

Isotype-specific regulation of human lymphocyte production of immunoglobulins by sustained exposure to vasoactive intestinal peptide

Avi Hassner, MD, Matthew S. Lau, MD, Edward J. Goetzl, MD, and Daniel C. Adelman, MD San Francisco, Calif.

Exposure of lymphocytes to nanomolar to micromolar concentrations of vasoactive intestinal peptide (VIP) for 1 to 3 days only modestly suppressed or enhanced the production of IgA and IgM, but not IgG. The effects of twice daily additions of 10^{-12} to 10^{-7} mol/L VIP for up to 18 days on pokeweed mitogen-stimulated peripheral blood mononuclear cells (PBMCs) from normal human subjects was examined by quantifying the production of IgG, IgM, and IgA. The maximum suppression of IgG by 10^{-9} mol/L VIP was $79\% \pm 33\%$ (mean $\pm$ SD) (range, 41% to 97%; $p < 0.015$) on day 9 and $84\% \pm 1\%$ (range, 74% to 96%; $p < 0.0001$) on day 14 and was significant at 6×10^{-10} to 4×10^{-9} mol/L VIP. Suppression of IgM production by 10^{-9} mol/L VIP was significant and was observed first on day 5 and persisted through day 14. VIP did not alter IgA production or affect the proliferation or viability of PBMCs. The production of IgE by interleukin-4 stimulated PBMCs was enhanced consistently in two subjects but not in two other subjects. The duration of exposure to nanomolar concentrations of VIP is thus a critical determinant of its immunoregulatory effect, as manifested by late suppression of production of IgG and IgM and concurrent enhancement of production of IgE in some subjects. (J ALLERGY CLIN IMMUNOL 1993;92:891-901.)

Key words: Vasoactive intestinal peptide, immunoglobulin isotype regulation, neuropeptides

Vasoactive intestinal peptide (VIP₁₋₂₈) is a potent mediator of vascular, smooth muscle, epithelial, and glandular functions and has been recently shown to affect immunologic responses in both murine and human systems. VIP suppresses mouse lymphocyte proliferation with preferential inhibition of helper-inducer T-cell functions,¹ secretion of interleukin (IL)-2, and synthesis of

Abbreviations used

cAMP:	Cyclic adenosine monophosphate
IL:	Interleukin
PBMCs:	Peripheral blood mononuclear cells
PWM:	Pokeweed mitogen
VIP:	Vasoactive intestinal peptide

From the Division of Allergy and Immunology, Departments of Medicine and Microbiology, University of California, Medical Center, San Francisco.

Supported by grant AI 29912 from the National Institutes of Health.

Dr. Adelman was the Robert C. Siegel Fellow at University of California, San Francisco.

Dr. Hassner was a visiting scholar From the Department of Medicine, D, Tel Aviv Elias-Sourasky Medical Center, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, at the University of California, San Francisco.

Received for publication Dec. 15, 1992; revised May 6, 1993; accepted for publication May 6, 1993.

Reprint requests: Daniel C. Adelman, MD, Division of Allergy and Immunology, University of California, Medical Center, 533 Parnassus Ave. U-426, San Francisco, CA 94143-0724.

Copyright © 1993 by Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/48465

immunoglobulin and DNA.^{2, 3} Incubation of intestinal lymphocytes with VIP reduces expression of their VIP receptors and alters the normal tissue homing pattern.⁴ Short-term exposure of mouse peripheral blood mononuclear cells (PBMCs) or Peyer's patch lymphocytes to VIP for 24 to 72 hours only modestly enhances IgM and suppresses the production of IgA, but not IgG.^{2, 5, 6} In most of these studies the exposure of cells of the immune system to the immunoregulatory effects of VIP has been brief, and the effect of VIP on immunoglobulin production was assessed after only a few days. Because VIP is readily degraded by cellular and fluid-phase proteolytic enzymes, previous experiments with only one addition of VIP have evaluated principally the initial immunoregulatory effects.

