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Induction of aggregation of Raji human B-lymphoblastic cells by vasoactive intestinal peptide

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SUMMARY

Subsets of neurons in the thymic cortex, Peyer's patches and lymphoid tissues of the respiratory system deliver vasoactive intestinal peptide (VIP) at nanomolar concentrations. The possible effects of VIP on B-cell adhesiveness in these tissues were examined in studies of the homotypic aggregation (HA) of human B-lymphoblastoid cells of the Raji line, which express a mean of 27,950 VIP receptors/cell with a mean K_d of 0·8 nM. Mean HA, assessed microscopically, attained a maximum of 54% after 8 hr with 0·1 μg/ml of phorbol 12-myristate 13-acetate (PMA) ($P < 0·01$) and 31% after 24 hr with 10^{-8} M VIP ($P < 0·05$), as contrasted with 13% and 20% at the respective times in medium alone, and both stimuli also increased the mean size of aggregates. The presence of the phosphodiesterase inhibitor Ro 20-1724 permitted 10^{-9} M VIP, which had no effect alone, to raise the mean cyclic AMP content of Raji cells by more than 10-fold and concurrently to elevate mean HA from 55% in medium alone at 48 hr to 70% and from 55% at 72 hr to 68% ($P < 0·05$ for both). Monoclonal antibodies to lymphocyte function-associated (LFA-1) adhesive protein and to intercellular adherence molecule-1 (ICAM-1) suppressed significantly the HA of Raji cells induced by VIP and PMA. The effects of VIP on compartmental immunity in the lungs and intestines thus may be mediated in part by increases in lymphocyte adhesiveness, which could contribute to the regional accumulation of specifically immunocompetent cells.

INTRODUCTION

Vasoactive intestinal peptide (VIP) is a 28-amino acid neuromediator with potent physiological effects on blood vessels, smooth muscle, epithelial cells and glands.[1] At concentrations attained in some immune and other tissues, VIP inhibits proliferative responses of T lymphocytes and alters B-lymphocyte production of immunoglobulins with isotypic specificity *in vitro*.[2–4] The selective VIPergic innervation of thymic cortex, internodular regions of Peyer's patches and bronchial-associated lymphoid tissue[5] suggests that VIP also may have specific roles in the tissue localization and homing of lymphocytes. This possibility is supported by the redirection of homing of murine mesenteric lymph node-derived lymphocytes achieved by their *in vitro* exposure to VIP, in order to down-regulate VIP receptors prior to reintroduction of the lymphocytes into syngeneic hosts.[6] In the present study, nanomolar concentrations of VIP are shown to induce homotypic aggregation of human B-lymphoblastoid cells of the Raji line, which express high levels of specific receptors for VIP, by increasing the interaction between type 1 lymphocyte function-associated (LFA-1) adhesive protein and intercellular adherence molecule-1 (ICAM-1). Tissue-specific increases in B-cell association, mediated by VIP, are postulated to resemble those observed in the Raji cell model system and to contribute to the expression of compartmental immune responses.

Abbreviations: HA, homotypic aggregation; IBMX, 3-isobutyl-1-methylxanthine; ICAM-1, intercellular adhesion molecule-1; LFA-1, lymphocyte function-associated adhesive protein-1; PHM, peptide histidine methionine; PMA, phorbol 12-myristate 13-acetate; VIP, vasoactive intestinal peptide.

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MATERIALS AND METHODS

Human B-lymphoblastoid cells of the Raji line[7] were cultured in RPMI medium (UCSF Cell Culture Facility) supplemented with 10% (v:v) foetal bovine serum (FBS) (Hyclone, Logan, UT), 100 U/ml of penicillin G and 100 μg/ml of streptomycin at 37° in 5% CO_2:95% air. Subcultures were prepared every 3–4 days to maintain a density of less than 2×10^5 Raji cells/ml. Phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co., St Louis, MO), forskolin and 3-isobutyl-1-methylxanthine (IBMX) (Sigma), purified synthetic secretin, peptide histidine methionine (PHM-27) and glucagon (Peninsula Laboratories, Inc., San Carlos, CA) and mouse monoclonal antibodies to

CD18 and CD11a chains of LFA-1, ICAM-1 and very late antigen-4 (VLA-4) (AMAC, Inc., Westbrook, ME) were purchased from the specified suppliers. VIP and VIP_{10-28} were synthesized by standard solid-phase techniques, purified by high-performance liquid chromatography and analysed by amino acid sequence analysis as described previously.[8] Ro 20-1724 and Ro 15-2041 were generous gifts from Hoffman LaRoche, Inc. (Nutley, NJ).

