 52242, USA

(Correspondence should be addressed to J V Weinstock, Gastroenterology–Hepatology Division, Department of Internal Medicine, 4607JCP, University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242, USA)

Abstract

Somatostatin is part of an immunoregulatory circuit that helps limit interferon- γ (IFN γ) production at sites of chronic inflammation. In murine schistosomiasis, parasite eggs induce focal, chronic granulomatous inflammation in the liver and intestines. These granulomas produce somatostatin 1–14 and express somatostatin receptor subtype number 2 (SSTR₂), which is the exclusive somatostatin receptor present in this inflammation. Granuloma and splenic macrophages as well as macrophage cell lines make somatostatin. There appears to be no other inflammatory cell source of the peptide. Various inflammatory mediators induce this expression, whereas substance P inhibits somatostatin production. Somatostatin can suppress IFN γ secretion from T cells via interaction with the SSTR₂ receptor expressed on these cells. Other cells within the granuloma also display SSTR₂. The effect of somatostatin on these other cell types remains unknown. The thymus of normal mice has a complete somatostatin regulatory circuit. The thymic epithelial and dendritic cells make somatostatin. Like the granulomas of murine schistosomiasis, the thymus expresses only SSTR₂. Somatostatin likely has an important role in thymic T cell education and selection.

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Introduction

Granulomas are the consequence of a normal immune response to some persistent noxious substances like mycobacteria or helminthic ova that invade the host. The purpose of this granulomatous response is to check the spread of persistent micro-organisms and to encase substances injurious to host tissue. Such granulomas form over several days and can endure for many years. All granulomas are governed by complex immunoregulatory circuits that allow destruction or confinement of the inciting factor, while limiting the immune response to avoid unnecessary tissue injury. Experimental models of granulomatous inflammation like murine schistosomiasis permit detailed analysis of the basic immune mechanisms involved in adaptive immunity and chronic immunoregulation.

Murine schistosomiasis is a particularly useful model for studying the unique features of chronic maintenance stage inflammation. Animals infected with the helminthic parasite *Schistosoma mansoni* form discrete, focal granulomas around the parasite eggs that deposit in the liver and intestines. Each granuloma lasts for many weeks and can be readily isolated from host tissue. After dissociation with collagenase, they yield large numbers of leukocytes, which are the immune cells from the site of chronic inflammation (1). Schistosome

granulomas are type 2 helper T cell (Th2) responses composed of about 50% eosinophils, 30% macrophages, 10% T cells and other immune cell types. The granulomas make predominantly Th2-type cytokines like interleukin (IL) 4 and IL5. Although these granulomas are Th2 responses, they contain large numbers of T cells that can produce the Th1 cytokine interferon- γ (IFN γ) (2). However, these granulomas produce little IFN γ because they also produce other important regulatory factors that limit IFN γ expression.

Substance P (SP) and somatostatin (SST) are two short polypeptide molecules found throughout the body. They are produced in nerves and endocrine cells at mucosal surfaces.

In murine schistosomiasis, both SP and SST participate in the regulation of IFN γ secretion from T cells. SP increases, while SST decreases the IFN γ response (3, 4). They modulate the rate of IFN γ production from T cells, particularly when T cells are exposed to a suboptimal antigen stimulation. Exposing the cells to physiological concentrations of SP can neutralize the inhibitory effect of SST on IFN γ production. Similarly, an antibody that specifically blocks one of the five specific SST receptors (SSTR₂) abrogates this regulation (5). This suggests that SST regulates IFN γ via interaction with SSTR₂.

It is important to regulate IFN γ synthesis at sites of inflammation because this molecule controls macrophage

activation, antigen presentation to T cells, helper T cell development, and aspects of B cell immunoglobulin subclass switching. An overly exuberant IFN γ response could lead to extensive tissue injury, whereas a suboptimal IFN γ response may promote microbial invasion.

