

# IN VITRO ALTERATION OF RECEPTORS FOR VASOACTIVE INTESTINAL PEPTIDE CHANGES THE IN VIVO LOCALIZATION OF MOUSE T CELLS

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Although the mechanisms by which the nervous system can influence immune responses are poorly understood, the concept that certain neuropeptides may regulate immunological responses through direct effects on lymphoid cells has been suggested by in vitro investigations from a number of laboratories. For example, beta-endorphin has been found to modulate mitogen responses (1), immunoglobulin synthesis (2), and spontaneous cytotoxicity expression by human peripheral blood mononuclear cells (3). Receptors for beta-endorphin have also been demonstrated on human lymphoid cells (4). Another neuropeptide, neurotensin, has been shown to bind to and modulate the phagocytic behavior of mouse peritoneal macrophages (5), and a partially homologous neuropeptide, substance P, has been shown to stimulate human T lymphocyte proliferation (6).

An immunoregulatory role has also been suggested for vasoactive intestinal peptide (VIP).<sup>1</sup> VIP is a 28-amino acid neuropeptide that is found within neurons of the central and peripheral nervous system (7-9). VIP-containing nerves are abundant in the vagus and splanchnic nerves (10, 11) and in mucosal tissues such as the gastrointestinal and upper respiratory tracts, salivary glands, and the male and female genital tracts (12, 13). Human peripheral blood mononuclear cells (14, 15), human peripheral blood T cells (16), and a human T cell-derived culture line (Molt 4b) (17) have all been demonstrated to possess specific binding sites for VIP. Specific receptors for VIP have also been demonstrated on mouse T cells (18) and in the presence of VIP a dose-dependent inhibition of the in vitro response of mouse lymphocytes to T cell mitogens has been found (18).

It has been proposed that a prime means through which neurophysiological factors might influence the immune system is by regulation of the migration of lymphocytes through lymphoid tissue (19). The object of the investigations reported here was to test the hypothesis that receptors for VIP on mouse T cells contribute to their migratory properties. Because there is, at present, no known

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<sup>1</sup> *Abbreviations used in this paper:* BSA, bovine serum albumin; C, complement; FCS, fetal calf serum; HEV, high endothelial venule; <sup>125</sup>I-VIP, radioiodinated VIP; K<sub>D</sub>, dissociation constant; MLN, mesenteric lymph node; PP, Peyer's patches; VIP, vasoactive intestinal peptide.

way in which to selectively disrupt VIP-containing nerves *in vivo*, the strategy chosen in the present studies was to perturb the expression of VIP receptors on T cells and to examine the effect of this perturbation on the subsequent migration of T cells *in vivo*.

It is well established that the interaction of target cells with a number of hormone and neurotransmitter ligands can alter the receptor characteristics of the target cells (20). This can occur in at least two ways. First, receptor occupancy over short periods of time may modify the behavior of unoccupied receptors for the ligand. Such cooperativeness between receptors is unlikely with the VIP receptors of mouse T cells, because the dissociation of labeled VIP has been found to be independent of the concentration of VIP available to the lymphocytes (18). A second mechanism can occur in a number of target cells (20–26), whereby binding capacity for a ligand and the subsequent response of cells to the ligand can be modified by more prolonged ligand exposure of the cells.

The results obtained in the present studies show that *in vitro* incubation of T cells in the presence of VIP leads to dose-dependent alterations in the expression of VIP receptors and changes in the ability of the treated T cells to localize in mesenteric node and Peyer's patch lymphoid tissues of recipient animals.

### Materials and Methods

*Mice.* Female BALB/c mice 6–8 wk of age were purchased from Canadian Breeding Laboratories (Montreal, P.Q.).

*Vasoactive Intestinal Peptide.* Synthetic porcine VIP was purchased from Peninsula Laboratories (San Carlos, CA). Radiiodinated VIP was prepared from native porcine VIP by the lactoperoxidase method and repurified by ion-exchange chromatography as previously described (15).

*Cell Suspensions.* Single cell suspensions of lymphocytes from mesenteric lymph nodes (MLN) were prepared at room temperature by gentle teasing of the tissues in RPMI 1640 medium (Gibco Canada Ltd., Burlington, Ontario) containing 5% fetal calf serum (FCS; Gibco). The cell suspensions were filtered through a 2-cm column of glass wool to remove debris, and the number of viable cells in suspension was determined by their ability to exclude 0.2% eosin. Cell suspensions were enriched for T cells by using nylon wool columns according to the method of Julius et al. (27).

*Cell Incubation.* Cells to be used in incubations were washed three times in RPMI 1640 and resuspended at  $2 \times 10^6$  cells/ml in RPMI 1640 supplemented with 10% FCS, penicillin (100 U/ml, Gibco), streptomycin (100 ug/ml, Gibco), 4 mM L-glutamine (Sigma Chemical Co., St. Louis, MO), 1 mM sodium pyruvate (Gibco), and 1 mM nonessential amino acids (Gibco). Cells were incubated in 25-cm<sup>2</sup> flasks (Falcon 3013; Falcon Plastics Co., Oxnard, CA) at 37°C in an atmosphere of 5% CO<sub>2</sub>.

*Binding Studies.* Binding studies were carried out using  $7.5 \times 10^{-11}$  M labeled VIP in the presence or absence of unlabeled VIP in binding medium consisting of RPMI 1640 containing 5% bovine serum albumin (BSA; Sigma) as previously described (18). Briefly, cells for the binding assay were washed three times in RPMI 1640 at 37°C, resuspended in the binding medium at  $5 \times 10^6$  cells/ml, and then incubated with radiolabeled VIP for 60 min at room temperature. Cell-associated, labeled peptide was separated by centrifugation at 6,500 g for 1 min and then washed three times with binding medium at 4°C to remove unbound label, and the radioactivity of the cell sediments and supernatants was determined. Specific binding was measured as the difference between cell-bound radioactivity found in the absence and presence of  $10^{-7}$  M unlabeled VIP as a competitor (18, 28).

