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Vasoactive intestinal peptide stimulates immunoglobulin production and growth of human B cells

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SUMMARY

The effect of vasoactive intestinal peptide (VIP) on human lymphoblastoid B cell lines and tonsil B cells was studied. VIP increased immunoglobulin production and proliferation by lymphoblastoid B cell line, GM-1056, in a dose-dependent manner. As little as 10^{-12} M of VIP was effective, and higher concentrations of VIP induced an approximately five-fold increase in IgA production. Moreover, this enhancement was blocked by VIP antagonist. Similarly, VIP enhanced IgM and IgG production by other lymphoblastoid B cell lines, CBL and IM-9, respectively. In contrast to VIP, another neuropeptide substance P (SP) or somatostatin failed to enhance immunoglobulin production and thymidine uptake. VIP also enhanced IgA production and thymidine uptake by purified tonsil B cells. However, in contrast to B cell lines, VIP failed to enhance IgM and IgG production by tonsil B cells. SP or somatostatin failed to enhance immunoglobulin production or thymidine uptake by tonsil B cells. These results indicate that VIP acts as B cell stimulatory factor and that VIP may also have preferential effect on IgA production on tonsil B cells.

Keywords vasoactive intestinal peptide B cell responses isotype-specific IgA production

INTRODUCTION

A regulatory loop between the immune and the neuroendocrine systems has been suggested. From the viewpoint of this neuro-immuno-endocrine axis, several attempts have been made to determine the bi-directional effects of neuropeptides on the immune regulation [1–3].

Vasoactive intestinal peptide (VIP) is a 28 amino acid neuropeptide that has been isolated from the intestine [4], the brain [5], upper respiratory and nasal mucosa, salivary glands, and the male and female genital tracts [6,7]. It is also identifiable in human eosinophils, polymorphonuclear and mononuclear leucocytes [8–12]. VIP is known as a potent stimulant of mucous secretion, vasodilatation, and smooth muscle relaxation in bronchus and many other organs [13].

According to recent studies, VIP also has effects on the immune regulation. In the murine immune system, VIP inhibited the proliferative responses of lymphocytes to the T cell mitogens [14,15]. VIP also altered preferentially IgA synthesis by lymphocytes from gastrointestinal tissues and spleen [3]. In the human immune system, VIP inhibited the proliferative response of T lymphocytes to mercuric chloride [16], and inhibited natural killer (NK) cell function [17]. Specific high affinity receptors for VIP have been identified on human peripheral blood lymphocytes, T cells and B cells [18–22] and on a number of human-derived lymphoid cell lines including T cell [23,24] and B cell [24,25] lineages. However, there are few studies of the effects of VIP on B cell responses in the human system. We therefore studied the effects of VIP on B cell responses.

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MATERIALS AND METHODS

Reagents

VIP (porcine sequence) and VIP antagonist [D-p-chloro-Phe-6,leu17]-VIP (porcine sequence) were purchased from Sigma Chemical Co. (St Louis, Mo). Substance P (SP) and somatostatin were purchased from Peptide Institute (Osaka, Japan). Neuropeptides were initially diluted in PBS to 10^{-5} M sterilized filtering through a millipore filter, and stored at −4°C until used. The culture medium was complete RPMI which consisted of RPMI 1640 (M.A. Bioproducts, Walksville, Md) containing 10% heat-inactivated fetal calf serum (FCS) (Irvine Scientific), 2 mM glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin.

B cell lines

GM-1056 is an EBV-transformed IgA producing human lymphoblastoid line obtained through the NIGMS human genetic mutant cell repository (Camden, NJ) [26]. CBL#3 (CBL) is an EBV-transformed IgM producing human lymphoblastoid line (a kind gift from Dr Christel H. Uittenbogaart, Department of Microbiology and Immunology and Jonsson Comprehensive

Cancer Center, UCLA, Ca.) [27]. IM-9 is an IgG producing human plasma cell line obtained from Japanese Cancer Research Resources Bank (Tokyo, Japan) [28]. All cell lines are mycoplasma free and maintained in complete RPMI.

B cell preparations

Tonsils (kind gifts from Dr K. Nagahara, Department of Otolaryngology, National Kyoto Hospital, Kyoto, Japan) were obtained from non-atopic donors with chronic tonsillitis during tonsillectomy. The tonsils were disrupted and mononuclear cells (MNC) were obtained using buoyant density centrifugation on a Ficoll-Hypaque gradient [29]. B cells were obtained from MNC by sheep erythrocyte rosetting, depleting T cells. This B cell fraction contained < 1% T cells [26].