Quantification of binding of [^{125}I]VIP by Raji cells

Raji cells were washed three times and resuspended at a density of 10^7/ml in Dulbecco's phosphate-buffered saline (PBS) with 0·9 mM calcium, 0·4 mM magnesium, 0·1 g/100 ml of recrystallized bovine serum albumin (BSA) (Sigma) and 25 mM HEPES (pH 7·4) with 0·1 mM DL-thiorphan (Peninsula Labs). Each replicate 12 × 75 mm polyethylene test-tube received 0·2 ml of the suspension with 2×10^6 Raji cells, $0–10^{-6}$ M non-radioactive VIP and 50,000 c.p.m. (12·5 fmol) of [^{125}I]Tyr_{10}–VIP (2000 Ci/mmol; Amersham Corp., Arlington Heights, IL). After incubation for 30 min at 22°, the bound and unbound [^{125}I]VIP were resolved by centrifugation of the Raji cells through a cushion of phthalate oils, and were quantified by counting the pellet and an aliquot of the supernatant.[9] The data were analysed by the SCTFIT computer program,[10] to derive values for K_d and receptor number.

Assessment of Raji cell aggregation

Replicate 2-ml portions of suspensions of $10^5–10^6$ Raji cells/ml in RPMI–10% FBS–HEPES with penicillin and streptomycin were added to each compartment of 12-well polystyrene plates without or with a phosphodiesterase inhibitor, an antibody to a surface adherence protein, and either VIP, another synthetic peptide or PMA. After 1–72 hr at 37°, the cells were agitated by manual swirling of the plates for 1 min. Then 20 μl of the contents of each well was mixed with 1 μl of 1 g/100 ml trypan blue, and free cells and aggregates in 0·9 mm^3 were counted in a haemocytometer. Percentage aggregation under each condition was calculated from the formula: 100 × (1 − the ratio of the number of free cells counted in the test suspension to the number counted in the initial suspension); each mean value was derived from the results of triplicate samples. The distribution of diameters of 100 aggregates also was determined for some samples using the calibrated central grid of the haemocytometer.

To define the early aggregation induced by VIP, replicate 150 μl suspensions of $1·5 \times 10^6$ Raji cells in RPMI–10% FBS–HEPES were added to 96-well microtitre plates and incubated at 37° for 2 and 4 hr with VIP or PMA. Aggregation was quantified, as described for neutrophils,[11] by the net increase in absorbency of the Raji cell suspensions at 650 nm in an ELISA reader (Molecular Devices Corp., Menlo Park, CA). The decreases in absorbency were shown not to be due to cell adherence to the well, using the same methods as employed for neutrophils.[11]

Determination of Raji cell concentration of cyclic AMP

Raji cells from 75-cm^2 polystyrene flasks, which had been subcultured 2 days earlier, were washed twice and resuspended at $0·3 \times 10^6$/ml in protein-free RPMI, preincubated for 5 min in 0·1 mM IBMX, Ro 20-1724 or Ro 15-2041, and incubated for 1–120 min at 37° without or with VIP or forskolin. Raji cells were pelleted by centrifugation at 5000 *g* for 30 seconds at 4° and solubilized in 200 μl of 10 g/100 ml of trichloroacetic acid. The 10,000 *g* supernatant was extracted with ethyl ether to remove the trichloroacetic acid, dried *in vacuo* and redissolved in radioimmunoassay buffer for quantification of cyclic AMP (New England Nuclear-Dupont cyclic AMP radioimmunoassay kit, Boston, MA).