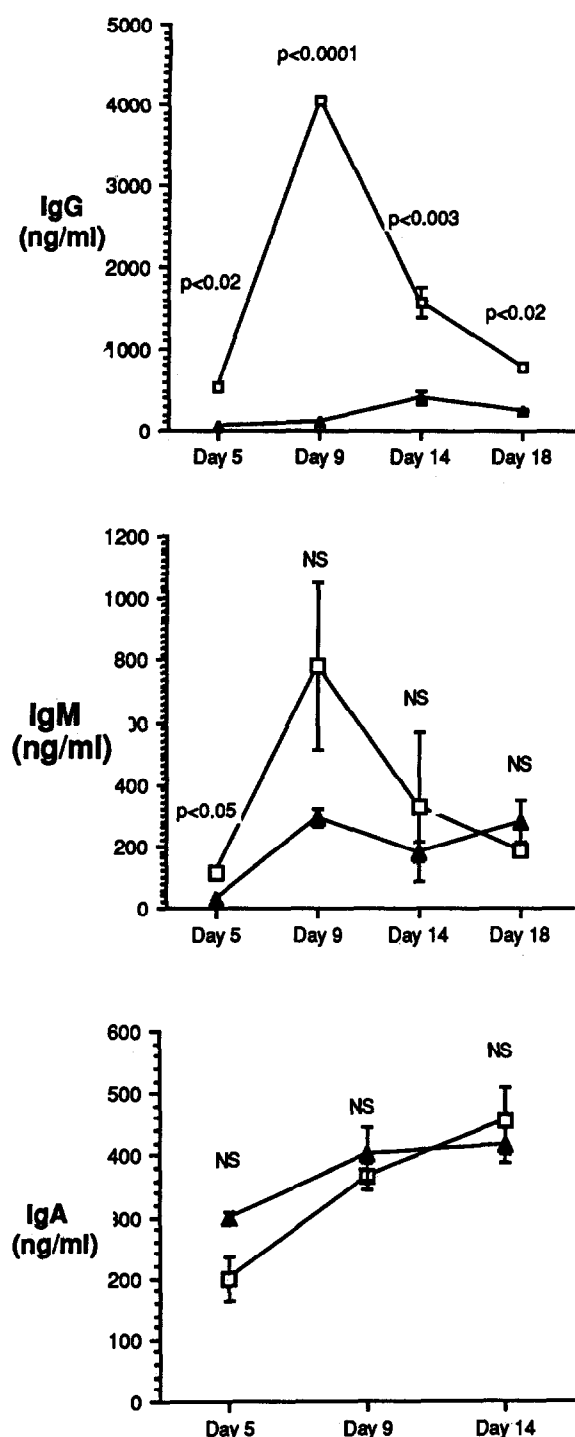


FIG. 1. Inhibition of IgG and IgM production by VIP₁₋₂₈. Each data point represents mean $\pm$ SD of three ELISA wells of culture supernatant. Significant inhibition of IgG production was observed on days 5 ($p < 0.003$), 9 ($p < 0.0001$), 14 ($p < 0.02$), and 18 ($p < 0.003$). Inhibition of IgM production was statistically significant on day 5 ($p < 0.02$), and there was no inhibition of IgA production. Open squares, control; solid triangles, VIP [10^{-9} mol/L].

We and others have previously shown that some murine and human cultured pre-B and T cell lines express high-affinity receptors for VIP with $K_{\text{dissociation}}$ values of 5 to 7 nmol/L.⁷⁻¹¹ In addition, pre-B cells isolated from patients with acute leukemia also express VIP receptors.¹² The identification of VIP receptors on B cells at several stages of differentiation suggests that the effects of VIP may encompass B-cell maturation, as well as immunoglobulin production, and may depend on more prolonged exposure to VIP.

We therefore examined the effects of long-term in vitro exposure of PBMCs to VIP on the production of a full range of immunoglobulin isotypes. Our results demonstrate that such prolonged exposure suppresses the production of IgG and IgM, but not of IgA, and enhances IgE production in some subjects.

METHODS

Preparation of PBMCs

PBMCs were obtained from heparinized whole blood of healthy volunteers by separation on a discontinuous Ficoll-Hypaque gradient (Pharmacia LKB Biotechnology AB, Uppsala, Sweden)¹³ and washed and resuspended in RPMI-1640 (Gibco BRL, Gaithersburg, Md.) supplemented with 10% fetal calf serum (lot 11111010 Hyclone Laboratories Inc., Logan, Utah) and 1% penicillin/streptomycin (complete RPMI - CRPMI) and incubated in Petri dishes (Falcon 1029 100 $\times$ 15 mm polystyrene, Becton-Dickinson, Lincoln Park, N.J.) at 37°C in a 5% CO₂ incubator for 40 minutes to remove adherent monocytes. Cells were then counted on a hemocytometer and qualitatively assessed to determine efficacy of monocyte depletion. In all experiments it was estimated that more than 98% of monocytes were removed by this procedure.

IgG, IgM, and IgA in culture supernatants

PBMCs (2×10^6) were cultured in 2 ml CRPMI (1×10^6 cells/ml) in duplicate culture tubes (Falcon 2063 12 $\times$ 75 mm polypropylene round bottom, Becton-Dickinson) with or without pokeweed mitogen (PWM) at 4 μ l/ml and with or without VIP₁₋₂₈ (Peninsula Laboratories, San Carlos, Calif.) added twice daily for up to 18 days. Control experiments were performed with VIP₄₋₂₈, VIP₁₀₋₂₈,¹⁴ and substance P (Peninsula Laboratories). On days 5, 9, 14, and 18 0.05 ml of supernatant was removed from each culture, and IgG, IgM, and IgA were subsequently assayed by standard immunoassay. The removal of 0.05 ml of supernatant at these times was not believed to significantly affect the remaining concentrations of cells, VIP concentration, or metabolic byproducts.

TABLE 1. Percent inhibition by VIP of IgG production by PWM-stimulated PBMCs

[VIP]	(Exp 1)		(Exp 2)	
	IgG (ng/mL)	% inhibition	IgG (ng/mL)	% inhibition
0	4066 ± 38	0	3122 ± 480	0
10 ⁻¹²	3907 ± 397	3.9	1987 ± 101	36.3
10 ⁻¹¹	2907 ± 65	28.5	ND	ND
10 ⁻¹⁰	2439 ± 118	40	2803 ± 91	10.2
10 ⁻⁹	116 ± 4	97.1	15 ± 10	99.5
10 ⁻⁸	3366 ± 239	17.2	2079 ± 119	33.4

PBMCs were treated for 9 days with different concentrations of VIP and stimulated once on day 0 with PWM (4 µg/ml). Results are means ± SD of the results of determinations of IgG concentrations in triplicate wells.

ND, Not done.

IgE in culture supernatants

For determination of IgE production, 1×10^6 PBMCs per milliliter were cultured separately for 9, 14, and 18 days in Iscove's modified Dulbecco's medium plus 10% fetal calf serum (lot 11111010, Hyclone Laboratories Inc.) and 1% penicillin/streptomycin in the absence or presence of 100 U/ml interleukin (IL)-4 (lot no. 1801-1, R&D Systems, Inc., Minneapolis, Minn.), added at day 0, and 10^{-12} to 10^{-9} mol/L VIP₁₋₂₈ added twice daily. Supernatants were assessed for IgE production on days 9, 14, and 18 by a standardized immunoassay. Control experiments with VIP₄₋₂₈, VIP₁₀₋₂₈, and substance P were performed as previously noted.

Assessment of cellular proliferation

To each replicate suspension of 1×10^6 PBMCs per milliliter 10 µCi of tritiated thymidine (Amersham Corporation, Arlington Heights, Ill.) was added on days 5, 9, 14, and 18 for the last 16 to 20 hours. Cells were then harvested onto glass filter paper for liquid scintillation counting. Radioactivity was measured in a liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

Radioimmunoassay for VIP

Replicate 5 and 50 µl aliquots of each sample were diluted to 0.1 ml with radioimmunoassay buffer and incubated with 0.1 ml of buffer containing 12,000 to 15,000 cpm of iodine-125-labeled VIP (Amersham Corp.) and 0.1 ml of a dilution of rabbit anti-VIP antibody for 2 hours at room temperature and 16 hours at 4° C. The radiolabeled VIP had a specific activity of 2000 Ci/mmol. Nonradiolabeled VIP was used to prepare a standard curve in the range of 0.001 to 0.3 pmol per tube. Suspensions of preformed complexes of goat anti-rabbit IgG (Pel-Freeze Biologicals, Inc., Rogers, Ark.) and normal rabbit IgG in antibody excess were prepared as previously described.¹⁴ Two tenths milliliter of these complexes were added to each tube, and the incubation continued for 2 hours at room temperature. Then 0.7 ml of

cold radioimmunoassay buffer was added to each tube, which was mixed and centrifuged at 4000 g for 30 minutes at 4° C. The supernatant fluids were discarded, and the radioactivity in the pellets was quantified in a gamma counter. The mean quantity of VIP that displaced 50% of the radiolabeled peptides from immune complexes in standard curves was 0.010 pmol.

