The Effect of Somatostatin on the Production of Human Interferons by Mononuclear Cells (43065)

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Abstract. **Somatostatin (SMS) is a tetradecapeptide which can inhibit the secretion of a number of peptides produced by the endocrine or nervous systems. SMS 201-995 (octreotide) is a somatostatin analogue with very potent somatostatin activities. We have been investigating the effects of both SMS and octreotide on the production of human interferon (IFN). We obtained human peripheral blood mononuclear cells from normal donors and induced them to produce IFN in the presence or absence of a number of peptides possessing somatostatin activities. SMS and octreotide were shown to inhibit the secretion of INF-γ but not IFN-α. Concentrations of 10^{-6} *M* were shown to decrease yields when Concanavalin A or phytohemagglutin were used as the inducer. Higher concentrations had a progressively greater effect. No effects were observed on IFN-γ production if interleukin 2, ionomycin, or various natural antigens were used to induce the cells. The 28-amino acid form of somatostatin had some effects on gamma IFN yields but the first 14-amino acid fragment of this peptide moiety did not. No effect of any of these compounds was observed on IFN bioactivity. These studies indicate SMS may have some regulatory action on the secretion of immunomodulators *in vitro* but the concentrations required are well above those encountered under physiologic circumstances, suggesting SMS may not play an important regulatory role governing such secretion *in vivo*.** [P.S.E.B.M. 1990, Vol 194]

Investigators have long sought substances that might provide communication between the endocrine, nervous, and immune systems. In fact it has been known for some time that endocrine influences can modulate immune function (1). Furthermore, lymphocytes appear to be capable of secreting endocrine substances (2) lending support to the concept that cooperation occurs between the endocrine and immune systems. Similarly, the nervous system is felt through a variety of pathways to regulate some of the immune functions of the body (3–5). In the past, others have described the ability of certain peptide components of the neuroendocrine system to exert regulatory influences on cells of the immune system (6). Furthermore, somatostatin (SMS), a neuropeptide, which is found naturally in 14- and 28-amino acid moieties, has been reported by several authors to inhibit T cell proliferation (7–9) and influence other immune functions (10, 11). SMS 201-995 (octreotide) is a somatostatin analogue that has recently been introduced into the clinic for the treatment of certain neoplastic diseases (12). It is a very potent analogue and achieves high serum levels after intravenous administration (13), but its effects on the immune system are not yet well studied and could possibly differ from those of the parent compound. Since SMS is known to influence other aspects of immunologic function and clinical application of an SMS analogue is at hand, it seemed important to examine the effects of both this agent and its analogue on the generation of cytokines especially gamma interferon.

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Materials and Methods

Reagents. Phytohemagglutinin (PHA) and concanavalin A (Con A) were purchased from Difco Inc. (Detroit, MI). Recombinant interleukin 2 (IL-2) was kindly provided by Cetus Inc. (Emoryville, CA). Ionomycin was purchased from Calbiochem (San Diego, CA). Staphylococcal enterotoxin A (SEA) was the gift of Dr. G. Murthy, U.S. Food and Drug Administration, Cincinnati, OH. Diphtheria-tetanus toxoid was purchased from Connaught Laboratories (Swift Water, PA)

and influenza vaccine (Fluogen) was obtained from Parke-Davis (Morris Plains, NJ). Streptokinase-streptodornase was a gift from Lederle Laboratories, (Pearl River, NY). The 14-amino acid somatostatin moiety (SMS 14), the first 14-amino acid fragment of the 28-amino acid form of somatostatin (SMS 28), and the 28 amino-acid somatostatin moiety (SMS 28) were all obtained from Sigma Inc. (St. Louis, MO). The synthetic somatostatin analog SMS 201-995 was the gift of Sandoz Inc. (East Hanover, NJ). Fetal bovine serum was purchased from Gibco (Grand Island, NY) and RPMI 1640 medium was obtained from Irvine Scientific (Irvine, CA). Penicillin and streptomycin were purchased from Pfizer Inc. (New York, NY). Recombinant human α-, γ-interferons were the gift of Hoffman La Roche (Nutley, NJ). Antiserum to human γ-interferon was obtained from Interferon Sciences (New Brunswick, NJ) and to human α-interferon from the National Institutes of Health (Bethesda, MD).