Cell culture system

GM-1056, CBL and IM-9 cells were cultured in 96-well flat-bottomed microplates at a density of 5×10^3 cells, 2×10^3 cells and 1×10^3 cells/ 200 μl/ well, respectively. Cells were cultured with varying concentrations of VIP or other factors for 2–4 days at 37°C in a humidified atmosphere of 5% CO_2, and then pulsed with 1 μCi [methyl-^{3}H-] thymidine per well 8 h before harvest. Each culture was set up in triplicate or tetraplicate. The amounts of IgG, IgM and IgA in the culture supernatant were determined by ELISA, and thymidine uptake was determined simultaneously [26].

Purified B cells (1×10^5 cells/200 μl per well) were cultured in 96-well U-shaped microplates for 14 days with or without VIP. The amounts of IgG, IgM and IgA in culture supernatant were measured by ELISA as above. Tonsil B cells were also cultured for 2 days and pulsed as above for 16 h before harvest, and thymidine uptake was determined. The sensitivities of the ELISA assays were 0·3 ng/ml for IgG, IgM and IgA.

RESULTS

VIP enhances IgA production and thymidine uptake by GM-1056 cells

As shown in Fig. 1a, VIP enhanced IgA production by GM-1056 cells in a dose-dependent fashion. VIP at concentrations above 10^{-12} M enhanced IgA production significantly. Higher concentrations (10^{-11}–10^{-8} M) made this enhancement reach a plateau, inducing an approximately five-fold increase in IgA production by GM-1056 cells. VIP also enhanced thymidine uptake by GM-1056 cells in a similar dose-dependent fashion (Fig. 1b). VIP at 10^{-12} M enhanced incorporating of ^{3}H-thymidine significantly. Higher concentrations (10^{-11}–10^{-8} M) made this enhancement reach a plateau, inducing an approximately five-fold increase. In contrast to VIP, another neuropeptide, SP or somatostatin enhanced neither IgA production nor thymidine uptake (Fig. 1). Moreover, this enhancement of IgA production and thymidine uptake at 10^{-9} M of VIP was completely blocked by 10^{-6} M of VIP antagonist, [D-p-chloro-Phe6,Leu17]-VIP, and 70% blocked by 10^{-9} M of VIP antagonist, while the addition of the VIP antagonist did not affect control IgA production and thymidine uptake of GM-1056 cells (data not shown). The kinetics of VIP-induced augmentation of IgA production and thymidine uptake by GM-1056 cells are shown in Fig. 2. Significant increase in both IgA production and thymidine uptake were observed on day 3 in culture, with the greatest enhancement occurring on day 4.

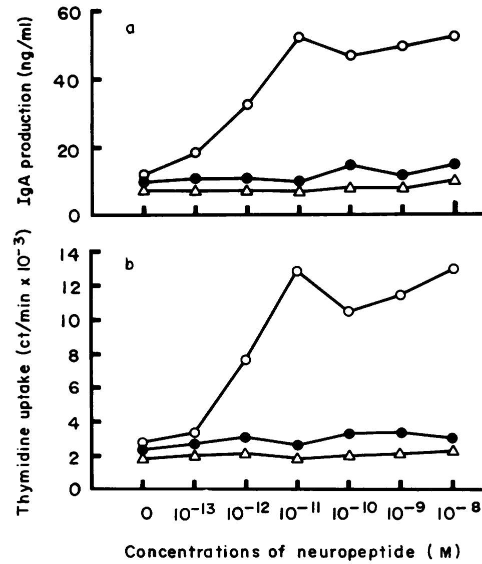

Fig. 1. Effect of VIP on IgA production and thymidine uptake by GM-1056 cells. a, GM-1056 cells were cultured with increasing concentrations of VIP (○), SP (●) or somatostatin (△). After 3 days of culture, IgA production was measured; b, GM-1056 cells were cultured with increasing concentrations of VIP (○), SP (●) or somatostatin (△). After 3 days of culture, thymidine uptake was measured. Results are the means of triplicate cultures. s.d. were < 15%.