The effect of SP and SST on IFN γ secretion is substantial, since they also modulate immunoglobulin-G2a (IgG2a) production (3, 6). IgG2a expression is heavily dependent on IFN γ stimulation. Octreotide, an SST receptor (SSTR) agonist, given to mice *in vivo* at the time of initial schistosome egg deposition, will completely abrogate granuloma IgG2a secretion (6). This was shown using a pulmonary model of granulomatous inflammation in which schistosome ova were embolized to the lungs. Splenocytes depleted of T cells make IgG2a in response to recombinant (r) IFN γ , but make no IFN γ endogenously. In the absence of T cells, neither SP nor SST modulate rIFN γ -induced IgG2a secretion. These and other experiments suggest that SP and SST modulate B cell IgG2a secretion in murine schistosomiasis through a direct interaction with the T cells that make IFN γ rather than through mechanisms that directly regulate B cell function.

In our animal model, we currently have no evidence that SST down-modulates expression of other T cell-derived cytokines. Unfractionated splenocytes or granuloma cells cultured *in vitro* make normal amounts of IL4, IL5, IL10, transforming growth factor- β (TGF β), and transforming nerve factor- α (TNF α) after SST exposure. This may infer that SST regulates T cell IFN γ secretion in a highly selective fashion. However, cytokines derive from many different cell sources. Unlike IFN γ , which is mostly of CD4+ T cell origin, the IL4, IL10 (2), TGF β and many other mediators emanating from dispersed granuloma cells or splenocytes come mostly from non-T cells (unpublished observations). Thus, further experiments are required using purified T cells and T cell lines to thoroughly address this issue.

SST production in murine granulomas

Immune cells from dispersed schistosome granulomas secrete SST (7). They contain the 117 amino acid precursor peptide preprosomatostatin (ppSST). In the brain and the intestine, SST is released from its precursor protein as either a 14 or a 28 amino acid moiety. The granulomas only make the 14 amino acid species.

If granulomas make SST, they must express the ppSST mRNA. We showed that inflammatory cells isolated from murine schistosome granulomas do indeed produce mRNA for ppSST (8). The sequence of granuloma ppSST mRNA is identical to that predicted by the sequence obtained from murine genomic DNA. We therefore conclude that granuloma cells synthesize SST. Granuloma cells from at least three mouse strains (C57BL/6, CBA and B129) express SST mRNA. Thus,

production of SST at sites of chronic inflammation is a property universal to the murine species.

Macrophages are the likely source of this ppSST. Adherent granuloma and spleen cells from infected mice, which comprise mostly macrophages, express ppSST mRNA. Three macrophage cell lines (p388D1, J774 and RAW 264.7) express ppSST mRNA by RT-PCR (8). Various B cell and T cell lines do not express ppSST transcripts. Granuloma cells and splenocytes from schistosome-infected B cell deficient J_HD mice express ppSST mRNA. These observations, in addition to immunohistochemical (9) and radioimmunoassay (RIA) evidence, strongly suggest that macrophages synthesize and secrete SST, and are the predominant source of this molecule within granulomas.

Rag-1 mice are deficient in T and B cells. Splenocytes from Rag-1 mice contain macrophages, dendritic cells, neurokinin (NK) cells, myeloid cells and mast cells. Splenocytes from Rag-1 mice also express SST probably derived from the splenic macrophage or dendritic cell population (8).

In murine schistosomiasis, egg deposition begins about 6 weeks after initiation of the infection. It is the eggs that deposit in the liver and intestines that induce the Th2 response and the eosinophilic granulomas characteristic of this infection.

Splenocytes from mice colonized with schistosomes constitutively express ppSST mRNA, which is not evident in splenocytes from normal, uninfected animals. Splenocytes from infected mice do not express ppSST transcripts until onset of egg deposition and granuloma development. Animals exposed just to non-viable schistosome ova will express ppSST mRNA in their spleens suggesting that the ova are responsible for induction of ppSST in the spleen (8).

The spleens in murine schistosomiasis contain no ova or granulomas. This suggested that the induction of ppSST mRNA in the spleen may result from exposure to circulating inflammatory mediators produced in granulomas at sites distant from the spleen. Subsequent experiments showed that various inflammatory mediators could quickly induce (≤ 4 h) ppSST mRNA in splenocytes from normal, uninfected mice. Such mediators included rIL10, rIFN γ , rTNF α , prostaglandin E₂ (PGE₂), vasoactive intestinal polypeptide (VIP) and dibutyryl cAMP (8). Cytokines that had no effect were TGF β and rIL4. Moreover, 4 h of exposure to anti-CD3 to induce T cell activation did not stimulate expression of ppSST transcripts.