RESULTS

The characteristics of the Raji cell receptor for VIP were determined first to permit selection of the range of VIP concentrations expected to affect Raji cell aggregation. The specific binding of [^{125}I]VIP by Raji cells, defined as the difference between total binding in the absence of non-radioactive VIP and non-specific binding in the presence of 10^{-6} M VIP, was assessed at 22° for a range of cell concentrations and times. With 12·5 fmol of [^{125}I]VIP in each 0·2-ml suspension of Raji cells, mean specific binding (±SD, $n=3$) at 10, 30, 60 and 90 min, respectively, was 1482±98, 4012±210, 3767±190 and 3801±92 c.p.m. for 3×10^6 Raji cells/ml, 4298±410, 11,032±530, 11,904±716 and 9833±606 c.p.m. for 10^7 Raji cells/ml, and 8599±480, 22,005±1226, 20,171±1158 and 20,582±1714 c.p.m. for 3×10^7 Raji cells/ml. With 37·5 fmol of [^{125}I]VIP in each 0·2 ml of suspension of Raji cells, mean specific binding ($n=3$) at 10, 30 and 60 min, respectively, was 2922±86, 7243±654 and 7249±598 c.p.m. for 3×10^6 Raji cells/ml, 6130±222, 20,407±1054 and 19,143±1074 c.p.m. for 10^7 Raji cells/ml, and 13,145±708, 36,906±2192 and 37,249±1524 c.p.m. for 3×10^7/ml. The plateau of specific binding was attained by 30 min under each condition, at which time the non-specific binding ranged from 8% to 23% of total binding. To examine the reversibility of binding, replicate 0·2-ml suspensions of 10^7 Raji cells/ml were incubated at 22° for 60 min with 12·5 fmol of [^{125}I]VIP, washed twice and resuspended in 0·2 ml with 10^{-6} M non-radioactive VIP. The mean residual specific binding detected at 10, 30, 60 and 90 min, relative to 100% at time 0, was 82%, 36%, 18% and 16% ($n=3$), indicating near total reversibility of specific binding. For the standard incuba-

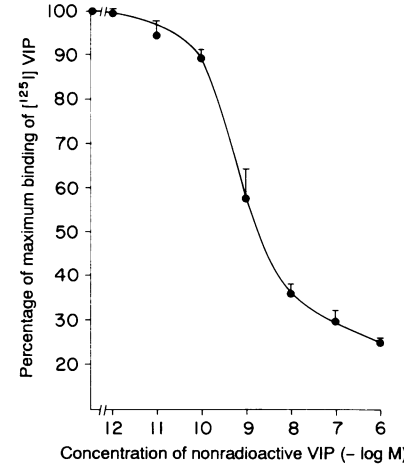

Figure 1. VIP concentration dependence of the competitive inhibition of binding of [^{125}I]VIP to Raji cells. Mean±SD of the results of three separate binding studies carried out in duplicate, $K_d = 0·8 \pm 0·7$ nM. Receptors/Raji cell = 27,950±3074.

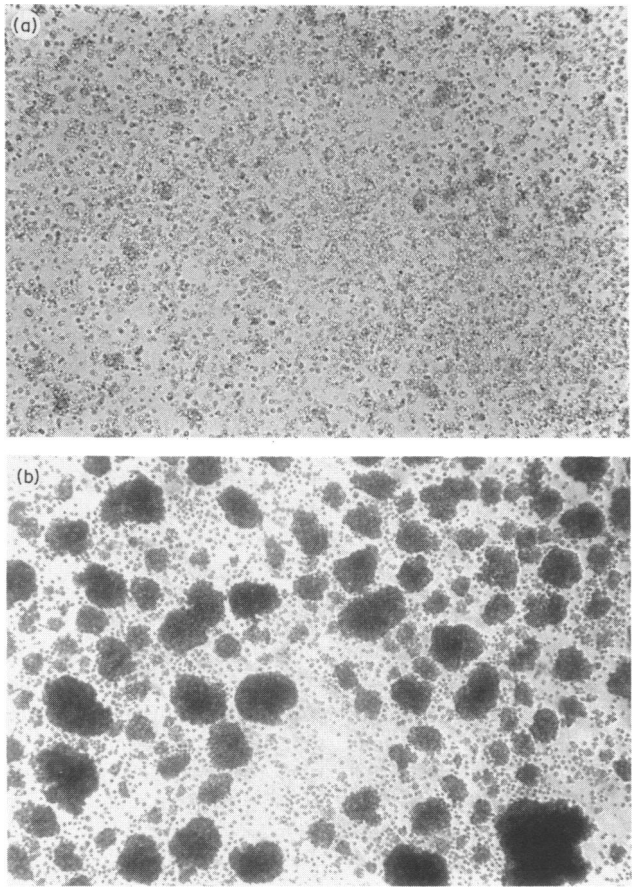

Figure 2. Aggregation of Raji cells by VIP. (a) Spontaneous aggregation of 10^6 Raji cells/ml in medium alone after 24 hr. (b) Aggregation of 10^6 Raji cells/ml by 10^{-8} M VIP after 24 hr. Magnification ×80. The photographic technique was selected to display optimally the differences in extent of aggregation.