ELISA for immunoglobulin isotypes

IgG, IgM, IgA, and IgE concentrations in culture supernatants were measured by ELISA, as previously described.¹⁵ The anti-IgE monoclonal antibodies CIA 7.12 and CIA 4.15 (kindly provided by Andrew Saxon, MD) have been shown to demonstrate no cross-reactivity with other immunoglobulin isotypes.¹⁵ For the IgE assays a phosphatase-labeled goat anti-human IgE antibody was used (Kirkegaard and Perry Laboratories, Gaithersburg, Md., Lot no. MAO 9-5).

Analysis of cell surface phenotype

Fluorescein isothiocyanate- or phycoerythrin-labeled anti-IgG1 (myeloma X63 Ag.8xBalb/c Spleen, anti CD3, clone SP2/0. Ag 1.4xBalb/c spleen; anti-CD19 [phycoerythrin-labeled] myeloma X63 Ag.8xBalb/c spleen [all from AMAC Inc., Westbrook, Maine]) were used to stain PBMCs by two-color flow cytometry. Specifically, 1×10^6 cells were incubated in 100 ml phosphate-buffered saline (PBS) and fluorescein isothiocyanate- and phycoerythrin-labeled monoclonal antibodies for 30 minutes at 4° C, washed three times with PBS, resuspended in 1 ml PBS with 0.2% propidium-iodine (Sigma Chemical Co., St. Louis, Mo.) and analyzed by a modified FACS-4 (three lasers) flow cytometer (Becton-Dickinson). Five thousand cells were counted.

Presentation of results and statistical analysis

Results of the effects of VIP on IgG, IgM, and IgA production are expressed as the percent inhibition with the equation:

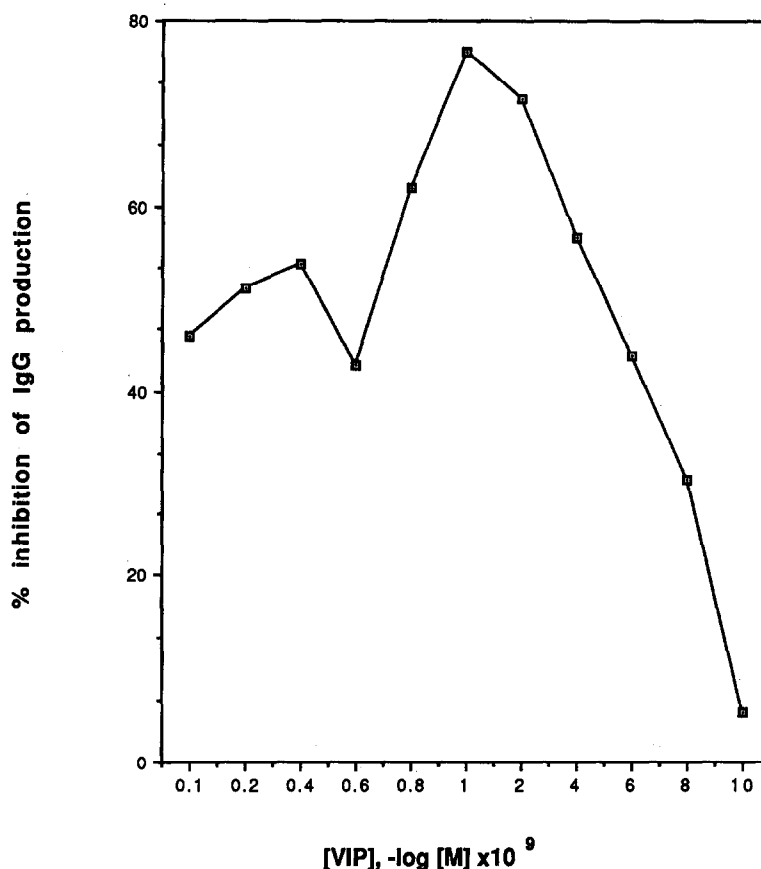


FIG. 2. VIP concentration dependence of inhibition of IgG production by PWM-stimulated PBMCs on day 9. At VIP concentrations of 6×10^{-10} through 4×10^{-9} mol/L a significant inhibition of IgG production was observed.

Percent inhibition =

$$1 - \frac{\text{Ig in the experimental sample (ng/mL)}}{\text{Ig in the untreated control ng/mL}} \times 100$$

Statistical analysis was performed with a paired Student's *t* test, with the Statview II statistical software package (version 1.03, Abacus Concepts, Inc., Berkeley, Calif.).

RESULTS

Effect of VIP on IgG, IgM, and IgA production

To examine the effects of prolonged exposure to VIP, in six separate experiments with blood mononuclear cells isolated from six different subjects, VIP was added twice daily at concentrations ranging from 1×10^{-12} to 10^{-8} mol/L to cultured PBMCs. Suppression of IgG and IgM production by VIP was apparent by day 5 and persisted for 18 and 14 days, respectively. Fig. 1 is representative of the effect of VIP on PBMCs from one subject. In all subjects studied, optimal suppression of IgG

and IgM production occurred at 10^{-9} mol/L VIP (Fig. 1 and Table I, experiment 1). IgG production was suppressed significantly on days 5 to 18, with a maximal effect on days 9 and 14 ($p < 0.0001$ and $p < 0.003$, respectively). Suppression of IgM also was observed at these times, but statistical significance was attained only on day 5 ($p < 0.05$). VIP showed no statistically significant suppressive effect on IgA production (Fig. 1).