**Complement-mediated Cytotoxicity.** Cells to be used in cytotoxicity assays were washed three times in RPMI 1640 at 37°C and resuspended in RPMI 1640 containing 0.3% BSA. Monoclonal anti-Thy-1.2 and anti-Lyt-2.2 antibodies were purchased from New England Nuclear Corp. (LaChine P.Q.) Monoclonal anti-Lyt-1.2 antibody was purchased from Cedarlane Laboratories (Hornby, Ontario). These were used in cytotoxicity assays at final dilutions of 1/200, 1/1,600, and 1/30, respectively, for  $2.5 \times 10^6$  cells/0.5 ml. These dilutions were found to be maximally effective in producing cytotoxicity of thymus cells from these animals. Cytotoxicity was developed in the presence of low toxicity rabbit complement (C) (Cedarlane Laboratories) at a final dilution of 1/10.

**Localization Studies.** Cells to be used for in vivo localization were washed three times with RPMI 1640 at 37°C and resuspended in RPMI 1640 containing 5% FCS. Cell labeling was carried out by incubation with ( $^{51}\text{Cr}$ ) sodium chromate (New England Nuclear) at  $50 \mu\text{Ci}/10^8$  cells/ml at 37°C for 30 min. The labeled cells were washed three times in RPMI 1640 without FCS and injection doses of  $5 \times 10^6$  labeled cells were given through a lateral tail vein in 0.4-ml volumes. Multiple samples of each injection dose were kept for counting of the administered radioactivity. At intervals after cell transfer, recipient mice were killed by cervical dislocation and the abdomen immediately opened. The aorta and vena cava were cut and a measured volume of blood collected. Mesenteric node and Peyer's patches lymphoid tissues of the recipients were dissected and immediately weighed in tared containers. The in vivo localization of labeled lymphocytes was expressed as the percentage of the injected dose of radioactivity found per gram of tissue, per milliliter of blood or per organ.

**Analysis of Data.** Results were expressed as the mean  $\pm$  standard deviation for replicas of experiments or groups of animals and compared by Student's *t* test. Tracer dilution studies of  $^{125}\text{I}$ -VIP binding were assessed by Scatchard analysis (29).

## Results

### *Alteration of VIP Receptors with Incubation*

When T-enriched populations of mesenteric node lymphocytes were incubated in the presence of  $10^{-7}$  M VIP, there was a decrease in the ability of the cells to specifically bind  $^{125}\text{I}$ -labeled VIP (Table I). Alteration in specific binding of VIP to the cells was time dependent, and was well developed by 18 h of incubation (Table I). This alteration of VIP binding was investigated by studying the binding properties of T-enriched populations that had been incubated in the presence or absence of  $10^{-7}$  M VIP for 18 h. Suspensions of cells incubated in the presence

TABLE I  
*Effect of Incubation with VIP on the Specific Binding of VIP to T-Enriched Lymphocytes\**

Time of incubation <i>h</i>	Specific VIP binding (% control)
3	61.6 $\pm$ 5.5
6	42.1 $\pm$ 5.8
18	33.4 $\pm$ 5.2
42	27.7 $\pm$ 7.5

\* Nylon wool effluent MLN cells were incubated for the times shown in the presence or absence (control cells) of  $10^{-7}$  M VIP. The cells were harvested and washed and the specific binding of  $^{125}\text{I}$ -VIP was determined. The results are the means  $\pm$  SD of three experiments.

of  $10^{-7}$  M VIP bound much less VIP throughout a wide range of VIP concentrations than did cells incubated without the addition of VIP (Fig. 1). Scatchard analyses (Table II) of these tracer dilution experiments suggested that there was a marked alteration in the density of VIP binding sites in these two types of cell suspensions, but there was no significant alteration of the affinity of the cells for VIP.

When T-enriched populations of MLN lymphocytes were incubated with different concentrations of VIP, there was a dose-dependent decrease in the ability of the incubated cells to specifically bind  $^{125}\text{I}$ -VIP (Fig. 2).

Thus, in vitro exposure of T cells to VIP induces a time-dependent and dose-dependent down regulation of the expression of VIP receptor sites on the incubated lymphocytes.

*Phenotypes of Incubated Lymphocyte Populations.* The expression of T cell-associated surface markers by the incubated cell populations was examined (Table

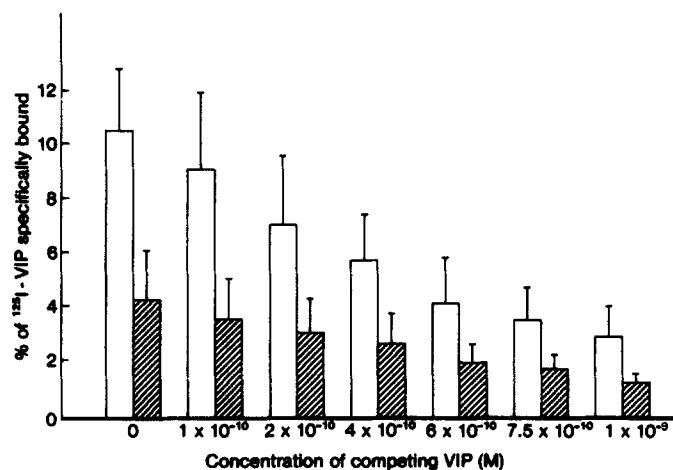


FIGURE 1. Binding of VIP to T-enriched populations of MLN lymphocytes incubated with or without VIP. Nylon wool effluent cells were incubated for 18 h in the presence (hatched areas) or absence (open areas) of  $10^{-7}$  M VIP, harvested, washed, and incubated with  $^{125}\text{I}$ -VIP in the presence of different concentrations of unlabeled VIP for 60 min at  $22^\circ\text{C}$ , and the percent of the added  $^{125}\text{I}$ -VIP specifically bound to the cells was determined. The results are the means ( $\pm$  SD) of triplicate determinations from four experiments with each type of cell suspension.

TABLE II  
VIP Binding Characteristics of T-Enriched Lymphocytes Incubated with or without VIP\*

Incubation	$K_D$ ( $10^{-10}$ M)	Sites/cell
No VIP	$2.6 \pm 0.7$	$5,200 \pm 970$
$10^{-7}$ M VIP	$3.5 \pm 1.5$	$2,810 \pm 520$

\* Results are the means  $\pm$  SD for the parameters determined from Scatchard analyses of four independent experiments with each type of cell suspension. Nylon wool effluent MLN cells were incubated for 18 h in the concentration of VIP shown.