It is notable that rIFN γ induces splenic adherent cells to transcribe ppSST. This is probably a direct effect since rIFN γ (200 U/ml) rapidly induces ppSST transcripts in the RAW 264.7 macrophage cell line (Fig. 1). SST inhibits secretion of IFN γ by antigen-stimulated T cells (5). Thus, IFN γ can induce a negative feedback circuit by augmenting macrophage SST production.

Substance P is another short polypeptide present at mucosal surfaces and at sites of chronic inflammation.

Figure 1 RAW 264.7 macrophage cells express ppSS mRNA after 4 h of exposure to 200 U rIFN γ . Total RNA from RAW 264.7 cells was extracted and analysed by RT-PCR for ppSS transcripts as previously described (11).

As indicated above, both SP and SST help regulate T cell IFN γ secretion. SP enhances while SST decreases the IFN γ response (10). Because SP and SST have opposing immunoregulatory functions, it was determined if SP could influence SST production at sites of inflammation.

SP prevents other inflammatory mediators from inducing ppSST mRNA expression in splenocytes from normal, uninfected mice (11). SP also down-modulates ongoing ppSST mRNA expression in granulomas of schistosome-infected animals. This inhibition in mRNA productions results in a decrease in SST peptide synthesis within the inflammatory cells. The SP effect is potent, requiring only 10^{-9} mol/l SP to nearly completely suppress SST expression. Specific SP receptor antagonists completely inhibit this regulation implying that SP operates through the authentic NK-1 SP receptor, which is expressed on immunocytes. SP blocks ppSST mRNA productions in *Rag*-1 splenocytes, suggesting that neither T nor B cells are needed for this receptor-dependent, regulatory process. It appears likely that SP works directly on the macrophages to limit SST synthesis.

While SP blocks leukocyte SST production, IL4 can prevent this suppression (11). IL4 is the Th2 cytokine that induces most of the phenotypic characteristics of a Th2 response. IL4 also helps block development of IFN γ -producing T cells. Conversely, expression of IFN γ early in an inflammatory response helps limit development of IL4-producing T cells. Thus, SP and SST have opposing effects on Th1/Th2 development through their reciprocal influence on IFN γ production. In the face of SP, IL4 can allow continued SST production, which helps prevent IFN γ secretion from T cells. This in turn further helps development of the Th2 response.

SSTR subtype expression in murine granulomas

Low concentrations of SST or octreotide (10^{-9} mol/l) strongly inhibit T cell IFN γ secretion suggesting that this effect is mediated by a specific SSTR. Mice and humans express five different receptors for SST (SSTR $_{1-5}$), each encoded by separate genes (12). We showed that whole unfractionated granuloma cells, as well as T cells (Thy1.2+ or CD4+) isolated from granulomas of schistosome-infected mice, express mRNA only for SSTR $_2$ (5, 13). We developed a series

of highly sensitive and specific quantitative PCR assays for each SSTR subtype to demonstrate this point. Since schistosome granulomas consist of B cells, mast cells, fibroblasts, eosinophils, plasma cells and macrophages, this suggests that none of these cell types express SSTR $_1$, 3, 4 or 5. Splenocytes from normal or schistosome-infected animals do contain cells expressing SSTR $_3$ and SSTR $_4$ mRNA. The cellular source of these transcripts among dispersed splenocytes is unknown. While human and mouse leukocytes express predominantly SSTR $_2$, rat leukocytes favour SSTR $_3$ and SSTR $_4$ (14).