Representative values from two experiments in which PBMCs from two subjects were treated with 10^{-11} to 10^{-8} mol/L VIP are reported in Table I (the subject represented in experiment 1 is also shown in Fig. 1). All concentrations of VIP studied suppressed IgG production effectively after 9 days of continuous exposure (Table I), although maximal inhibition of IgG production was observed at 10^{-9} mol/L in both experiments. In a separate experiment, 0.2 log-fold dilutions of VIP (1×10^{-10} to 1×10^{-8} mol/L) were added to

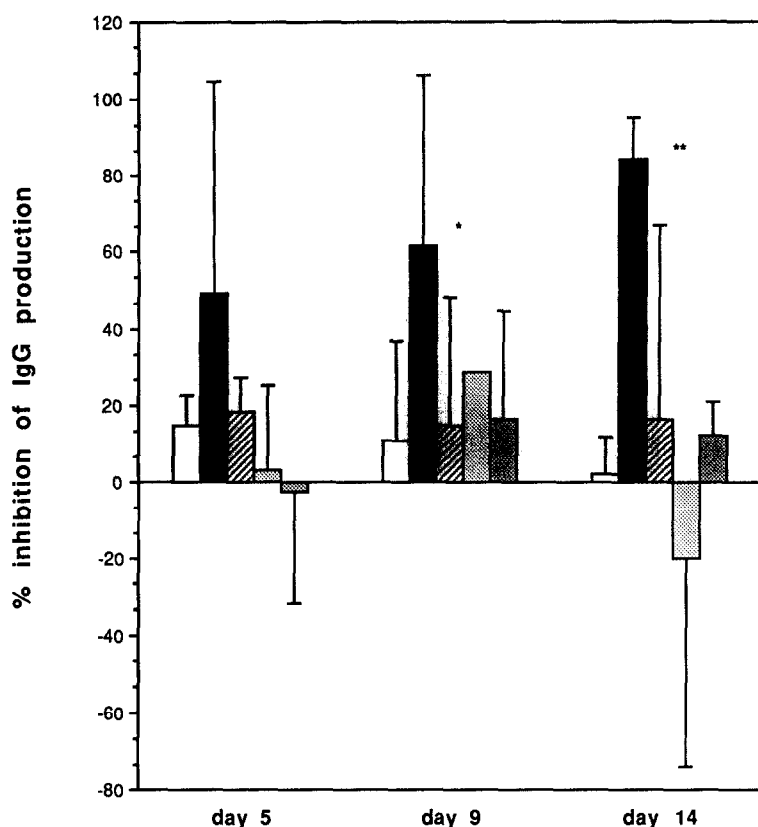


FIG. 3. Percent inhibition of IgG production. PBMCs treated with PWM and VIP at concentrations ranging from 10^{-8} through 10^{-12} mol/L. Open bar, 1×10^{-8} mol/L; solid bar, 1×10^{-9} mol/L VIP; cross-hatched bar, 1×10^{-10} mol/L VIP; coarse stippled bar, 1×10^{-11} mol/L VIP; fine stippled bar, 1×10^{-12} mol/L VIP. Each data point represents mean $\pm$ SD of inhibition (IgG in treated cells as a fraction of control cells) of IgG production in three to five individual (consecutive) experiments. Significant inhibition was observed at VIP concentration of 10^{-9} mol/L on day 9 (* $p < 0.015$) and on day 14 (** $p < 0.0001$).

PBMC cultures for 9 days in order to more closely define the dose-response relationship of VIP suppression of IgG production (Fig. 2). There was substantial inhibition of IgG production at VIP concentrations ranging from 4×10^{-10} through 6×10^{-9} mol/L. VIP failed to suppress IgG, IgM, or IgA production by unstimulated PBMCs (data not shown).

In these experiments untreated PBMCs from each donor served as controls; data are thus presented as the percentage suppression of immunoglobulin production compared with untreated cells (0% suppression). Maximal significant inhibition of IgG production by 10^{-9} mol/L VIP was observed on days 9 ($79.2\% \pm 27\%$; range, 41% to 97%; $p < 0.02$) and 14 ($84.4\% \pm 9\%$; range, 74% to 96%; $p < 0.001$) at 1×10^{-9} mol/L VIP (Fig. 3). Statistically significant inhibition was not observed at higher or lower concentrations.

Effect of VIP on IL-4-induced IgE production

To examine the effects of VIP on IgE production, we used a modification of a culture system previously described¹⁶ in which mixed PBMCs were stimulated on day 0 with IL-4 to evoke production of IgE. In this system PBMCs were cultured in media with optimal concentrations of recombinant IL-4 (data not shown) with or without twice daily addition of VIP. These cells were cultured in duplicate tubes under four culture conditions: (1) untreated, (2) VIP alone, (3) IL-4 alone, and (4) IL-4 with VIP. On day 18, PBMCs from all subjects responded to IL-4 with statistically significant increases in IgE production.

The effects of twice daily additions of VIP on IL-4-induced IgE production was donor-dependent in four subjects and differed from the suppression of PWM-evoked IgG and IgM production (Fig. 4). The production of IgE by IL-4-