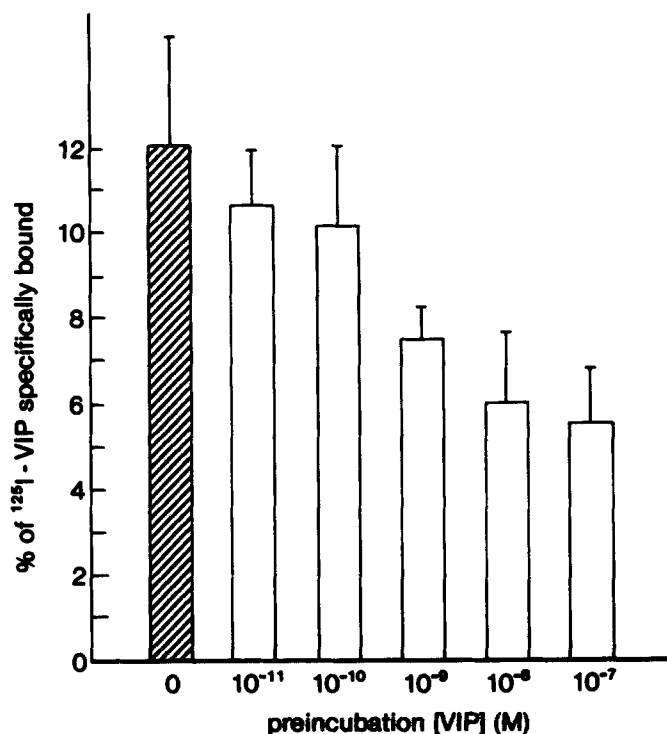


FIGURE 2. Specific binding of <sup>125</sup>I-VIP to T-enriched MLN lymphocytes incubated with different concentrations of VIP. Nylon wool effluent cells were incubated for 18 h in the absence of VIP (hatched area) or in the presence of the concentrations of VIP shown (open areas). The cell suspensions were harvested, washed, and the specific binding of VIP was assessed. The results are the means ( $\pm$  SD) of triplicate determinations from three experiments with each condition.

TABLE III  
Surface Markers of T-Enriched Lymphocytes Incubated with or without VIP\*

Antiserum	Percent of cells killed: incubation	
	-VIP	+VIP
Anti-Thy-1.2	93.5 $\pm$ 2.5	94.8 $\pm$ 4.3
Anti-Lyt-1.2	83.5 $\pm$ 4.1	84.7 $\pm$ 2.6
Anti-Lyt-2.2	10.2 $\pm$ 3.2	11.5 $\pm$ 3.7

\* Nylon wool effluent MLN cells were incubated for 18 h in the presence or absence of 10<sup>-7</sup> M VIP, harvested, washed, and tested in C-mediated cytotoxicity assays for the markers shown. The results are the means  $\pm$  SD for four experiments.

III). There was no difference in the proportion of cells which were killed by Thy-1.2, Lyt-1.2, or Lyt-2.2 monoclonal antisera plus C in suspensions which had been incubated in the presence or absence of 10<sup>-7</sup> M VIP. Therefore, the decreased density of VIP receptors expressed by the cell suspensions preincubated in the presence of the peptide is not accompanied by changes in the

expression of these major structural determinants of the T cells in the suspensions.

*In Vivo Localization of Incubated T-enriched Populations.* To examine the effect that in vitro-induced alterations in VIP receptor expression may have on the in vivo localization of the cells, populations of mesenteric node T cells that had been incubated for 18 h in the presence or absence of  $10^{-7}$  M VIP were labeled with  $^{51}\text{Cr}$  and injected into recipient animals. When the organ distribution of these labeled cell populations was assessed 1 h after cell transfer, there was a marked decrease in the recovery of VIP-pretreated cells in the mesenteric lymph node (MLN) and Peyer's patches of the recipient animals (Fig. 3). In contrast, there was no difference in the availability of VIP-treated or untreated incubated cells in the blood of the recipients and there was no difference in the recovery of these different cell populations in other major organs of the recipients (Table IV).

At 18 h after cell transfer, the recovery of injected cells in the blood, spleen, liver, and lungs was less than that found at 1 h after cell transfer (Table V), while further accumulation of the injected radioactivity had occurred in the mesenteric nodes and Peyer's patches (Table V). There was no longer any significant difference, however, in the accumulation of the differently treated incubated cells in any of the tissues examined ( $p > 0.05$ ) (Table V).

When populations of MLN T cells that had been incubated with varying concentrations of VIP were labeled and transferred, the 1 h localization of those cells in mesenteric node and Peyer's patches was decreased in a dose-dependent manner (Fig. 4), but there was no alteration in the way in which these cell

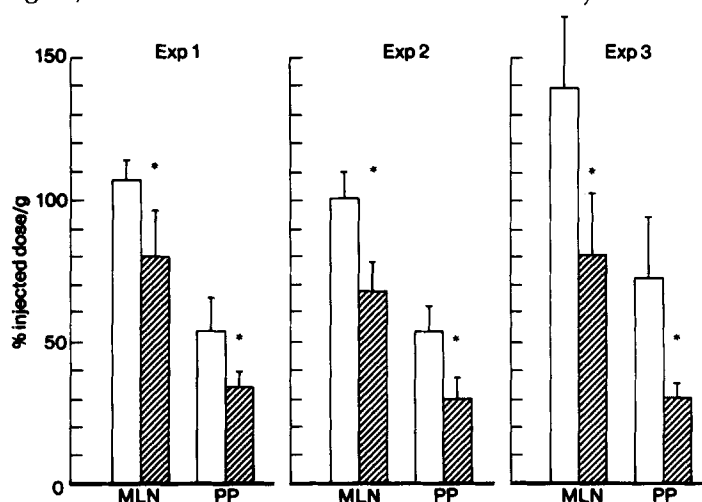


FIGURE 3. Localization 1 h after cell transfer of T-enriched MLN lymphocytes incubated with or without VIP. Nylon wool effluent cells were incubated for 18 h in the presence (hatched areas) or absence (open areas) of  $10^{-7}$  M VIP, harvested, washed, and labeled with  $^{51}\text{Cr}$  and transferred to recipients. The results are the means ( $\pm$  SD) for four or five animals in each group for the localization in mesenteric nodes (MLN) and Peyer's patches (PP) in three experiments. \* $P < 0.05$  for comparison between cells incubated in the presence or absence of VIP.