About 10% of the granuloma cell population is T cells. To determine if inflammatory cells other than T cells express this receptor transcript, we depleted T cells from the granuloma cell population by anti-Thy directed complement lysis. Depletion of T cells, as confirmed by flow analysis, removed only about 50% of the SSTR $_2$ transcripts present in the granuloma cell population as measured by quantitative, competitive PCR assay. Thus, inflammatory cells other than T cells express SSTR $_2$ mRNA as well. Well-characterized macrophage-like cell lines (P388D1, J774A1) and B cell lines (38C-13 and CH12.LX) all express SSTR $_2$ transcripts (5). These experiments suggest that SSTR $_2$ has a broad cellular distribution.

Alternative splicing of the SSTR $_2$ transcript produces two isoforms of this receptor designated SSTR $_{2a}$ and SSTR $_{2b}$. The SSTR $_2$ transcript is processed as a single exon without splicing. The SSTR $_2$ transcript is generated by excising the 341 bp intron that contains the code for the C-terminal end of SSTR $_{2a}$. Thus, the two receptor isoforms differ only in the intracellular C-terminal sequence. This may alter signal transduction or receptor regulation.

Granuloma cells express both isoforms of SSTR $_2$. Using a quantitative competitive PCR assay, we showed that SSTR $_{2a}$ mRNA is the dominant isoform expressed by inflammatory cells (5). Ninety-nine per cent of inflammatory cell SSTR $_2$ is SSTR $_{2a}$ transcripts. It is likely that the dominance of SSTR $_{2a}$ mRNA reflects dominant production of the SSTR $_2$ protein. It remains possible that rare cells express predominantly SSTR $_{2b}$, but T cell, B cell and macrophage cell lines that we tested all expressed chiefly SSTR $_2$ mRNA.

SS likely inhibits IFN γ release by acting through SSTR $_2$. We tested this by blocking SSTR $_2$ with a specific antibody raised against a peptide sequence only present on the extracellular N-terminus of SSTR $_2$. This antiserum abrogated SST and octreotide-mediated regulation of IFN γ both in dispersed granuloma cell and splenocyte cultures. These experiments also confirmed that splenocytes and granuloma cells express SSTR $_2$ protein (5).

The D1.1 Th1 cell line secretes IFN γ upon cross-linking of the T cell receptor with anti-CD3 and anti-CD4. Either SST or octreotide can inhibit this IFN γ release. This showed that SST can act directly on T cell SSTR $_2$ receptors to down-modulate IFN γ synthesis (5).

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Figure 2 Macrophages and perhaps other types of antigen-presenting cells (APC) can make SST upon stimulation with IFN γ , IL10, VIP and some other inflammatory mediators. Substance P can prevent induction of SST and down-modulate ongoing expression of this molecule. IL4 can negate the SP effect. SST acts on T cells through SSTR₂ to down-modulate antigen-induced IFN γ production. Substance P, acting through its unique receptor (NK1), up-regulates IFN γ expression and neutralizes the effect of SST on IFN γ secretion.

However, we did not rule out that SST could effect IFN γ production indirectly through action on B cells or macrophages, which also express this receptor.

Since inflammatory mediators like LPS, PGE₂, IFN γ , TNF α and IL10 induce SST production, we determined if they could modulate SSTR₂ mRNA expression. Exposure to various inflammatory mediators did not alter SSTR_{2a} or SSTR_{2b} mRNA expression in splenocytes, granuloma cells or various cell lines. Thus, we found no evidence of SSTR₂ regulation at the transcriptional level in inflammatory cells (5). Post-transcriptional regulation is still a possibility.

In humans, SSTR-specific scintigraphy using octreotide detects thymomas (15), lymphomas (16), accessory spleens (17) and granulomatous inflammation (18). Octreotide is highly selective for SSTR₂ and SSTR₅. Thus, it is likely that one or both of these SST subtypes are expressed by human leukocytes *in vivo*.

Role of SST in the murine thymus

The thymus is the primary site for T lymphopoiesis. It receives T cell progenitors that undergo maturation and education before mature, competent T cells are released into the peripheral circulation. Thymocyte maturation involves a complex series of events involving interactions between the T and non-T cell elements of this organ. The final result of this process is the release of mature T cells that have antigen receptors carefully selected to recognize particular foreign antigens without inducing autoimmune disease. Cells expressing inappropriate antigen receptors are killed within the thymus through the process of apoptosis. T cell progenitors enter the thymus through the outer cortical region and gradually move inward into the medullary region encountering macrophages and various thymic

epithelial cell types. The T cells undergo molecular rearrangement of their antigen receptors and begin expressing various cell surface molecules important for cell activation and cell-to-cell communication.