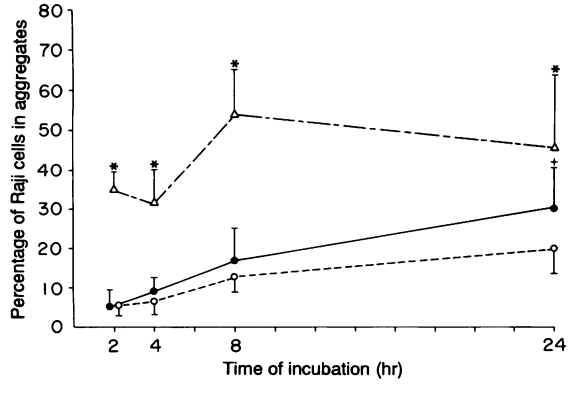

Figure 3. Time-course of homotypic aggregation of Raji cells. Mean ± SD of the results of six separate aggregation studies conducted in duplicate. The level of significance of the difference between aggregation of 2.5×10^5 Raji cells/ml by 10^{-8} M VIP (●) or 0·1 μg/ml of PMA (△) and in medium alone (○) was determined by a two-sample Student's *t*-test and is shown by $*P < 0.01$ or $\dagger P < 0.05$.

Table 1. VIP concentration dependence of stimulation of Raji cell homotypic aggregation

	Time of incubation (hr)			
	2	4	8	24
Control	5±2	6±3	13±4	18±8
VIP (10^{-9} M)	4±2	7±2	13±6	21±9
VIP (10^{-8} M)	5±4	9±4	17±8	31±11†
VIP (10^{-7} M)	2±1	5±2	13±6	24±11†
VIP (10^{-6} M)	4±1	11±4	13±5	18±7
PMA (0·1 μg/ml)	35±4*	32±9*	54±11*	43±1*
Secretin (10^{-8} M)	5±1	7±1	13±2	19±2
Glucagon (10^{-8} M)	4±1	8±2	14±2	17±2

Each value is the mean percentage of aggregation ± SD of the results of four to eight studies, except for secretin and glucagon where the values are mean ± range for the results of two studies. Raji cell concentrations were $2.0–2.6 \times 10^5$/ml. The levels of significance of differences between values with a stimulus and the control value were calculated by a paired Student's *t*-test and the *P*-values of <0.05 (†) and <0.01 (*) are depicted by the respective symbols.

Table 2. Size distribution of Raji cell aggregates

	Area of aggregates (mm²)			
	<0·06	0·06–0·125	0·125–0·25	>0·25
Control	46±3	39±8	15±6	0
VIP	40±9	33±4	27±7†	0
PMA	45±6	23±10†	15±1	17±11*

Each value shown is the mean ± SD of the results of three separate studies with Raji cell concentrations of $2.4–3.0 \times 10^5$/ml. The calculation and depiction of *P*-values are as in Table 1. VIP was used at a concentration of 10^{-8} M and PMA at 0·1 μg/ml.

tion conditions of 30 min at 22°, when specific binding was maximal, VIP inhibited the binding of [^{125}I]VIP to 10^7 Raji cells/ml in a concentration-dependent relationship (Fig. 1). A computer-based analysis[10] of the binding data revealed 27,950 ± 3074 receptors (mean ± SD) per Raji cell with a K_d of 0·8 ± 0·7 nM. The specificity of VIP binding was reflected in the respective mean K_d values (± SD) of 27 ± 9 nM, 4·6 ± 1·5 nM and 38 ± 14 nM for the structurally related peptides VIP_{10-28}, PHM-27 and secretin. The characteristics of VIP binding thus were similar to those of other lymphocytes with VIP receptors,[2,4,9] except for the 3- to 10-fold higher affinity of the Raji cell receptors.