TABLE IV  
Tissue Distribution 1 h after Cell Transfer of T-Enriched Lymphocytes Incubated with or without VIP\*

Tissue	Treatment (±) VIP	Experiment		
		1	2	3
Blood/ml	-	1.7 ± 0.2	1.4 ± 0.1	1.5 ± 0.5
	+	1.6 ± 0.9	1.1 ± 0.1	1.4 ± 0.2
Spleen	-	29.2 ± 4.4	35.2 ± 2.8	32.6 ± 1.2
	+	28.1 ± 3.5	34.9 ± 2.9	34.2 ± 3.6
Liver	-	14.5 ± 1.5	11.0 ± 0.5	12.5 ± 0.3
	+	15.0 ± 1.6	10.1 ± 0.2	14.9 ± 2.0
Lung	-	7.3 ± 1.2	7.1 ± 1.1	6.7 ± 0.9
	+	10.7 ± 1.2	6.1 ± 1.1	7.4 ± 1.8
Small intestine	-	1.6 ± 0.5	0.9 ± 0.2	1.5 ± 0.2
	+	1.7 ± 0.7	1.2 ± 0.4	1.5 ± 0.5
Large intestine	-	2.6 ± 0.7	1.5 ± 0.3	2.3 ± 0.4
	+	2.6 ± 0.7	1.2 ± 0.5	2.2 ± 0.7

\* Nylon wool effluent MLN cells were incubated for 18 h in the presence or absence of  $10^{-7}$  M VIP, harvested, washed, and labeled with  $^{51}\text{Cr}$  and transferred to recipients. Results are the means ± SD of the percentage of the injected dose per milliliter (blood) or the percentage of the injected dose per organ for four or five animals in each group. The small intestine excludes Peyer's patches; the large intestine includes cecum.

TABLE V  
Tissue Distribution 18 h after Cell Transfer of T-Enriched Lymphocytes Incubated with or without VIP\*

Tissue	Treat- ment (±) VIP	Experiment		
		1	2	3
Blood/ml	-	0.8 ± 0.4	0.6 ± 0.2	0.9 ± 0.2
	+	0.4 ± 0.2	0.6 ± 0.2	1.2 ± 0.4
Mesenteric node /g	-	191.3 ± 32.7	244.7 ± 55.7	264.0 ± 20.4
	+	164.1 ± 27.0	217.5 ± 28.2	241.5 ± 32.5
Peyer's patch/g	-	62.1 ± 6.7	77.1 ± 4.3	78.5 ± 5.3
	+	55.1 ± 6.3	59.4 ± 13.6	59.7 ± 11.1
Spleen	-	21.8 ± 2.1	24.1 ± 1.3	23.0 ± 1.7
	+	22.9 ± 1.1	22.3 ± 2.5	23.2 ± 1.4
Liver	-	9.9 ± 1.2	7.6 ± 0.4	8.1 ± 0.6
	+	11.0 ± 1.1	8.5 ± 0.9	9.6 ± 1.0
Lung	-	1.5 ± 0.5	1.3 ± 0.2	1.9 ± 0.4
	+	1.9 ± 0.4	1.8 ± 0.4	2.1 ± 0.8
Small intestine	-	1.3 ± 0.7	0.7 ± 0.2	1.6 ± 0.2
	+	1.2 ± 0.4	0.9 ± 0.4	1.4 ± 0.5
Large intestine	-	2.9 ± 0.5	1.8 ± 0.9	2.9 ± 0.7
	+	2.5 ± 0.6	1.7 ± 0.4	3.6 ± 0.6

\* Nylon wool effluent MLN cells were incubated for 18 h in the presence or absence of  $10^{-7}$  M VIP, harvested, washed, and labeled with  $^{51}\text{Cr}$  and transferred to recipients. The results are the means ± SD of the percentage of the injected dose per milliliter (blood), the percentage of the injected dose per gram for mesenteric node and Peyer's patches, or the percentage of the injected dose per organ for four or five animals in each group. The small intestine excludes Peyer's patches, the large intestine includes the cecum.

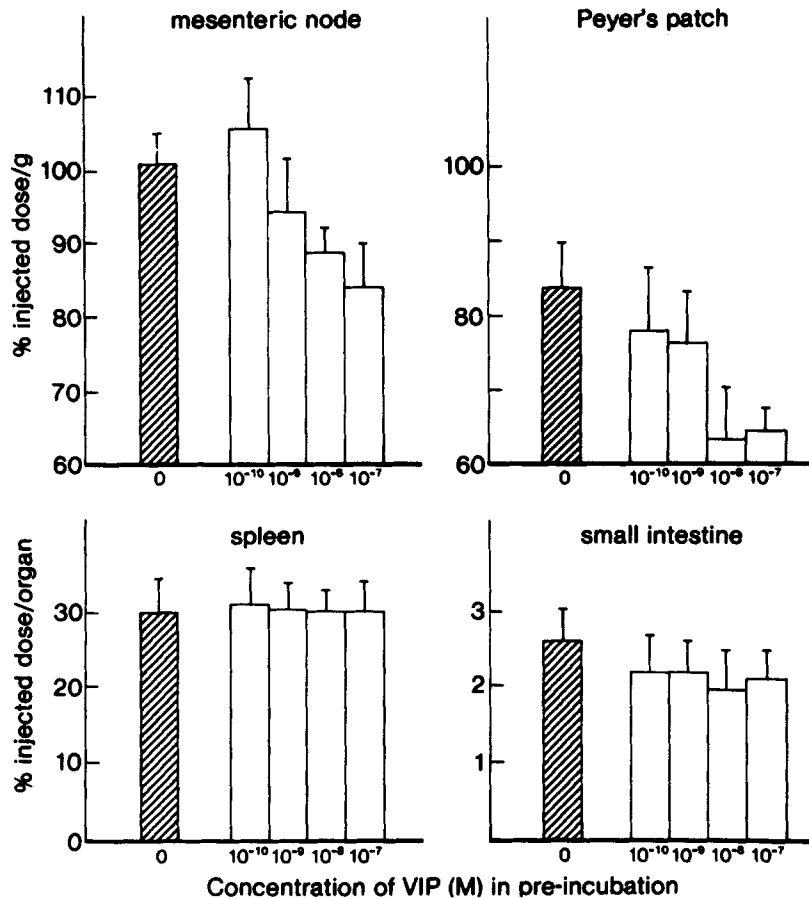


FIGURE 4. Localization 1 h after cell transfer of T-enriched MLN lymphocytes incubated in the presence of different concentrations of VIP. Nylon wool effluent cells were incubated for 18 h in the absence (hatched areas) of VIP or in the presence of the concentrations of VIP indicated (open areas). The incubated cells were harvested, washed, labeled, and transferred to recipients, and then their distribution in different organs assessed. The results are the means ( $\pm$  SD) for three to four animals in each group.

populations localized in the spleen and small intestine (Fig. 4). Preincubation of the cells in different concentrations of VIP also did not affect the amounts of cell-associated radioactivity available in the blood or recovered from other major organs of these recipients (data not shown).