We have shown that the murine thymus expresses SST. As shown by PCR, HPLC peptide purification and RIA, this organ contains ample amounts of ppSST mRNA and SST peptide. The molecule is stored in cells bearing surface proteins characteristic of thymic dendritic cells. However, it appears that epithelial cells may also express it. The highest concentration of these SST-producing cells is in the thymic medulla and at the cortical-medullary junction (unpublished observations). Our data support the previous observations associating SST with murine thymic dendritic cells (19) and rat thymus (20, 21).

Dispersed thymocytes express SSTR₂ mRNA, but lack transcripts for SSTR₁, 3, 4 and 5. Experiments using a mutant mouse lacking SSTR₂ showed that the thymus will not express other SST receptor subtypes in compensation for loss of the SSTR₂ receptor. The thymus in this animal contains about four times more ppSST mRNA transcripts and SST product. This abundant expression of SST remains restricted to thymic dendritic and epithelial cells, but there are a larger number of such cells expressing SST. The compensatory increase in thymic SST upon loss of SSTR₂ suggests that the thymus has a feedback regulatory circuit governing the rate of SST production.

Thymic dendritic and epithelial cells express MHC surface proteins that present antigen to T cells. The intimate association of T cells with these antigen-presenting cells provides the mechanism through which T cells acquire the potential for MHC-restricted recognition and are selected for their capacity to distinguish self from non-self. In addition to SST, the thymic stromal cells make various other inflammatory mediators that further shape the growth and development of the T cell progenitors. Ongoing experiments using the SSTR₂ knockout mouse suggest that SST is essential for normal T cell development within the thymus.

The human thymus is a source of SST (22). Thymic epithelial cells express SST. The thymic tissue contains several SSTR subtypes (23). SSTR₂ is preferentially expressed in the human thymic medullary region. These data suggest that SST has a physiological role in the human thymus.

In conclusion, SST is expressed throughout the body and has many diverse functions. Its receptors are displayed on many cell types. Here we show yet another important role for SST. The immune system has a self-contained SST immunoregulatory circuit, which governs important aspects of an immune response (Fig. 2).