The addition of 10^{-9} M VIP to a suspension of 10^6 Raji cells/ml resulted in greater homotypic aggregation (HA) than in medium alone at 24 hr (Fig. 2). Although such results project a clear overall pattern, the Raji cell density of 10^6/ml decreased the accuracy of microscopic assessment of HA. Therefore, all

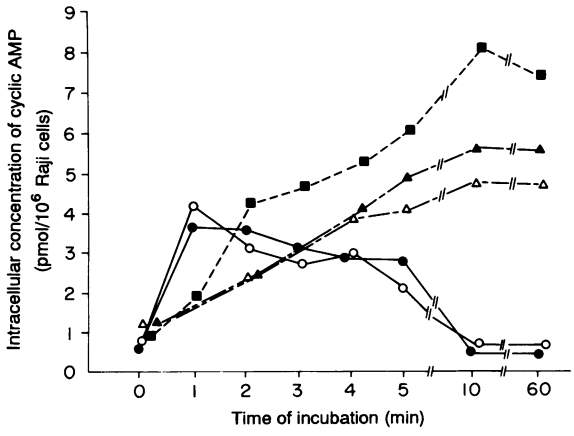

Figure 4. Time-course of VIP-induced increase in Raji cell concentration of cyclic AMP. Mean of the results of three separate studies of the effects of 10^{-9} M VIP alone (○) or with 0·01 mM each of the phosphodiesterase inhibitors IBMX (●), Ro 20-1724 (▲), or Ro 15-2041 (△), in contrast to 10 μM forskolin (■) on cyclic AMP concentration in suspensions of Raji cells. The differences between the concentrations of cyclic AMP when VIP was present alone and when Ro 20-1724 or Ro 15-2041 were added with VIP were significant at 5 min ($P<0·05$) and at 10 and 60 min ($P<0·01$), as determined by a paired Student's *t*-test.

Table 3. Time-course of homotypic aggregation of Raji cells with phosphodiesterase inhibition

	Time of incubation (hr)					
	1	2	10	24	48	72
Control	26±5	32±7	46±8	57±5	55±5	55±4
VIP	28±14	33±8	52±9	65±10	70±9†	68±6†
PMA	55±5	75±7	92±5	92±5	92±4	92±5
Secretin	27±5	ND	50±5	ND	53±3	56±4
Glucagon	28±3	ND	46±7	ND	53±5	58±5

Each value is the mean percentage of aggregation ± SEM of the results of three experiments, except for secretin and glucagon where each value is the mean ± range for the results of two studies. The concentrations of Raji cells were 2·3–2·8 × 10^5/ml. ND, not done. The calculation and depiction of *P*-values are as in Table 1. VIP, secretin and glucagon were used at a concentration of 10^{-9} M and PMA at 0·1 μg/ml. Phosphodiesterase inhibition was achieved with 0·1 mM Ro 20-1724. The increases in HA elicited by PMA are significant with $P<0·01$ at all time-points.

subsequent studies of the induction of aggregation by VIP were carried out at a Raji cell density of 2–3 × 10^5/ml. Under these conditions, PMA resulted in significantly greater HA than in medium alone after only 2 hr, with maximal stimulation by 8 hr (Fig. 3). VIP attained a lower maximal effect than PMA, that was significant only after 24 hr at 10^{-8} M and 10^{-7} M, but not 10^{-9} M (Fig. 3, Table 1). A second addition of the same concentration of VIP to Raji cell suspensions after 8 hr of incubation in two studies did not change the level of HA observed at 24 hr. Neither secretin nor glucagon, of the same peptide family as VIP, affected HA of Raji cells at 10^{-8} M (Table 1). The size of Raji cell aggregates was also increased by stimulation, with different patterns for VIP and PMA (Table 2). The number of aggregates >0·25 mm² was higher and of 0·06–0·125 mm² lower after 24 hr with PMA. In contrast, the number of aggregates of 0·125–0·25 mm² was higher with VIP.

The initial induction of cellular association by VIP was examined with an optical density assay,[11] which is more sensitive than microscopic assessment but does not permit calculation of the percentage of Raji cells involved in aggregates. The mean per cent decreases in absorbency of the Raji cell suspensions elicited by 0·1 μg/ml of PMA and 10^{-8} M VIP, respectively, were 36 (range 32–40, $n=3$) and 14 (range 13–16) after 2 hr and 42 (range 38–44) and 25 (range 23–27) after 4 hr. It is not possible to relate these values meaningfully to the characteristics of aggregates determined microscopically.