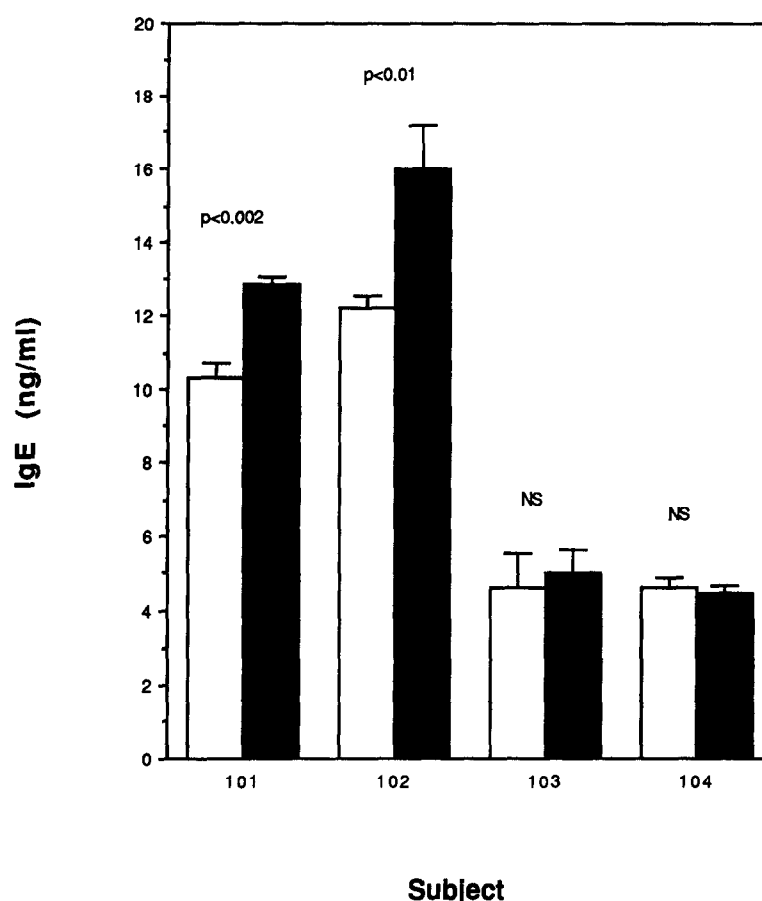


FIG. 4. Effect of VIP₁₋₂₈ [10^{-9} mol/L] (solid bar) compared with untreated controls (open bar) on IL-4 (100 U/ml)-induced IgE production (nanograms per milliliter). Each bar represents the mean of duplicate cultures of 1×10^6 cells/ml, incubated for 18 days. Statistically significant enhancement of IgE production by VIP is noted in subjects 101 and 102, but no significant effect was noted in subjects 103 and 104.

stimulated PBMCs of two subjects was enhanced by 10^{-9} mol/L VIP at 18 days. IgE produced by similarly stimulated PBMCs of two other subjects (subjects 103 and 104) was not altered by 10^{-9} mol/L VIP (Fig. 4).

As with the other isotypes of immunoglobulin, the VIP dose-response relationship for enhancement of IgE production by PBMCs of the two responsive subjects was relatively narrow, with optimal effects at 10^{-9} mol/L (Fig. 5). In three separate experiments conducted over a 10-week period, mixed lymphocytes from subject 101 were cultured in the presence of IL-4 with and without a range of concentrations of VIP. There was reproducible enhancement of IgE production by 10^{-9} mol/L VIP (Fig. 6). Similarly, subject 103 consistently failed to respond to VIP (data not shown) over the same period of time. These results suggest that the IgE responses of IL-4-

stimulated PBMCs to VIP may be a stable characteristic of each donor.

In control experiments VIP₄₋₂₈ (10^{-5} to 10^{-9} mol/L), VIP₁₀₋₂₈ (10^{-5} to 10^{-9} mol/L), and substance P (2×10^{-4} mol/L) had no effect on IgE production by IL-4-stimulated PBMCs from the same donors.

Effect of VIP on cellular proliferation

To determine whether the observed suppression of IgG and IgM production was secondary to possible antiproliferative effects of VIP, PWM-stimulated PBMCs were treated with VIP for 1, 6, 10, and 15 days and pulsed with tritiated thymidine for the last 16 to 20 hours. In three experiments with PWM-stimulated PBMCs from three different donors, serial log-fold varying concentrations of VIP encompassing the ranges with optimal effects on immunoglobulin production were

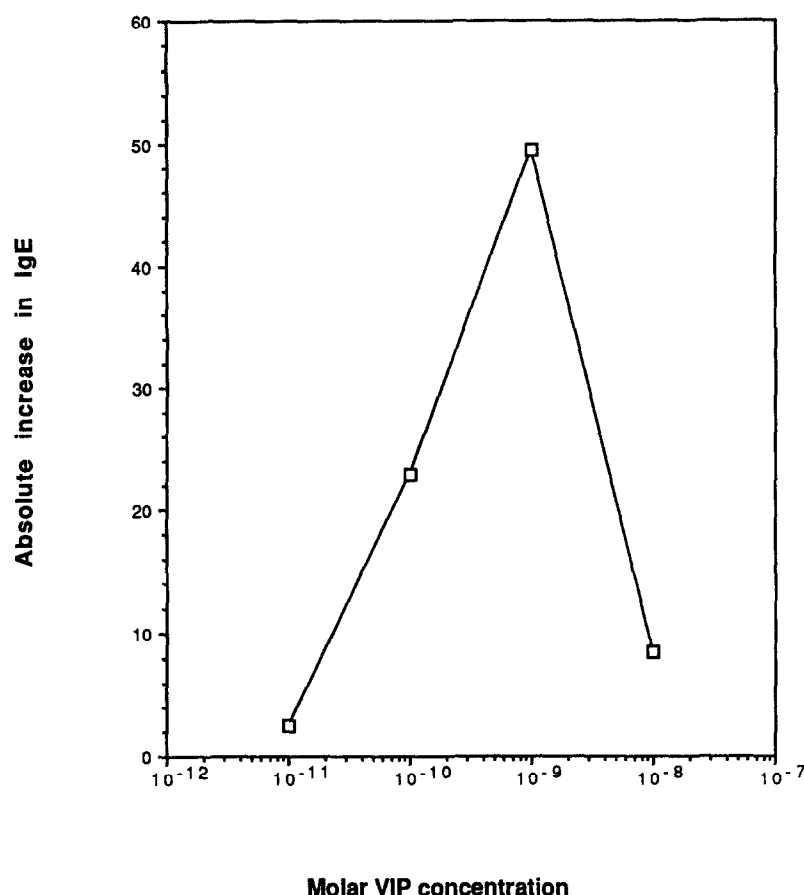


FIG. 5. Dose-response enhancement of IL-4-induced IgE production by VIP₁₋₂₈ in subject 101. The data are presented as the difference (Δ) in IgE production between VIP-treated cultures and untreated controls. Maximal VIP-induced enhancement of IgE production is noted at a VIP concentration of 1×10^{-9} mol/L.

added twice daily and had no significant biologic effect on the maximal proliferative response observed on day 5 (Fig. 7). VIP also failed to affect the proliferative responses of PWM-unstimulated PBMCs, compared with untreated controls (data not shown). These data demonstrate that the immunoglobulin suppressive effects of VIP are not secondary to inhibition of PWM-induced proliferation.