In other experiments, the early time course of the distribution of T-enriched populations that had been incubated for 18 h in the presence or absence of  $10^{-7}$  M VIP was examined (Fig. 5). Although there was a rapid decline in the amounts of injected radioactivity recovered from the blood of the recipients, the availability of blood-borne label with the differently treated cell populations was equivalent (Fig. 5). The accumulation of injected radioactivity in the spleens of the recipients was also similar with both groups of treated cell populations, but there was a marked difference in the rate at which the VIP-pretreated cells



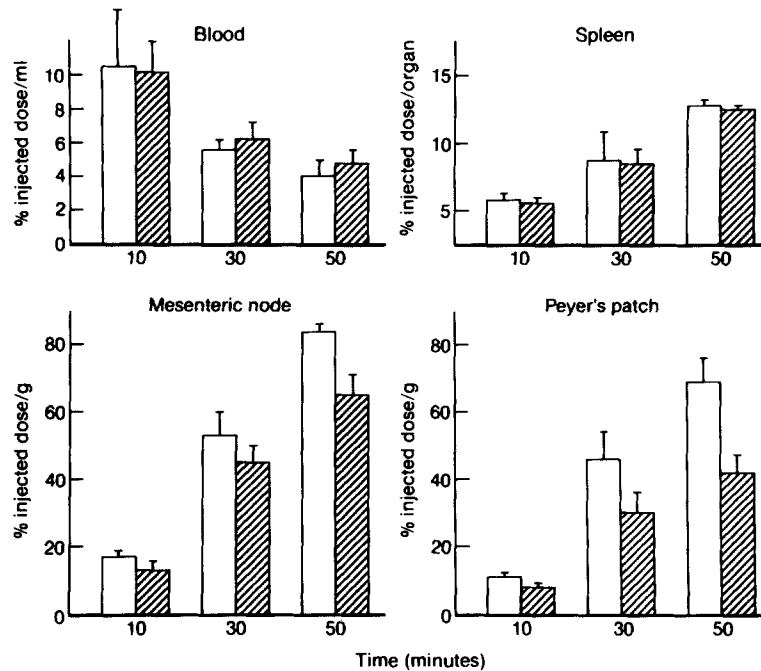


FIGURE 5. Early time course of the tissue accumulation of T-enriched MLN lymphocytes incubated with or without VIP. Nylon wool effluent cells were incubated for 18 h in the absence (open areas) or presence (hatched areas) of  $10^{-7}$  M VIP, harvested, washed, labeled, and transferred to recipients, and their distribution assessed at the times indicated after cell transfer. The results are the means ( $\pm$  SD) for three to four animals per group.

accumulated in the mesenteric nodes and Peyer's patches of the recipients (Fig. 5).

Thus, preincubation of T cells with the peptide VIP leads to a reduced ability of the cells to migrate, at early times after cell transfer, into the mesenteric node and Peyer's patches of recipient animals.

### Discussion

The purpose of this study was to examine the hypothesis that receptors for VIP affect the in vivo migration of T cells. The experiments demonstrate that incubation of suspensions of T cells in the presence of VIP results in a concomitant decrease in the binding capacity of the incubated cells for VIP and a selective decrease in the early in vivo localization of the treated cells in the mesenteric nodes and Peyer's patches of syngeneic recipients.

It is important that the altered localization of the VIP-treated cell populations was not associated with changes in the expression of Thy-1 or Lyl markers by the cells (Table III) because not only are T and B cells known to differ in their migratory properties (30), but it has been shown that subpopulations of T cells defined on the basis of Lyl-1 (31) and Lyl-2 (32) surface markers show differences in migration.

The altered localization of VIP-pretreated cells in the mesenteric node and Peyer's patches at early times after cell transfer is consistent with the notion that

cellular receptors for VIP contribute to events controlling the entry of T cells into these tissues. The principal factors that appear to influence the entry of lymphoid cells into tissues are the concentration of cells in the blood stream, the delivery of blood flow to the tissues, and the interaction of the cells with the vascular endothelium (33–35).

In these experiments, there was no difference in the availability of VIP-pretreated or control cells in the blood of the recipients (Tables IV and V, Fig. 5), and there was no apparent difference in the way in which the differently treated cells were retained by organs such as the lungs and the liver (Tables IV and V). Thus, variations in the presence of the differently treated cell populations in the blood stream cannot account for the observed differences in their localization.

VIP acquired its name because it has vasodilatory properties (36). Even though the incubated cell populations were carefully washed at 37°C under conditions that lead to the rapid dissociation of cell-bound VIP (18), it is conceivable that the VIP-pretreated cells might contain small amounts of intracellular peptide that could produce hemodynamic changes in the recipient animals. In this regard, it has been postulated that receptor-mediated ligand internalization plays a critical role in the down regulation of surface receptors of a variety of target cells (22–26), but it is not known whether such internalization is necessary for, or indeed plays any role in this process (21). It is unlikely, however, that the localization changes observed here are due to hemodynamic alterations secondary to the transfer of peptide by such means because a number of studies have shown that it requires the infusion of large amounts of VIP to produce transient increases in the flow of blood in the splanchnic vasculature of experimental animals (36, 37) or a redistribution of intestinal blood flow in rodents (38). Furthermore, if increased blood flow occurred to tissues served by these vessels, then increased, rather than decreased, localization of cells would be expected in the affected tissues (33, 39).

Thus, it is likely that the induced changes in migration of the differently treated cells reflect alterations in the way in which the cells interact with the vascular endothelium of MLN and Peyer's patches. The specialized high endothelium of the postcapillary venules (HEV) of lymph nodes and Peyer's patches is recognized as the site through which lymphocytes leave the blood to enter these tissues (40, 41). Numerous investigations of both the *in vitro* adherence of lymphocytes to these endothelial structures and the *in vivo* migration of lymphocytes (42–44) have suggested that the interaction of HEV with specific cell-surface components of lymphocytes mediates their migration into these lymphoid organs.