To understand better the basis for the aggregating activity of VIP, the alterations in Raji cell concentrations of cyclic AMP induced by VIP were examined without and with phosphodiesterase inhibition. At 1 min after the introduction of 10^{-10} M, 10^{-9} M, and 10^{-8} M VIP, the mean concentration of cyclic AMP (±SD) in Raji cells was 0·4±0·2, 4·2±1·3 and 6·0±2·4 pmol/10^6 Raji cells, respectively, as contrasted with 0·7±0·4 pmol/10^6 Raji cells without VIP ($n=3$). At 60 min after the addition of the same concentrations of VIP, the mean concentration of cyclic AMP (±SD) was 0·6±0·3, 0·7±0·2 and 2·7±1·5 pmol/10^6 Raji cells, in contrast to 0·6±0·5 pmol/10^6 Raji cells without VIP ($n=3$). The presence of the cyclic AMP phosphodiesterase inhibitors Ro 20-1724 and Ro 15-2041, but not IBMX, increased significantly the level of cyclic AMP evoked by 10^{-9} M VIP at 10 min, with no detectable increase for 10^{-9} M VIP alone (Fig. 4). The elevation in cyclic AMP concentration evoked by 10^{-9} M VIP with phosphodiesterase inhibition reached a plateau at 10 min, which lasted at least 60 min and was of a magnitude approximately 2/3 that attained by an optimal concentration of forskolin. The lesser increase in cyclic AMP concentration observed at 1 min was no different for VIP alone than for VIP with IBMX, but was prevented by the two Ro phosphodiesterase inhibitors.

In the presence of 0·1 mM Ro 20-1724, which permitted 10^{-9} M VIP to increase significantly and persistently the concentration of cyclic AMP in Raji cells (Fig. 4), the total HA of Raji cells was enhanced significantly by 10^{-9} M VIP after 48 and 72 hr (Table 3). The induction of aggregates of 0·125–0·25 mm², which is a preferential effect of VIP (Table 2), was increased from respective control percentage values of 8±3, 12±5, 14±5 and 16±7 at 10, 24, 48 and 72 hr to 11±4, 26±7, 29±8 and 34±23 ($P<0·05$ for 24, 48 and 72 hr) by 10^{-9} M VIP. In contrast, neither secretin nor glucagon, at 10^{-9} M, affected the HA of Raji cells. The spontaneous HA of Raji cells and that evoked by PMA were both more rapid and greater in magnitude than in medium without a phosphodiesterase inhibitor (Tables 1 and 3). Monoclonal antibodies capable of specifically antagonizing the activities of cell-surface adhesive proteins were used to identify the proteins mediating VIP-enhanced HA of Raji cells. Anti-LFA-1 directed either to the CD11a or CD18 chains altered the pattern of HA elicited by VIP in the absence of phosphodiesterase inhibition (Table 4a). Anti-LFA-1 specific for the CD18 chain both suppressed the extent of HA and reduced the number of larger aggregates, whereas anti-CD11a only reduced the number of larger aggregates attributable to VIP. The same pattern of inhibitory effects of the two antibodies to LFA-1 were

Table 4. LFA-1 and ICAM-1 mediation of Raji cell aggregation

Stimulus	Antibody	Percentage of Raji cells in aggregates	Size of aggregates (mm^2)			
			<0·06	0·06–0·125	0·125–0·25	>0·25
(a) *Without PDE inhibition*						
VIP	0	31	38	32	30	0
VIP	αCD11a	20	43	35	22†	0
VIP	αCD18	17†	49*	39	12*	0
Control	0	18	47	40	13	0
PMA	0	43	42	24	17	17
PMA	αCD11a	39	44	29	19	8†
PMA	αCD18	25*	49†	35*	15	1*
Control	0	18	47	40	13	0
(b) *With PDE inhibition*						
VIP	0	68	36	29	35	0
VIP	αICAM-1	15*	100*	0*	0*	0
VIP	αCD18	22*	83*	17†	0*	0
Control	0	55	48	38	14	0
PMA	0	92	42	33	15	10
PMA	αICAM-1	25*	100*	0*	0*	0*
PMA	αCD18	49*	74*	26	0*	0*
Control	0	53	56	34	9	1

Each value is the mean of the results of three separate experiments with $2·1–3·0 \times 10^5$ Raji cells/ml. The Student's *t*-test and symbols used to depict the levels of significance are the same as for Table 1. The concentration of VIP was 10^{-8} M for (a) and 10^{-9} M for (b). PMA was used at 0·1 μg/ml. Phosphodiesterase inhibition was achieved with 0·1 mM Ro 20-1724.