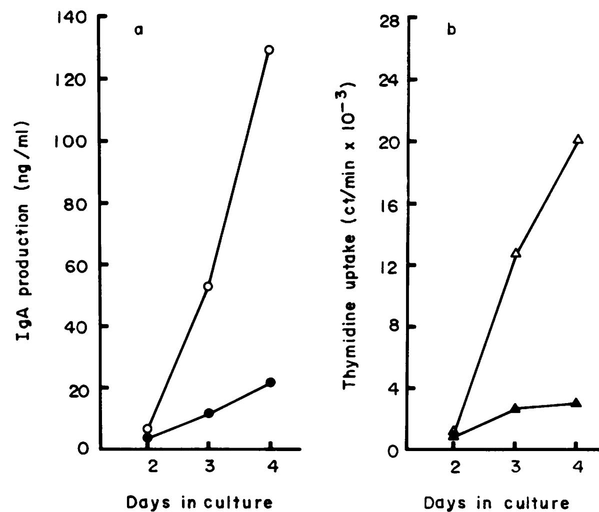

Fig. 2. Kinetics of VIP effect on GM-1056. GM-1056 cells were cultured with VIP (10^{-11} M) for 2 to 4 days, and IgA production and thymidine uptake were measured. a, IgA production was measured after culture with (○) or without (●) VIP, b, Thymidine uptake was measured after culture with (△) or without (▲) VIP. Results are the means of triplicate cultures. s.d. were < 15%.

VIP enhances immunoglobulin production and thymidine uptake by CBL and IM-9 cells

We then tested the effect of VIP on other B cell lines, CBL and IM-9. As shown in Table 1, VIP enhanced immunoglobulin production and cell proliferation in both cell lines. VIP at 10^{-10}

Table 1. Effect of VIP on CBL and IM-9

Factor	CBL IgM (ng/ml)	CBL Thymidine (ct/min)	IM-9 IgG (ng/ml)	IM-9 Thymidine (ct/min)
Medium	13·2	850	9·2	341
VIP 10^{-11} M	17·1 (30)*	2014 (137)	11·3 (23)	ND
VIP 10^{-10} M	31·0 (135)	2185 (157)	15·6 (70)	648 (90)
VIP 10^{-9} M	ND	ND	22·6 (146)	1078 (216)

CBL and IM-9 cells were cultured with increasing concentrations of VIP. After 3 days of culture, immunoglobulin production and thymidine uptake were measured. Results are the means of triplicate cultures. s.d. were < 15%.

* Percentage of enhancement of control is indicated in parentheses.
ND, not done

M induced a two- to three-fold enhancement in IgM production and thymidine uptake by CBL cells, and 10^{-9} M VIP induced a two- to three-fold enhancement in IgG production and thymidine uptake by IM-9 cells. As in the case of GM-1056, SP or somatostatin at concentrations from 10^{-13} to 10^{-8} M, enhanced neither immunoglobulin production nor thymidine uptake by CBL or IM-9 cells (data not shown).

VIP enhances IgA production and cell proliferation in cultures of tonsil B cells

The effect of VIP on tonsil B lymphocytes was then studied. As shown in Fig. 3a, unstimulated tonsil B cells cultured in the medium alone produced $10{\cdot}8 \pm 0{\cdot}8$ ng/ml of IgA, and 10^{-10} M VIP augmented IgA production to $16{\cdot}5 \pm 1{\cdot}2$ ng/ml (53% enhancement). In contrast, VIP failed to enhance IgG and IgM production. As shown in Fig. 3b, VIP also induced a three-fold and four-fold enhancement in thymidine uptake by tonsil B cells at 10^{-11} M and 10^{-12} M concentration, respectively. In other experiments, there are 100–300% enhancements of IgA production and 100–160% enhancements of thymidine uptake, depending on donors (Table 2). The difference of optimal concentrations in IgA production and thymidine uptake may indicate that VIP actively enhances both immunoglobulin production and proliferation depending on its concentration. In contrast to VIP, SP or somatostatin at concentrations of 10^{-13}–10^{-8} M had no effects on immunoglobulin production and proliferation of tonsil B cells (data not shown).

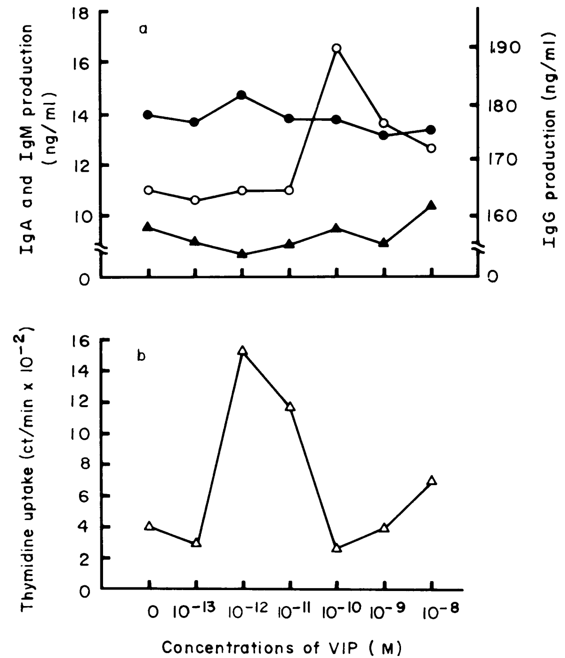

Fig. 3. Effect of VIP on tonsil B cells. Tonsil B cells were cultured with various concentrations of VIP, and immunoglobulin production and thymidine uptake were measured on day 14 and 3 of culture, respectively. a, IgA (○) IgM (●) and IgG (▲) production were measured after culture with or without VIP; b, Thymidine uptake (△) was measured after culture with or without VIP. Results are the means of triplicate cultures. s.d. were < 15%.