The molecular basis for the interaction of lymphocytes with HEV is not yet fully understood, but it has been shown that a factor isolatable from rat thoracic duct lymph can inhibit the binding of lymphocytes to lymph node HEV *in vitro* (45, 46). Furthermore, an antiserum against this inhibitory factor binds to rat thoracic duct lymphocytes, and treatment of lymphocytes with F(ab)<sub>2</sub> or Fab fragments of the antiserum selectively decreased the *in vivo* accumulation of B and T cells in lymph nodes, but not Peyer's patches, of recipient animals (47). Other investigators have described a monoclonal antibody specific for a lympho-

cyte surface molecule of murine B and T cells (48). Pretreatment of mouse lymphocytes with this antibody blocked the binding of the lymphocytes to peripheral lymph node HEV *in vitro* and inhibited the accumulation of the cells in peripheral lymph nodes *in vivo*, but had no effect on the interaction of lymphocytes with Peyer's patches, either *in vitro* or *in vivo* (48). While it is possible that the peptide-treated cells investigated here had experienced concurrent alterations in both their VIP receptors and other specific cell surface components influencing their migration, the most direct hypothesis by which to interpret the treatment-dependent decreases in VIP receptor expression and migration into MLN and Peyer's patches is that the presence of VIP receptors contributes to the way in which T cells interact with the specialized vascular endothelium of those organs.

The spleen, in contrast to lymph nodes and Peyer's patches, does not contain recognizable HEV, and alteration of the surface of lymphocytes with, for example, trypsin has little or no effect on the entry of lymphocytes into the spleen (49, 50). Trypsin treatment decreases the interaction of lymphocytes with HEV *in vitro* (51), however, and their accumulation in HEV-containing organs *in vivo* (49, 50). The finding that altered VIP receptor expression of T cells is associated with changes in the early localization of those cells in mesenteric node and Peyer's patches without allied changes in the localization in the spleen, therefore, is consistent with the notion that the VIP receptors may play a permissive role in the interaction of T cells with HEV in these organs.

The precise means by which T cell receptors for VIP could affect the interaction of T cells with HEV is not known. VIPergic nerve fibers have been identified in close proximity to small blood vessels in a number of tissues (52–55). It is possible that local release of VIP from VIPergic nerve endings may occur near HEV or within other elements of the microvasculature of mesenteric nodes and Peyer's patches. Occupancy of their VIP receptors by the neuropeptide may alter the state of activation of the T cells or facilitate the presentation of other surface components on the T cells mediating their interaction with HEV.

These considerations emphasize how little is known about the innervation, particularly the peptidergic innervation, of lymphoid tissues, but the concept that VIP receptors on T cells influence the interaction of these cells with HEV, and thus influence the migration of T cells, is consistent with other observations. First, the number of VIP receptors expressed by thymocytes is low compared with T cells from secondary lymphoid organs (18). It has been found that the binding of thymocytes to HEV *in vitro* is much lower than that displayed by T cells from lymph nodes (56), and thymocytes localize less well in lymph nodes and Peyer's patches than do T cells from secondary lymphoid tissues (57, 58). Second, T cells from different secondary lymphoid organs appear to have similar migratory properties (59, 60), as well as similar HEV-binding capabilities (44) and the expression of VIP-binding sites by T cells is uniform throughout the secondary lymphoid organs (18).

The finding that no difference in the tissue localization of the pretreated cell populations was apparent at 18 h after cell transfer may reflect the superposition of other processes that affect the retention and redistribution of lymphocytes at later times. Alternatively, the altered receptor properties induced during the in

vitro incubation may have reverted after prolonged periods in vivo. Further studies will be required to determine whether the induced receptor alterations are reversible in vivo.

It is not known to what extent these experimental animal studies model the behavior of T cells in humans, but human peripheral blood T cells have been shown to have specific receptors for VIP (16). If local interactions with VIP can affect the migration of human T cells, this could have important pathological as well as physiological implications. For example, abnormalities of VIP-containing nerves have been identified in the mucosa and submucosa of the intestine of patients with Crohn's disease as well as in the vicinity of granulomas of Crohn's affected bowel (61). Disruption of the local interaction of VIP with T cells in these regions may contribute, therefore, to the lymphocytic infiltrate and the pathophysiology of this disease.

### Summary

The capacity of T lymphocytes exposed in vitro to the neuropeptide vasoactive intestinal peptide (VIP) to bind VIP in vitro and to migrate to different tissues in vivo has been studied. VIP treatment of T cells resulted in a time- and dose-dependent loss of the ability of T cells to specifically bind radioiodinated VIP. Altered binding was due to a decrease in the expression of cellular receptors for VIP on the treated cells rather than an alteration in the affinity of the cells for the neuropeptide. Alteration of VIP receptor expression was not associated with a change in the expression of Thy-1, Lyt-1, or Lyt-2 surface markers by the treated cells. VIP treatment of T cells in vitro resulted, however, in a dose-dependent decrease in the ability of the treated cells to localize in mesenteric lymph nodes (MLN) and Peyer's patches of recipient animals at early times after cell transfer, and this was due to a selective decrease in the rate of accumulation of the treated cells in these tissues. There was no alteration in the distribution of VIP-treated cells in the blood, spleen, liver, or other major organs of the recipient animals. It is concluded that the presence of VIP receptors on T cells facilitates the entry of T cells into MLN and Peyer's patches in vivo, and it is proposed that this effect is mediated by T cell-VIP interactions in the vicinity of the specialized endothelium of those tissues.

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### References

1. McCain, H. W., I. B. Lamster, J. M. Bozzone, and J. T. Gribic. 1982. Beta-endorphin modulates human immune activity via nonopioid receptor mechanisms. *Life Sci.* 31:1619.
2. Johnson, H. M., E. M. Smith, B. A. Torres, and J. E. Blalock. 1982. Regulation of the *in vitro* antibody response by neuroendocrine hormones. *Proc. Natl. Acad. Sci. USA.* 79:4171.
3. Mathews, P. M., C. J. Froelich, W. L. Sibbitt, and A. D. Bankhurst. 1983. Enhancement of natural cytotoxicity by beta-endorphin. *J. Immunol.* 130:1658.