Table 5. Enhancement by VIP of Raji cell aggregation induced by a monoclonal antibody to LFA-1

	Time of incubation (hr)		
	2	4	8
Medium control	6±4	14±6	17±7
VIP (10^{-8} M)	6±4	11±3	15±5
NKI-L16 (20 ng/ml)	9±3†	21±4‡	23±7
VIP (2 hr+NKI-L16)	19±5**	26±8†	29±6*

Each of the values presented is the mean percentage of aggregation ± SD of the results of six studies, except for VIP alone which is of five studies. Raji cell concentrations were $2·2–2·6 \times 10^5$/ml. The levels of significance of differences between results with a stimulus and those for medium alone were calculated by a paired Student's *t*-test and respective symbols depict the *P*-values of 0·06 (‡), <0·05 (†), <0·01 (*), and <0·001 (**).

observed for PMA in the absence of phosphodiesterase inhibition. With phosphodiesterase inhibition by 0·1 mM Ro 20-1724, both anti-LFA-1 specific for CD18 and anti-ICAM-1 suppressed the extent of HA and the formation of larger aggregates evoked by VIP and PMA (Table 4b). At similar concentrations, monoclonal antibodies to VLA-4 had no effect on VIP- or PMA-induced aggregation of Raji cells.

The capacity of VIP to induce LFA-1/ICAM-1-mediated HA of Raji cells was examined further using a mouse monoclonal antibody to a unique activation epitope of LFA-1, termed NKI-L16,[12] which evokes HA of lymphocytes by stabilizing the activated configuration of LFA-1 required for binding to ICAM-1. In preliminary analyses, 20 ng/ml of NKI-L16 alone increased the HA of Raji cells maximally to a mean of 142% of medium control ($n=4$) after 8 hr and 70–80% of the effect was apparent by 2 hr, as has been reported for other cultured lines of lymphocytes.[12] In additional studies at 2, 4 and 8 hr, the marginally significant increase in HA of Raji cells attained by this low concentration of NKI-L16 was most apparent after 2 and 4 hr (Table 5). A 2-hr preincubation of Raji cells with VIP, at a concentration which did not alone elicit aggregation, enhanced substantially that achieved by NKI-L16. The enhancing effect of VIP was greatest at 2 hr, but also was significant at 4 and 8 hr (Table 5).

DISCUSSION

VIP enhances the aggregation of Raji cells (Figs 2 and 3, Table 3) to an extent dependent on the initial concentration of VIP (Table 1). The necessity for higher concentrations of VIP to attain significant increases in aggregation, than would be predicted from the 0·8 nM K_d of the VIP receptors expressed by Raji cells (Fig. 1), appears to be a functon of the lack of a persistent rise in Raji cell concentration of cyclic AMP at less than 10^{-8} M VIP (Fig. 4). When a phosphodiesterase inhibitor capable of preserving the rise in cyclic AMP evoked by 10^{-9} M VIP (Fig. 4) is included in the Raji cell suspension, significant aggregation is elicited by 10^{-9} M VIP that has no effect without phosphodiesterase inhibition (Table 3). Other immunological

and pharmacological agonists are known to elicit homotypic aggregation of lymphocytes through cyclic AMP-dependent mechanisms.[13,14] In three of the current studies, introduction of only the Ro 20-1724 or Ro 15-2041 inhibitor of cyclic AMP phosphodiesterase significantly increased the basal level of homotypic aggregation of Raji cells (Table 3). Other biochemical and cellular processes are also likely to influence aggregation during the long time-course required for an optimal response.

The critical roles for LFA-1 and ICAM-1 in both VIP- and PMA-induced aggregation of Raji cells were revealed by the inhibitory effect of monoclonal antibodies to either adhesive protein (Table 4). The induction of aggregation of Raji cells by VIP may involve a conformational change in LFA-1 similar to that stabilized by binding of the NKI-L16 monoclonal antibody, which aggregates Raji cells (Table 5) and other lymphocytes by activating LFA-1.[12] Enhancement of NKI-L16-stimulated aggregation of Raji cells by a concentration of VIP that lacks aggregating activity in the first 8 hr, supports an LFA-1/ICAM-1-dependent mechanism of action of VIP.

The effects of VIP on Raji cells suggest that the immunological relevance of VIP-induced aggregation of normal B cells relates to the development of compartmental immune responses in the gastrointestinal and respiratory tracts. In these locations, a single exposure to nanomolar concentrations of VIP may increase the avidity of resident B cells for migrating B cells sufficiently to augment the local accumulation of B cells and thereby enhance the production of antibodies in that site. This hypothesis predicts that the regional administration of a VIP antagonist or antibody to VIP would diminish the accumulation of B cells after antigenic challenge and suppress the local production of antibodies. However, the concurrent capacity of VIP to inhibit directly the production of some isotypes of antibodies[14,15] would be removed by these perturbations and thus may preclude this approach to experimental verification.