Peripheral Blood Mononuclear Cells. Human peripheral blood mononuclear cells (PBMC) were obtained as the buffy coat fraction from normal human donors. PBMC were separated on Ficoll-Hypaque gradients (14), washed, and adjusted to a final concentration of 2.5×10^6 cells in RPMI 1640 supplemented with 10% fetal bovine serum, 250 units of penicillin, and 150 μg/ml of streptomycin.

Mitogenic Stimulation of PBMC. PBMC, separated as above, were stimulated with either 15 μg/ml (unless otherwise indicated) of PHA or Con A, 500 units of IL 2, or 1 μM ionomycin. In the case of the natural antigens, 0.8 μg/ml of SEA or an appropriate dilution of each of the following microbiologic mitogens was used: streptokinase-streptodornase, tetanus-diphtheria toxoid, or influenza vaccine. Preliminary experiments determined the optimal concentrations of mitogens used. All of the natural antigens except SEA were dialyzed against phosphate-buffered saline prior to use to remove preservatives. The SEA did not contain these agents. Incubation of the cells was then carried out in 15-ml plastic tubes in a 5% CO_2 incubator at 37°C for 3 days. At the end of that time, the cells were removed by centrifugation, tested for viability using trypan blue, and exposed to [^{3}H]thymidine to assess the proliferative responses as described below. PBMC were used for assay only if the viability of the cells was 95% or greater. All supernatants were harvested from the cells, dialyzed overnight, and frozen for subsequent assay. In those experiments where various reagents were added to the cells, final dilutions of the substances were made just prior to addition to the PBMC. Appropriate controls were performed for each test sample. Controls were simultaneously prepared and treated in every way identically to the test samples except for the absence of the test reagent.

Proliferative Responses. Proliferative responses were determined by pulsing stimulated and unstimulated PBMC at a concentration of 1×10^6 cells/ml for 4 hr in a CO_2 incubator with [^{3}H]thymidine (0.5 μCi/well) having a specific activity of 6.7 Ci/mM (ICN, Irvine, CA). After pulsing, the cells were harvested using a multisample automatic cell harvester. The [^{3}H]thymidine incorporation was measured using a liquid scintillation counter.

Induction of Human α-Interferon. Human PBMC separated as described above were adjusted to a concentration of 1×10^7 cells/ml. These cells were added to 15-ml plastic centrifuge tubes and poly(rI:rC) was added along with DEAE D as described in detail in the past (15). After a 30-min incubation, the cells were washed, RPMI 1640 medium supplemented as described above was added and incubation carried out in a CO_2 incubator overnight. The following morning, the supernatants were harvested after centrifugation and frozen for subsequent assay. In those experiments where the effects of somatostatin on the production of human α-interferon were being investigated, control and test aliquots of PBMC were processed simultaneously and under identical circumstances save for the presence or absence of the test reagent. Somatostatin was added to both the inducing solution (poly(rI:rC) with DEAE-D) and subsequent incubation media (supplemented RPMI 1640) in the indicated concentrations.

Interferon Assays. Interferon (IFN) was assayed as previously reported (16). Briefly WISH cells were grown to confluence at the bottom of individual wells of 96-well microtiter plates (Costar, Boston, MA). Serial dilutions of samples were added, allowed to incubate with the cells overnight, and then removed. After the cells were washed, they were challenged with encephalomyocarditis virus and reincubated for an additional 24 hr. At that time the cells were examined by light microscopy for cytopathic effect. The reciprocal of the last dilution of the sample where cytopathic effect was inhibited by 50% was termed the titer of the sample. A standard IFN-α preparation titered against NIH α standard G023-901-530 and a γ standard titered against NIH γ standard Gg23-901-530 were included in all assays. In this system, the NIH α standard titers 7200 units and the γ standard titers 16,000 units. All results are expressed in international reference units.