4. Hazum, E., K. J. Chang, and P. Cautrecasas. 1979. Specific nonopiate receptors for beta-endorphins. *Science (Wash. DC)*. 205:1033.
5. Bar-Shavit, Z., S. Terry, S. Blumberg, and R. Goldman. 1982. Neurotensin-macrophage interaction: specific binding and augmentation of phagocytosis. *Neuropeptides*. 2:325.
6. Payan, D. G., D. R. Brewster, and E. J. Goetzl. 1983. Specific stimulation of human T lymphocytes by substance P. *J. Immunol.* 131:1613.
7. Hokfelt, T., R. Elde, O. Johansson, A. Ljungdahl, M. Schultzberg, K. Fuxe, M. Goldstein, G. Nilsson, B. Pernow, L. Terenius, D. Ganten, S. L. Jeffcoate, J. Rehfeld, and S. I. Said. 1978. Distribution of peptide-containing neurons. *In Psychopharmacology: a generation of progress*. M. A. Lipton, A. Dimascio, and K. F. Killam, editors. Raven Press, New York. p. 39.
8. Polak, J. M., and S. R. Bloom. 1978. Peptidergic nerves of the gastrointestinal tract. *Invest. Cell Pathol.* 1:301.
9. Sundler, F., R. Hakanson, and S. Leander. 1980. Peptidergic nervous systems in the gut. *Clin. Gastroenterol.* 9:517.
10. Lundberg, J. M., T. Hokfelt, G. Nilsson, L. Terenius, J. Rehfeld, R. Elde, and S. Said. 1978. Peptide neurons in the vagus, splanchnic and sciatic nerves. *Acta Physiol. Scand.* 104:499.
11. Gilbert, R. F. T., P. C. Emson, J. Fahrenkrug, C. M. Lee, E. Penman, and J. Wass. 1980. Axonal transport of neuropeptides in the cervical vagus nerve of the rat. *J. Neurochem.* 34:108.
12. Larsson, L. I., J. M. Polak, R. Buffa, F. Sundler, and E. Solcia. 1979. On the immunocytochemical localization of the vasoactive intestinal peptide. *J. Histochem. Cytochem.* 27:936.
13. Polak, J. M., and S. R. Bloom. 1982. Distribution and tissue localization of VIP in the central nervous system and seven peripheral organs. *In Vasoactive Intestinal Peptide*. S. I. Said, editor. Raven Press, New York. p. 107.
14. Guerrero, J. M., J. C. Prieto, F. L. Elorza, R. Ramirez, and R. Goberna. 1981. Interaction of vasoactive intestinal peptide with human blood mononuclear cells. *Mol. Cell. Endocrinol.* 21:151.
15. Ottaway, C. A., C. Bernaerts, B. Chan, and G. R. Greenberg. 1983. Specific binding of vasoactive intestinal peptide to human circulating mononuclear cells. *Can. J. Physiol. Pharmacol.* 61:664.
16. Danek, A., M. S. O'Dorisio, T. M. O'Dorisio, and J. M. George. 1983. Specific binding sites for vasoactive intestinal polypeptide on nonadherent peripheral blood lymphocytes. *J. Immunol.* 131:1173.
17. Beed, E. A., S. O'Dorisio, T. M. O'Dorisio, and T. S. Gagarella. 1983. Demonstration of a functional receptor for vasoactive intestinal polypeptide on Molt 4b T lymphoblasts. *Reg. Peptides*. 6:1.
18. Ottaway, C. A., and G. R. Greenberg. 1984. Interaction of vasoactive intestinal peptide with mouse lymphocytes: specific binding and the modulation of mitogen responses. *J. Immunol.* 131:417.
19. Cunningham, A. J. 1981. Mind, body, and immune response. *In Psychoneuroimmunology*. R. Ader, editor. Academic Press, New York. p. 609.
20. Tell, G. P., F. Haour, and J. M. Saez. 1978. Hormonal regulation of membrane receptors and cell responsiveness: a review. *Metabolism*. 27:1566.
21. King, A. C., and P. Cuatrecasas. 1981. Peptide hormone-induced receptor mobility, aggregation, and internalization. *N. Engl. J. Med.* 305:77.
22. Tsuruhara, T., M. L. Dufau, S. Cigorruga, and K. J. Catt. 1977. Hormonal regulation

- of testicular luteinizing hormone receptors: effects on cyclic AMP and testosterone responses in isolated Leydig cells. *J. Biol. Chem.* 252:9002.
23. Vlodaysky, I., K. D. Brown, and D. Gospodarowicz. 1978. A comparison of the binding of epidermal growth factor to cultured granulosa and luteal cells. *J. Biol. Chem.* 253:3744.
  24. Heldin, C. H., B. Westermark, and A. Wasteson. 1979. Desensitization of cultured glial cells to epidermal growth factor by receptor down-regulation. *Nature. (Wash. DC)*. 282:419.
  25. Josefsberg, Z., B. I. Posner, B. Patel, and J. J. M. Bergeron. 1979. The uptake of prolactin into female rat liver: concentration of intact hormone in the Golgi apparatus. *J. Biol. Chem.* 254:209.
  26. Marshall, S., and J. W. Olefsky. 1980. Effects of insulin incubation on insulin binding, glucose transport, and insulin degradation by isolated rat adipocytes: evidence for hormone-induced desensitization at the receptor and post-receptor level. *J. Clin. Invest.* 66:763.
  27. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived lymphocytes. *Eur. J. Immunol.* 3:645.
  28. Hollenberg, M. D., and P. Cuatrecasas. 1979. Distinction of receptor from nonreceptor interactions in binding studies. In *The Receptor*, Vol. 1. R. D. O'Brien, editor. Plenum Press, New York. p. 193.
  29. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* 51:660.
  30. Parrott, D. M. V., and P. C. Wilkinson. 1981. Lymphocyte locomotion and migration. *Prog. Allergy*. 28:193.
  31. Carroll, A. M., Y. Reisner, and M. deSousa. 1982. Lyt phenotype and lectin binding properties of mouse lymphocytes which enter lymph nodes. *Adv. Exp. Med. Biol.* 149:161.
  32. Kraal, G., I. L. Weissman, and E. C. Butcher. 1983. Differences in *in vivo* distribution and homing of T cell subsets to mucosal *vs* nonmucosal lymphoid organs. *J. Immunol.* 130:1097.
  33. Ottaway, C. A., and D. M. V. Parrott. 1981. A method for the quantitative analysis of lymphoid cell migration experiments. *Immunol. Lett.* 2:283.
  34. Ottaway, C. A. 1982. The efficiency of entry of lymphoid cells into lymphoid and non-lymphoid tissues. *Adv. Exp. Med. Biol.* 149:219.
  35. Ottaway, C. A., R. G. Bruce, and D. M. V. Parrott. 1983. The *in vivo* kinetics of lymphoblast localization in the small intestine. *Immunology*. 49:641.
  36. Said, S. I., and V. Mutt. 1972. Isolation from porcine intestinal wall of a vasoactive octacosapeptide related to secretin and to glucagon. *Eur. J. Biochem.* 28:199.
  37. Matsuo, Y., and A. Seki. 1978. The coordination of gastrointestinal hormones and the autonomic nerves. *Am. J. Gastroenterol.* 69:21.
  38. Holliger, C., M. Radzyner, and M. Knoblauch. 1983. Effects of glucagon, vasoactive intestinal peptide, and vasopressin on villous microcirculation and superior mesenteric artery blood flow of the rat. *Gastroenterology*. 85:1036.
  39. Ottaway, C. A., and D. M. V. Parrott. 1979. Regional blood flow and its relationship to lymphocyte and lymphoblast traffic during a primary immune reaction. *J. Exp. Med.* 150:218.
  40. Gowans, J. L., and E. J. Knight. 1964. The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. B Biol. Sci.* 159:257.
  41. Marchesi, V. T., and J. L. Gowans. 1964. The migration of lymphocytes through the endothelium of venules in lymph nodes: an electron microscopy study. *Proc. R. Soc. Lond. B Biol. Sci.* 159:283.