The ability of VIP to affect the interactions of B cells with T cells, as well as those of lymphocytes with endothelial cells, also should be investigated prior to any examination of the *in vivo* significance of the current findings. This is a particularly important line of study in view of the observations that LFA-1 on B cells is activated through CD40[13] and Fc receptors.[14] Further, the activation of LFA-1 by immune mechanisms and possibly VIP has central roles in transepithelial migration of T cells[16] as well as enhancement of antigen presentation by B cells to T cells.[17] Although not yet an integrated view, the current evidence that VIPergic nerves may affect B-lymphocyte traffic and interactions adds to our understanding of the range of possible neurogenic mechanisms for regulating compartmental immune responses.

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REFERENCES

1. Said S.I. & Mutt V. (1988) *Vasoactive Intestinal Peptide and Relates Peptides. Proceedings of the New York Academy of Sciences*, vol. 527. The New York Academy of Sciences, New York.
2. Ottaway C.A. (1987) Selective effects of vasoactive intestinal peptide on the mitogenic response of murine T cells. *Immunology*, **62,** 291.
3. Stanisz A.M., Befus D. & Bienenstock J. (1986) 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.* **136,** 152.
4. Hassner A., Lau M.S., Goetzl E.J. & Adelman D.C. (1993) Isotype-specific regulation of human lymphocyte production of immunoglobuline by sustained exposure to vasoactive intestinal peptide (VIP). *J. Allergy clin. Immunol.* (in press).
5. Ottaway C.A., Lewis D.L. & Asa S.L. (1987) Vasoactive intestinal peptide-containing nerves in Peyer's patches. *Brain, Behav. Immun.* **1,** 148.
6. Ottaway C.A. (1984) *In vitro* alterations of receptors for vasoactive intestinal peptide changes the *in vivo* localization of mouse T cells. *J. exp. Med.* **160,** 1054.
7. Pulvertaft R.J.V. (1964) Cytology of Burkitt's tumour (African lymphoma). *Lancet*, **1,** 238.
8. Goetzl E.J., Sreedharan S.P. & Turck C.W. (1988) Structurally distinctive vasoactive intestinal peptides from rat basophilic leukemia cells. *J. biol. Chem.* **263,** 9093.
9. Finch R.J., Sreedharan S.P. & Goetzl E.J. (1989) High-affinity receptors for vasoactive intestinal peptide on human myeloma cells. *J. Immunol.* **142,** 1977.
10. De Lean A., Hancock A.A. & Lefkowitz R.J. (1982) Validation and statistical analysis of a computer modeling method for quantitative analysis of radioligand binding data for mixtures of pharmacological receptor subtypes. *Mol. Pharmacol.* **21,** 5.
11. Shames R.S. & Goetzl E.J. (1993) Activation of human neutrophil LFA-1 (CD11a) by leukotriene B4. *Inflammation* (in press).
12. van Kooyk Y., Weder P., Hogervorst F., Verhoeven A.J., van Seventer G., te Velde A.A., Borst J., Keizer G.D. & Figdor C.G. (1991) Activation of LFA-1 through a Ca^{2+}-dependent epitope stimulates lymphoma adhesion. *J. Cell Biol.* **112,** 345.
13. Barrett T.B., Shu G. & Clark E.A. (1991) CD40 signaling activates CD11a/CD18 (LFA-1)-mediated adhesion in B cells. *J. Immunol.* **146,** 1722.
14. Dang L.H. & Rock K.L. (1991) Stimulation of B lymphocytes through surface Ig receptors induces LFA-1 and ICAM-1-dependent adhesion. *J. Immunol.* **146,** 3273.
15. Cheng P.P.-J., Sreedharan S.P., Kishiyama J.L. & Goetzl E.J. (1993) The SKW 6.4 line of human B lymphocytes specifically binds and responds to vasoactive intestinal peptide. *Immunology*, **79,** 64.
16. Kavanaugh A.F., Lightfoot E., Lipsky P.E. & Oppenheimer-Marks N. (1991) Role of CD11/CD18 in adhesion and transendothelial migration of T cells. *J. Immunol.* **146,** 4149.
17. Moy V.T. & Brian A.A. (1992) Signaling by lymphocyte function-associated antigen 1 (LFA-1) in B cells: enhanced antigen presentation after stimulation through LFA-1. *J. exp. Med.* **175,** 1.