Typing of Interferons. For purposes of identifying the specific IFN species present after induction with a given inducing agent, aliquots of IFN-containing supernatants were independently mixed with specific antisera to each IFN species. Quantities of antisera were selected to neutralize 100 units of IFN bioactivity and supernatants were adjusted to contain approximately 100 international IFN units. Incubation was carried out for 1 hr at 36°C with the specific antisera or with normal control serum. The IFN was then titered as described above and the amount of IFN bioactivity compared

between samples incubated in normal control serum or specific antiserum.

Effect of Somatostatin on IFN Bioactivity. Media containing appropriate concentrations of SMS-14, SMS 201-995, or SMS 28 were prepared and added to appropriate rows of microliter plates. Samples of recombinant human IFN-α and γ were then titered on media with the somatostatin moieties present or absent and the titers compared.

Statistical Analysis. Statistical analysis was performed using Student's paired t test.

Results

Initial studies using specific antisera verified that interferon induced by poly(rI:rC) was α-interferon and interferon induced by all other inducers employed in this study was γ-interferon.

Since SMS 201-995 is now available for clinical purposes, possesses greater inhibitory effects on peptide synthesis, and achieves higher serum levels than those observed with natural SMS, we tested this SMS analogue first. The range of concentrations employed varied from those encountered therapeutically to those well in excess of therapeutic levels. The effect of SMS 201-995 on the production of IFN-γ can be seen in Table I. A reduction in IFN yields was seen with PHA and Con A induction but not with IL-2 or ionomycin induction. Similarly, a panel of natural antigens were used to induce the cells. This panel included staphylococcal enterotoxin A, streptokinase-streptodornase influenza vaccine, and tetanus-diphtheria toxoid. No reduction of IFN-γ production as compared with controls occurred when induction was accomplished with these antigens in the presence of SMS 201-995 (10^{-5} and 10^{-6} M concentrations). Preincubation with SMS 201-995 for 24 hr prior to addition of the mitogen, daily addition of fresh SMS during the 3-day incubation or incubation in 3 mg/100 ml albumin rather than fetal bovine serum did not enhance the ability of SMS 201-995 to inhibit human IFN-γ production. In Table II the effect of different molecular forms of somatostatin on the production of human IFN-γ can be noted. Both the active 14-amino acid moiety of somatostatin and the 28-amino acid somatostatin reduced the yields of the IFN but the first 14-amino acid fragment of the SMS 28 did not. In Figure 1 the effect of varying the concentration of the inducer (PHA) was tested. It can be noted that WBC alone produced no IFN. The low concentration of PHA (5 μg/ml) induced a diminished yield of IFN as compared with the high (15 μg/ml or 25 μg/ml) concentrations of the inducer; however, at all three PHA concentrations the presence of 10^{-6} M SMS 201-995 was associated with a significant ($P < 0.05$) reduction in the production of IFN. Similar effects were seen with both PHA and Con A.

All of the various molecular forms of SMS tested for effects on the production of human IFN-γ were also tested for effect on the production of human IFN-α. No effect was appreciated and yields of human IFN-α remained undiminished by the presence of SMS 201-995 or the presence of either the 14- or 28-amino acid forms of SMS (data not shown). In examining the effect of SMS 201-995 SMS on the proliferative response as seen in Table I, it can be noted that both PHA and Con

Table I. Effect of SMS 201-995 (Octreotide) on the Production of γ-Interferon

Treatment	Log_2 mean IFN[a]	P Value[b] IFN	Proliferative response (cpm × 10^3)	P value[c] proliferative response
Cells	0.0	—		
PHA (25 μg/ml)	7.9 ± 2.0	—	3.8 ± 0.9	—
PHA + SMS 10^{-5} M	6.0 ± 2.0	<0.01	2.8 ± 0.7	<0.05
PHA + SMS 10^{-6} M	6.9 ± 2.0	<0.01	2.9 ± 0.7	NS[d]
PHA + SMS 10^{-7} M	7.9 ± 2.0	NS	3.2 ± 0.5	NS
Con A (25 μg/ml)	8.4 ± 3.1	—	5.7 ± 1.3	—
Con A + SMS 10^{-5} M	6.4 ± 3.2	<0.01	4.2 ± 1.8	<0.05
Con A + SMS 10^{-6} M	6.9 ± 3.2	<0.01	4.9 ± 1.9	NS
Con A + SMS 10^{-7} M	7.8 ± 3.2	NS	5.5 ± 1.9	NS
IL-2 (500 IU)	7.3 ± 2.6	—	3.2 ± 1.05	—
IL-2 + SMS 10^{-5} M	7.3 ± 2.8	NS	3.0 ± 0.8	NS
IL-2 + SMS 10^{-7} M	7.2 ± 2.4	NS	4.4 ± 0.6	NS
Ionomycin (10^{-6} M)	9.0 ± 2.0	—	1.9 ± 0.01	NS
Ionomycin + SMS 10^{-5} M	9.3 ± 2.0	NS	1.9 ± 0.01	NS
Ionomycin + SMS 10^{-6} M	9.3 ± 2.0	NS	2.6 ± 0.5	NS