42. Stamper, H. B., and J. J. Woodruff. 1976. Lymphocyte homing into lymph nodes: *in vitro* demonstration of the selective affinity of recirculating lymphocytes for high endothelial venules. *J. Exp. Med.* 144:828.
43. Stamper, H. B., and J. J. Woodruff. 1977. An *in vitro* model of lymphocyte homing. I. Characterization of the interaction between thoracic duct lymphocytes and specialized high endothelial venules of lymph nodes. *J. Immunol.* 119:772.
44. Stevens, S. K., I. L. Weissman, and E. C. Butcher. 1982. Differences in the migration of B and T lymphocytes: organ-selective localization *in vivo* and the role of lymphocyte-endothelial cell recognition. *J. Immunol.* 128:844.
45. Chin, Y.-H., G. D. Carey, and J. J. Woodruff. 1980. Lymphocyte recognition of lymph node high endothelium. I. Inhibition of *in vitro* binding by a component of thoracic duct lymph. *J. Immunol.* 125:1764.
46. Chin, Y.-H., G. D. Carey, and J. J. Woodruff. 1980. Lymphocyte recognition of lymph node high endothelium. II. Characterization of an *in vitro* inhibitory factor by antibody affinity chromatography. *J. Immunol.* 125:1770.
47. Chin, Y.-H., G. D. Carey, and J. J. Woodruff. 1982. Lymphocyte recognition of lymph node high endothelium. IV. Cell surface structures mediating entry into lymph nodes. *J. Immunol.* 129:1911.
48. Gallatin, W. M., I. L. Weissman, and E. C. Butcher. 1983. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature (Lond.)*. 304:30.
49. Woodruff, J. J., and B. M. Gesner. 1968. Lymphocytes: circulation altered by trypsin. *Science (Wash. DC)*. 161:176.
50. Ford, W. L., M. E. Smith, and P. Andrews. 1978. Possible clues to the mechanism underlying the selective migration of lymphocytes from the blood. In *Cell-Cell Recognition*. A. S. G. Curtis, editor. Cambridge University Press. p. 359.
51. Woodruff, J. J., I. M. Katz, L. E. Lucas, and H. B. Stamper. 1977. An *in vitro* model of lymphocyte homing. II. Membrane and cytoplasmic events involved in lymphocyte homing. *J. Immunol.* 119:1603.
52. Jessen, K. R., M. J. Saffrey, S. Noorden, S. R. Bloom, J. M. Polak, and G. Burnstock. 1980. Immunohistochemical studies of the enteric nervous system in tissue culture and *in situ*. Localization of vasoactive intestinal polypeptides (VIP), substance P, and enkephalin immunoreactive nerves in the guinea pig gut. *Neurosciences*. 5:1717.
53. Alm, P., J. Alumets, R. Hakanson, C. Owman, N. O. Sjöberg, F. Sundler, and B. Walles. 1980. Origin and distribution of VIP (vasoactive intestinal polypeptide)-nerves in the genitourinary tract. *Cell Tissue Res.* 205:337.
54. Uddman, R., J. Alumets, L. Edvinsson, R. Hakanson, and F. Sundler. 1981. VIP nerve fibres around peripheral blood vessels. *Acta Physiol. Scand.* 112:65.
55. Barja, F., and R. Mathison. 1982. Adrenergic and peptidergic (substance P and vasoactive intestinal polypeptide) innervation of the rat portal vein. *Blood Vessels*. 19:263.
56. Butcher, E. C., and I. L. Weissman. 1980. Cellular, genetic, and evolutionary aspects of lymphocyte interactions with high endothelial venules. *Ciba Found. Symp.* 71:265.
57. Goldschneider, I., and D. D. McGregor. 1968. Migration of lymphocytes and thymocytes in the rat. I. The route of migration from blood to spleen and lymph nodes. *J. Exp. Med.* 127:155.
58. Mitchell, J. 1972. Antigens in immunity. XVII. The migration of antigen-binding, bone marrow-derived and thymus derived spleen cells in mice. *Immunology*. 22:231.
59. Freitas, A. A., M. L. Rose, and D. M. V. Parrott. 1977. Mesenteric and peripheral lymph nodes in the mouse: a common pool of small recirculating T lymphocytes. *Nature (Lond.)*. 270: 731.

60. Freitas, A. A., M. Rose, and B. Rocha. 1980. Random recirculation of small T lymphocytes from thoracic duct lymph in the mouse. *Cell. Immunol.* 56:29.
61. Bishop, A. E., J. M. Polak, M. G. Bryant, S. R. Bloom, and S. Hamilton. 1980. Abnormalities of vasoactive intestinal polypeptide-containing nerves in Crohn's disease. *Gastroenterology.* 79:853.