Recovery of VIP from culture supernatant

Although VIP is degraded by cellular proteolytic enzymes, there was concern that twice daily additions might still lead to accumulation and higher than expected effective levels of VIP. Therefore on day 9, the concentrations of VIP in the culture media were determined by radioimmunoassay 1, 2, 4, 6, and 12 hours after the previous administration of VIP. By 12 hours, the VIP concentrations were never higher than 30%

of that attributable to dilution in medium alone in any of the culture supernatants (Table II).

Effect of VIP on the numbers of T and B cells in PBMC suspensions

PBMCs bearing specific surface markers were quantified by flow cytometry before and after 14 days of culture for subject 101 (experiment 2), for whom VIP reproducibly inhibited IgG and IgM production and enhanced IL-4-stimulated IgE production. The frequencies of CD4⁺ and CD8⁺ T lymphocytes and of CD23 on CD19⁺ B lymphocytes were the same with IL-4 or PWM alone as with these respective stimuli combined with VIP (Table III).

DISCUSSION

A series of studies was designed to assess the possibility that prolonged exposure of human mixed PBMCs to VIP *in vitro*, at concentrations

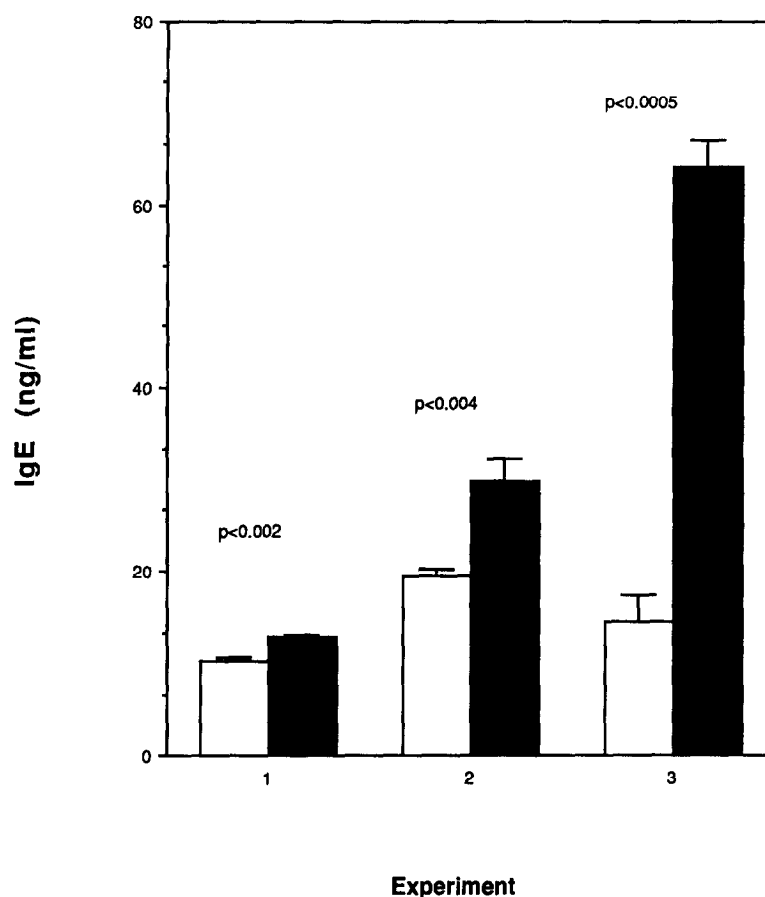


FIG. 6. VIP effect on IL-4-induced IgE production in subject 101 in three experiments on separate occasions. Effects of addition of VIP₁₋₂₈ [10^{-9} mol/L] (solid bar) are compared with untreated control cells (open bar), on IL-4 (100 U/ml)-induced IgE production (nanograms per milliliter). Each bar represents the mean of duplicate cultures of 1×10^6 cells/ml, incubated for 18 days. The functional response to VIP in this subject remains stable over time.

analogous to those detectable in the immune organs innervated by VIP-secreting nerves, might reproduce the effects of VIP on immunoglobulin production in situ. To more closely approximate the in vivo conditions of lymphocytes influenced by VIP, synthetic VIP was added to culture media twice daily for up to 18 days to achieve constant levels of approximately 3% to 30% of those expected after a single addition of VIP to medium alone (Table II), despite the expected lymphocytic binding and degradation of VIP.¹⁷

Twice daily addition of VIP to culture supernatants resulted in significant suppression of both IgG and IgM production measured on days 9 and 14. VIP suppressed the immunoglobulin production by PWM-stimulated PBMCs more than that by unstimulated PBMCs. The absence of changes in T-cell subsets suggests that direct alteration of PBMC composition or proliferative responses to PWM or IL-4 by VIP is unlikely. The absence of

generalized suppressive effects in lymphocytes (Fig. 7) supports this conclusion, although it is possible that VIP may be suppressing the proliferation of a subset of B cells that is destined to contribute to the IgM or IgG immunoglobulin responses; examining this possibility would be technically difficult.

The binding of radiolabeled VIP to cell surface receptors was not determined in this study because of the heterogeneity of cell types in mixed PBMC preparations. The greatest effects of VIP on immunoglobulin production were observed at nanomolar VIP concentrations, consistent with the dissociation constant in the nanometer range previously reported for most homogeneous populations of lymphocytes. The mechanisms by which VIP suppresses IgG and IgM production may include direct suppression of B-cell immunoglobulin synthesis, possibly by increases in cellular levels of cyclic adenosine monophosphate