[a] Interferon titer is the mean of 18 experiments on PBMC from nine individuals.
[b] P value for the difference in mean interferon titer with and without this concentration of SMS.
[c] P value for the difference in proliferative response with and without this concentration of SMS.
[d] NS, not significant.

Table II. Effect of Somatostatin Moieties on the Production of Leukocyte-Derived Interferons

Treatment		Log_2 IFN titer[a]	P[b]	Proliferative response[c] (cpm × 10^3)
WBC		0	—	0.5 ± 0.1
PHA		6.9 ± 1.1[d]	—	4.6 ± 0.8
PHA + SMS 28	10^{-5} *M*	6.3 ± 1.1	NS[e]	4.3 ± 1.4
(28-amino acid moiety)	10^{-6} *M*	5.8 ± 1.8	<0.05	4.0 ± 0.5
	10^{-7} *M*	6.9 ± 1.0	NS	4.5 ± 1.6
	10^{-8} *M*	6.9 ± 1.3	NS	4.7 ± 0.9
PHA + SMS 28	10^{-5} *M*	6.9 ± 1.3	NS	4.8 ± 1.7
fragment containing amino	10^{-6} *M*	6.8 ± 1.2	NS	5.4 ± 1.0
acids 1–14	10^{-7} *M*	6.7 ± 1.2	NS	5.3 ± 2.6
	10^{-8} *M*	6.9 ± 1.3	NS	4.5 ± 1.0
PHA + SMS 14	10^{-5} *M*	5.9 ± 1.3	<0.05	5.0 ± 0.3
(14-amino acid moiety)	10^{-6} *M*	6.0 ± 1.3	NS	4.7 ± 0.7
	10^{-7} *M*	6.1 ± 1.3	NS	4.6 ± 0.7
	10^{-8} *M*	6.2 ± 1.3	NS	4.5 ± 0.7

[a] Mean of eight experiments.
[b] *P* value for the difference in IFN titer with PHA alone and PHA with this concentration of test component.
[c] The proliferative responses in the presence of the various concentrations of each of the somatostatin moieties tested were not significantly different from the controls without somatostatin (i.e., PHA).
[d] ± indicates standard deviation.
[e] NS, not significant.