Table 2. Effect of VIP on tonsil B cells

Experiment	IgA production (ng/ml)	Thymidine uptake (ct/min)
1		
Medium	1·4	707
VIP 10^{-12}M	ND	1258 (78)
10^{-11}M	2·9 (107)*	1725 (143)
10^{-10}M	ND	ND
10^{-9}M	2·1 (50)	ND
2		
Medium	0·5	418
VIP 10^{-12}M	ND	848 (102)
10^{-11}M	1·7 (240)	1089 (160)
10^{-10}M	2·0 (300)	ND
10^{-9}	1·4 (180)	ND

Tonsil B cells were cultured with or without VIP, and immunoglobulin production and thymidine uptake were measured on days 14 and 3 of culture, respectively. The results of two other experiments in which donors of tonsils were different from one another are shown. Results are the means of triplicate cultures. s.d. were < 15%.

* Percentage of enhancement of control is indicated in parentheses.

ND, not done.

DISCUSSION

Our experiments show that VIP directly stimulated B cell lines to enhance immunoglobulin production and thymidine uptake, while other neuropeptides, SP and somatostatin failed to do so. Moreover, this enhancement is blocked by the VIP antagonist, indicating that this stimulation is specific to VIP. Among the three B cell lines, GM-1056 cells which produce IgA were most stimulated (five- to six-fold), while IgM-producing CBL cells and IgG-producing IM-9 cells were less stimulated (two- to three-fold). VIP also enhanced IgA production and thymidine uptake by unstimulated tonsil B cells without affecting IgG and IgM production. This is in agreement with the result that VIP modulated IgA production by mouse lymphocytes from spleen,

mesenteric lymph nodes and Peyer's patches while IgM production was less affected and IgG synthesis was unchanged [3]. The different effect of VIP on B cell lines and on tonsil B cells is not surprising. We have previously reported that the nerve growth factor specifically enhances IgG4 production by tonsil B cells while it enhances IgG, IgA and IgM production, respectively, from B cell lines [30,31]. Moreover, weak but significant enhancement (53%) of IgA production by tonsil B cells stimulated with VIP has also a precedent. Nerve growth factor induces IgG4 production slightly by unactivated resting B cells, while it greatly enhances IgG4 production by B cells activated *in vitro* by *Staphylococcus aureus* Cowan I (SAC) or by large B cells activated *in vivo* [30]. Therefore, the effect of VIP may depend on the maturational stage of B cells. Furthermore, enhancement of IgA production varies depending on donors; 100–300% enhancement of IgA production can be observed in some donors. We are currently investigating the effects of VIP on the tonsil small resting B cells with or without SAC activation and large activated B cells.

The mechanisms through which VIP modulates B cell responses remain to be elucidated. One possibility is that the effect is mediated by cyclic AMP as it is in other tissues [32–34]. However, this is unlikely since cyclic AMP failed to stimulate B cell lines (data not shown). Another possibility is that it is mediated by IL-6 or some other cytokine, since VIP is a potent inducer of IL-6 [35]. This is also unlikely, since these B cell lines were not responsive to IL-6 [36]. Moreover, IL-1β, IL-2, IL-4, IL-5, interferon-alpha (IFN-α), IFN-β or IFN-γ also failed to enhance immunoglobulin production by these B cell lines [36, 37]. Nevertheless, it is possible that VIP can induce some unknown cytokines which in turn stimulate B cell lines. Alternatively, VIP can directly stimulate B cell lines as nerve growth factor [31]. These possibilities are currently under investigation.

It is also of note that VIP enhanced IgA production and thymidine uptake by unstimulated tonsil B cells without affecting IgG and IgM production. IgA plays an important role in mucosal immunity and VIP is found at intestine, upper respiratory and nasal mucosa and genital tracts [4]. Moreover, the plasma VIP level is about 10^{-11} M in healthy persons, but the concentration of VIP in mucosal site is 100- to 1000-fold higher than in blood. Taken together these results may indicate that VIP acts as immunoregulatory factor at mucosal site.

We are currently studying whether the B cell lines or tonsil B cells have receptors for VIP and how the enhancement of B cell function and isotype-specific immunoglobulin secretion are produced by VIP. VIP may be an excellent reagent for study of IgA regulation.

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