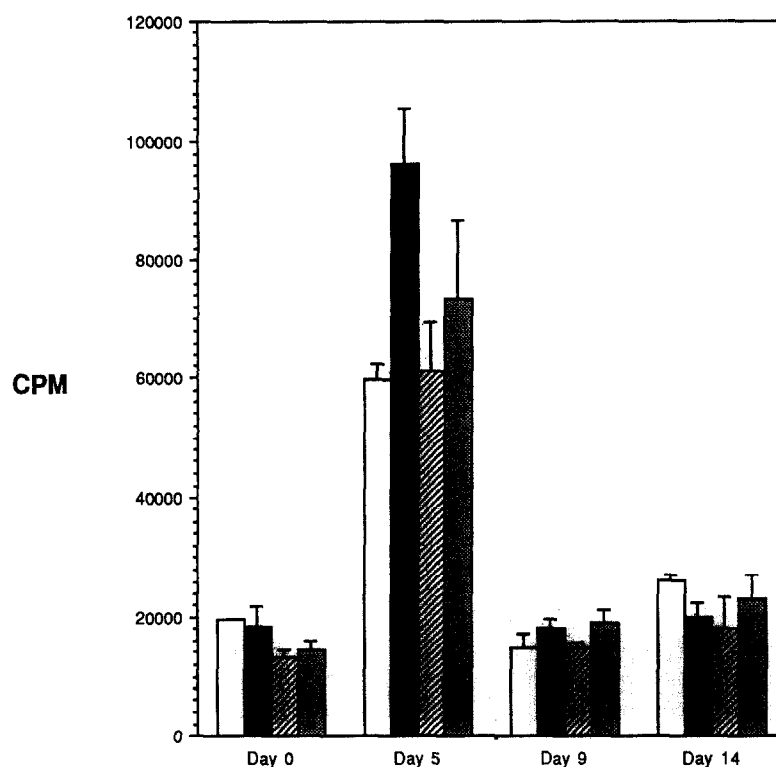


FIG. 7. Proliferative responses of PBMCs to treatment with PWM (4 μ g/ml) in the absence and presence of VIP at 10^{-8} through 10^{-10} mol/L. PBMCs were pulsed with 10 μ Ci/ml of tritiated thymidine on days 0, 5, 9, and 14 of culture. Each data point represents mean $\pm$ SD of counts per minute per 1×10^6 PBMCs in three independent experiments. No inhibition of cellular proliferation by VIP was observed at each point. *Open bar*, Control; *solid bar*, VIP [10^{-8} mol/L]; *cross-hatched bar*, VIP [10^{-9} mol/L]; *stippled bar*, VIP [10^{-10} mol/L].

TABLE II. Time course of recovery of VIP in PBMC culture supernatants*

	Hours after addition of VIP				
	1	2	4	6	12
No PBMCs†					
1 nmol/L VIP	0.8, 0.7	0.7, 0.6	0.6, 0.8	0.6, 0.8	0.7, 0.5
3 nmol/L VIP	2.6, 2.8	2.7, 2.8	2.6, 2.7	2.4, 2.7	2.3, 2.5
PBMC +					
1 nmol/L VIP	0.6, 0.6	0.4, 0.5	0.3, 0.4	0.2, 0.3	0.1, 0.04
3 nmol/L VIP	2.4, 2.5	2.1, 2.4	1.3, 1.7	0.9, 1.2	0.8, 0.5
PBMC + PWM +					
1 nmol/L VIP	0.7, 0.8	0.4, 0.3	0.2, 0.4	0.2, 0.2	0.1, 0.09
3 nmol/L VIP	2.5, 2.1	2.0, 2.2	1.2, 1.5	1.0, 1.0	0.9, 0.6

*Results shown of duplicate samples.

†Single addition of VIP on day 7.

(cAMP), or elaboration of inhibitory cytokines by regulatory T cells. Several investigators have shown that VIP exerts preferential inhibitory effects on some subsets of T cells,^{2, 4, 7, 18-20} but the underlying mechanisms have not been defined.

Recent evidence from our laboratory suggests that VIP may be specifically affecting the B lymphocyte. In a separate model system we have shown that SKW 6.4, an Epstein-Barr virus-transformed human B-cell line, expresses high-affinity receptors for VIP. Phorbol myristate acetate-

TABLE III. Effect of VIP on T and B lymphocyte subsets from subject 101

Cell surface marker	Day 0* (baseline)	Day 14					
		Medium alone	VIP†	PWM	IL-4	PWM + VIP	IL-4 + VIP
CD3	69	81	90	99	93	99	91
CD4/CD3	61	69	76	37	73	35	76
CD8/CD3	18	22	18	70	26	75	29
CD19	6	10	6	1	4	1	4
CD23/CD19	76	75	73	67	86	86	75

*Percentage of cells expressing each cell surface marker.

†VIP 10^{-9} mol/L, PWM 4 μ g/ml, IL-4 100 U/ml.

stimulated production of IgM by SKW 6.4 was suppressed up to 40% after 7 days of exposure to 10^{-12} to 10^{-9} mol/L VIP. Similar to our results in PBMCs, there was no demonstrable effect on unstimulated cells or cellular proliferation.²¹

Cytokines contribute to the regulation of immunoglobulin synthesis and secretion. In the murine immune system, two distinct subsets of CD4⁺ lymphocytes have been characterized phenotypically and functionally. The T_{H1} cell produces IL-2 and interferon- γ and the T_{H2} cell produces IL-4, IL-5, and IL-10. T_{H2} cells appear to promote preferential production of some immunoglobulin isotypes, whereas T_{H1} cells appear to inhibit the proliferation of the T_{H2} cells. Such subsets have been characterized only functionally, not phenotypically, in human beings.²² T-cell subset elaboration of cytokines may be modulated by neuropeptides, as demonstrated in several experimental models. For example, substance P functions as a cofactor to enhance the expression of specific IL-2 messenger RNA and IL-2 secretion by T lymphocytes,²³ to augment interferon- γ production by PBMCs and to stimulate the release of IL-1, tumor necrosis factor- α , and IL-6 by monocytes.^{24, 25} In this study, subject 101 demonstrated a reproducible functional IgE response to VIP and concomitantly showed changes in lymphocyte phenotype with this neuropeptide, which were similar to the effects of IL-4 or PWM alone (Table III). These data suggest that VIP may exert some of its effects on IgE production by enhancement of IL-4-dependent mechanisms.

Interestingly, it has recently been reported that prostaglandin E₂ may inhibit activation of some subsets of murine B lymphocytes while also functionally promoting IgE and IgG1 synthesis.²⁶ Although we did not observe inhibition of mitogen-induced cellular proliferation, we were studying

mixed PBMCs rather than homogeneous subsets of prostaglandin E₂-sensitive lymphocytes. These observations suggest that the enhancement of IgE synthesis by VIP observed in our experiments may be due in part to VIP-induced prostaglandin E₂ release, although this question has not yet been directly tested.