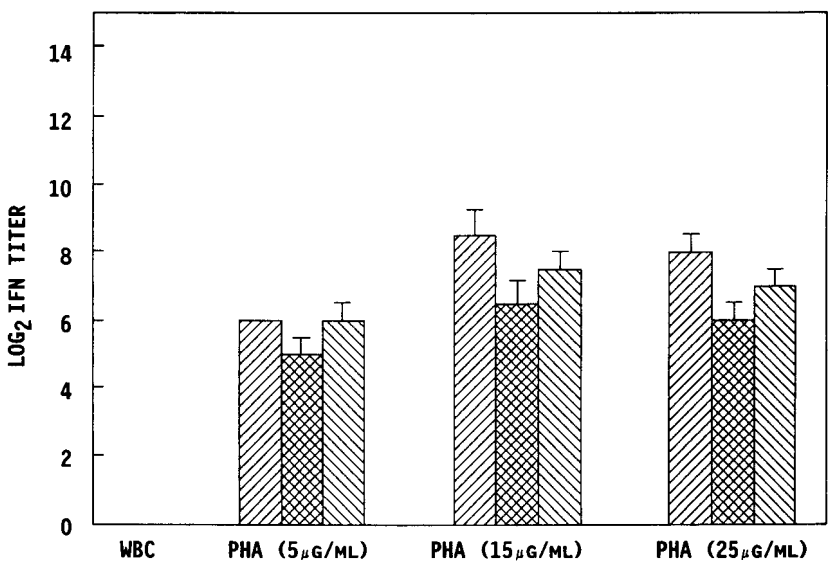

Figure 1. The effect of SMS 201-995 on IFN-γ production by different concentrations of PHA. For each concentration of PHA, three bars are shown. The first bar which is stripped from bottom to top is the titer of the IFN produced by PHA alone. The middle cross-hatched bar is the titer of the IFN produced by PHA in the presence of 10^{-6} *M* SMS 201-995. The third bar stripped from top to bottom is the titer of the IFN produced in the presence of 10^{-7} *M* SMS 201-995. The data are derived from a mean taken from six experiments. The error bar for the PHA (5 μg/ml) alone is deleted since in all six experiments the identical concentration of IFN was detected and hence the standard deviation was zero.

A at the higher concentrations utilized diminished proliferation to a statistically significant degree. Proliferation was not significantly inhibited with the other compounds (Table II).

While dialysis was employed to remove the compounds and prevent them from asserting any effect on the bioactivity of either IFN, separate studies demonstrated that neither compound influenced the titer of either recombinant human IFN-γ or IFN-α.

Discussion

Somatostatin is a peptide synthesized in many tissues of the body and can act as either a hormone or neurotransmitter (12). Encoded in the form of a single large precursor molecule, pre-prosomatostatin, the protein is cleaved to somatostatin-28 and somatostatin-14. These two peptides are known to have variable effects

in different tissues and may have different functions (12). Somatostatin is known to inhibit the secretion of various proteins including growth hormone and may act as either an autocrine, paracrine, or hormonal substance (12). SMS 201-995 is a long-acting synthetic analogue of somatostatin now available for clinical use.

Previous reports (7–9) have documented the fact that somatostatin may influence the immune system by inhibiting proliferation of T lymphocytes or effecting other specific immune functions (10, 11). Further transformed human lymphocyte lines have been shown to have a receptor for somatostatin (17).

Since somatostatin has the potential to inhibit the secretion of a number of other peptide molecules, it might be anticipated that it could have similar effects on components of the immune system and thus might inhibit the secretion of human IFN. Our results demonstrate that somatostatin-14, somatostatin-28, and the synthetic somatostatin analogue SMS 201-995 can all inhibit the secretion of human IFN-γ at high concentrations. The first 14-amino acid fragment of SMS 28 has no effect on IFN-γ production. This is not surprising since the last 11-amino acid sequence of SMS 28 and SMS 14 are identical, but the first 14-amino acids of the former have no comparable sequence in the latter (18). Of importance, however, is the fact that this inhibition only occurs when induction is accomplished with PHA or Con A and only at concentrations well above those encountered in the circulation (19). Yields of IFN-γ from PBMC were not diminished if IL-2, ionomycin, or any of a panel of natural antigens were used to induce the cells. Furthermore, the decrease in the production of IFN-γ in the presence of SMS 201-995 was associated with a reduction in the proliferative response of these cells.

The concentrations of all forms of somatostatin at which reductions in yields of IFN-γ occurred were so far above physiologic serum levels that it seems unlikely that this effect could occur *in vivo* unless isolated areas of the body exist where the concentration of somatostatin is well above that encountered in the circulation. This, if it ever occurs, would have to be in areas immediately adjacent to those cells actively secreting SMS. Thus, if there is any role for SMS in the regulation of γ-interferon production in the body, it would likely be a paracrine effect. This possibly is made more likely by the fact that certain cells of the immune system may produce SMS (20). The inability of SMS to diminish the secretion of IFN-γ when induced by natural antigens also suggests that the inhibition of IFN production by SMS may not be of major significance during infections. Finally, while somatostatin influenced the production of human IFN-γ under certain circumstances, this family of compounds did not effect the production of human IFN-α, at least when induced by poly(rI:rC).

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