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References

- 1 Elliott DE. Methods used to study immunoregulation of schistosome egg granulomas. *ImmunoMethods* 1996 **9** 255–267.
- 2 Rakasz E, Blum A, Metwali A, Elliott D, Li J, Ballas Z *et al.* Localization and regulation of IFN- γ production within the granulomas of murine schistosomiasis in IL-4 deficient and control mice. *Journal of Immunology* 1998 **160** 4994–4999.
- 3 Blum AM, Metwali A, Cook G, Mathew RC, Elliott D & Weinstock JV. Substance P modulates antigen-induced, IFN-gamma production in murine *Schistosomiasis mansoni*. *Journal of Immunology* 1993 **151** 225–233.
- 4 Blum AM, Metwali A, Mathew RC, Cook G, Elliott D & Weinstock JV. Granuloma T lymphocytes in murine *Schistosomiasis mansoni* have somatostatin receptors and respond to somatostatin with decreased IFN-gamma secretion. *Journal of Immunology* 1992 **149** 3621–3626.
- 5 Elliott DE, Li J, Blum AM, Metwali A, Patel YC & Weinstock JV. SST2A is the dominant somatostatin receptor subtype expressed by inflammatory cells, is widely expressed and directly regulates T cell IFN-gamma release. *European Journal of Immunology* 1999 **29** 2454–2463.
- 6 Blum AM, Metwali A, Mathew RC, Elliott D & Weinstock JV. Substance P and somatostatin can modulate the amount of IgG2a secreted in response to schistosome egg antigens in murine *Schistosomiasis mansoni*. *Journal of Immunology* 1993 **151** 6994–7004.
- 7 Weinstock JV, Blum AM & Malloy T. Macrophages within the granulomas of murine *Schistosoma mansoni* are a source of a somatostatin 1–14-like molecule. *Cellular Immunology* 1990 **131** 381–390.
- 8 Elliott DE, Blum AM, Li J, Metwali A & Weinstock JV. Preprosomatostatin messenger RNA is expressed by inflammatory cells and induced by inflammatory mediators and cytokines. *Journal of Immunology* 1998 **160** 3997–4003.
- 9 Weinstock JV, Elliott D, Metwali A, Blum A, Li J, Qadir K & Sandor M. Immunoregulation within the granulomas of murine *Schistosomiasis mansoni*. *Microbes and Infection* 1999 **1** 491–498.
- 10 Weinstock JV & Elliott D. The substance P and somatostatin interferon-gamma immunoregulatory circuit. *Annals of the New York Academy of Sciences* 1998 **840** 532–539.
- 11 Blum AM, Elliott DE, Metwali A, Li J, Qadir K & Weinstock JV. Substance P regulates somatostatin expression in inflammation. *Journal of Immunology* 1998 **161** 6316–6322.
- 12 Patel YC. Somatostatin and its receptor family. *Frontiers in Neuroendocrinology* 1999 **20** 157–198.
- 13 Elliott DE, Metwali A, Blum AM, Sandor M, Lynch R & Weinstock JV. T lymphocytes isolated from the hepatic granulomas of schistosome-infected mice express somatostatin receptor subtype II (SSTR2) messenger RNA. *Journal of Immunology* 1994 **153** 1180–1186.
- 14 ten Bokum AM, Lichtenauer-Kaligis EG, Melief MJ, van Koetsveld PM, Bruns C, van Hagen PM *et al.* Somatostatin receptor subtype expression in cells of the rat immune system during adjuvant arthritis. *Journal of Endocrinology* 1999 **161** 167–175.
- 15 Lastoria S, Palmieri G, Muto P & Lombardi G. Functional imaging of thymic disorders. *Annals of Medicine* 1999 **31** 63–69.
- 16 Vanhagen PM, Krenning EP, Reubi JC, Mulder AH, Bakker WH, Oei HY *et al.* Somatostatin analogue scintigraphy of malignant lymphomas. *British Journal of Haematology* 1993 **83** 75–79.
- 17 Lebtahi R, Cadiot G, Marmuse JP, Vissuzaine C, Petegnief Y, Courillon-Mallet A *et al.* False-positive somatostatin receptor scintigraphy due to an accessory spleen (see comments). *Journal of Nuclear Medicine* 1997 **38** 979–981.
- 18 Vanhagen PM, Krenning EP, Reubi JC, Kwekkeboom DJ, Bakker WH, Mulder AH *et al.* Somatostatin analogue scintigraphy in granulomatous diseases. *European Journal of Nuclear Medicine* 1994 **21** 497–502.
- 19 Throsby M, Homo-Delarche F, Chevenne D, Goya R, Dardenne M & Pleau JM. Pancreatic hormone expression in the murine thymus: localization in dendritic cells and macrophages. *Endocrinology* 1998 **139** 2399–2406.
- 20 Gomariz RP, Lorenzo MJ, Cacicedo L, Vicente A & Zapata AG. Demonstration of immunoreactive vasoactive intestinal peptide (IR-VIP) and somatostatin (IR-SOM) in rat thymus. *Brain, Behavior, and Immunity* 1990 **4** 151–161.
- 21 Fuller PJ & Verity K. Somatostatin gene expression in the thymus gland. *Journal of Immunology* 1989 **143** 1015–1017.
- 22 Ferone D, van Hagen PM, van Koetsveld PM, Zuijderwijk J, Mooy DM, Lichtenauer-Kaligis EG *et al.* *In vitro* characterization of somatostatin receptors in the human thymus and effects of somatostatin and octreotide on cultured thymic epithelial cells. *Endocrinology* 1999 **140** 373–380.
- 23 Ferone D, van Hagen PM, Colao A, Annunziato L, Lamberts SW & Hofland LJ. Somatostatin receptors in the thymus. *Annals of Medicine* 1999 **31** 28–33.

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