VIP stimulates T-lymphocyte production of IL-5,¹⁸ which may facilitate the enhancement of IgE production by IL-4. Our finding of an augmentation in IgE production by VIP suggests that its effects on the T_{H2}-type cell in human PBMCs may include increased elaboration of IL-5. VIP also activates adenylate cyclase and subsequent protein phosphorylation by means of a cAMP-dependent protein kinase.¹² Isotype switching to IgG1 and IgE in IL-4-treated murine B lymphocytes is positively regulated by increases in cAMP.²⁷ The effects on IgE production may thus reflect cAMP-dependent mechanisms, as well as enhancement of VIP or T-cell subset production of IL-5 and other immunoregulatory cytokines.

VIP appeared to elicit two different IgE responses from PBMCs exposed to IL-4, which were consistent over time in each donor. VIP enhanced IgE production in two subjects and had no effect in two other subjects. Although the relationship of IgE responses to atopy has been well established, a much larger study will need to be undertaken to examine the relationship of VIP effects on IgE production to baseline serum IgE concentrations, atopic status, specific allergic profile to environmental allergens, previous history of allergy immunotherapy, or other underlying metabolic disorders. It will be important to determine the factors that distinguish those subjects with VIP-enhanced IgE production and study the influences of VIP on the expression of IL-4, interferon- γ , and IL-10.

REFERENCES

- Krco CJ, Gores A, Go VL. Gastrointestinal regulatory peptides modulate in vitro immune reactions of mouse lymphoid cells. *Clin Immunol Immunopathol* 1986;39:308-18.
- Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferation by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. *J Immunol* 1986;136:152-6.
- Stanisz AM, Scicchitano R, Bienenstock J. The role of vasoactive intestinal peptide and other neuropeptides in the regulation of the immune response in vitro and in vivo. *Ann NY Acad Sci* 1988;527:478-85.
- Ottaway CA. In vitro alteration of receptors for vasoactive intestinal peptide changes the *in vivo* localization of mouse T cells. *J Exp Med* 1984;160:1054-69.
- Drew PA, Shearman DJC. Vasoactive intestinal peptide: a neurotransmitter which reduces human NK cell activity and increases Ig synthesis. *Aust J Exp Biol Med Sci* 1985;63:313-8.
- Ottaway CA. Vasoactive intestinal peptide as a modulator of lymphocyte and immune function. *Ann NY Acad Sci* 1988;527:486-500.
- Ottaway CA, Greenberg GR. Interaction of vasoactive intestinal peptide with mouse lymphocytes: specific binding and the modulation of mitogen responses. *J Immunol* 1984;132:417-23.
- Danek A, O'Dorisio MS, O'Dorisio TM, George JM. Specific binding sites for vasoactive intestinal peptide on non-adherent peripheral blood lymphocytes. *J Immunol* 1983;131:1173-7.
- Wood CL, O'Dorisio MS. Covalent cross-linking of vasoactive intestinal polypeptide to its receptors on intact human lymphoblasts. *J Biol Chem* 1985;260:1243-7.
- Calvo JR, Molinero P, Jimenez J, Goberna R, Guero JM. Interaction of vasoactive intestinal peptide (VIP) with rat lymphoid cells. *Peptides* 1986;7:177-81.
- Finch RJ, Sreedharan SP, Goetzl EJ. High-affinity receptors for vasoactive intestinal peptide on human myeloma cells. *J Immunol* 1989;142:1977-81.
- O'Dorisio MS, Shannon BT, Fleshman DJ, O'Dorisio TM. Identification of high affinity receptors for vasoactive intestinal peptide on human lymphocytes of B cell lineage. *J Immunol* 1989;142:3533-6.
- Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest* 1968;21(suppl. 97):77-89.
- Goetzl EJ, Sreedharan SP, Turck CW. Structurally distinctive vasoactive intestinal peptides from rat basophilic leukemia cells. *J Biol Chem* 1988;263:9083-6.
- Macy E, Kemeny M, Saxon A. Enhanced ELISA: how to measure less than 10 picograms of a specific protein (immunoglobulin) in less than 8 hours. *FASEB J* 1988;2:3003-9.
- Claassen JL, Levine AD, Buckley RH. Recombinant human IL-4 induces IgE and IgG synthesis by normal and atopic donor mononuclear cells. Similar dose response, time course, requirement for T cells, and effect of pokeweed mitogen. *J Immunol* 1990;144:2123-30.
- Goetzl EJ, Kodama KT, Turck CW, Schiogolev SA, Sreedharan SP. Unique pattern of cleavage of vasoactive intestinal peptide by human lymphocytes. *Immunology* 1989;66:554-8.
- Mathew RC, Cook GA, Blum AM, Metwali A, Felman R, Weinstock JV. Vasoactive intestinal peptide stimulates T lymphocytes to release IL-5 in murine schistosomiasis mansoni infection. *J Immunol* 1992;148:3572-7.
- Ottaway CA. Selective effects of vasoactive intestinal peptide on the mitogenic response of murine T cells. *Immunology* 1987;62:291-7.
- Söder O, Hellström PM. Neuropeptide regulation of human thymocyte, guinea pig T lymphocyte and rat B lymphocyte mitogenesis. *Int Arch Allergy Appl Immunol* 1987;84:205-11.
- Cheng PJ, Sreedharan SP, Robichon A, Goetzl EJ. Vasoactive intestinal peptide inhibits phorbol ester-enhanced IgM synthesis in a human B cell line [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:260.
- Robinson DS, Hamid Q, Ying S, et al. Predominant TH₂-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
- Calvo CF, Chavanel G, Senik A. Substance P enhances IL-2 expression in activated human T cells. *J Immunol* 1992;148:3498-504.
- Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science* 1988;241:1218-21.
- Wagner F, Fink R, Hart R, Dancygier H. Substance P enhances interferon-2 production by human peripheral blood mononuclear cells. *Regul Pept* 1987;19:355-64.
- Roper RL, Phipps RP. Prostaglandin E₂ and cAMP inhibit B lymphocyte activation and simultaneously promote IgE and IgG1 synthesis. *J Immunol* 1992;149:2984-91.
- Lycke N. Analysis of the second messenger systems involved in the synergistic effect of cholera toxin and interleukin-4 on B cell isotype-switching [Abstract]. *FASEB J* 1992;6:A1708.