

# Neuropeptides, intestinal immunological integrity and inflammatory bowel disease

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**CA OTTAWAY. Neuropeptides, intestinal immunological integrity and inflammatory bowel disease. *Can J Gastroenterol* 1993;7(2):76-82.** Although many aspects of immunoregulation are exerted through mechanisms that are autonomous to the immune system, it is established that complex and potent regulatory interactions link the nervous and immune systems in intact animals. The intestinal mucosa is a highly integrated organ which contains specialized subdivisions of both the nervous and immune systems and is expected, therefore, to be a specialized venue for neural-immune interactions. A principal means for this interaction is through the ability of lymphoid cells of the mucosa to recognize and respond to neuropeptide signals, such as the vasoactive intestinal peptide and substance P, released from local nerves. This report examines mechanisms by which neurophysiological regulation of the local activity and local accumulation of lymphoid cells can occur in the intestinal mucosa, and explores the pathophysiological implications of the pronounced disruptions of local nerves that occur in the mucosa of patients with IBD.

**Key Words:** *Inflammatory Bowel Disease, Intestinal immunoregulation, Mucosal nerves, Neuropeptides, Substance P, Vasoactive intestinal peptide*

## Neuropeptides, immunité immunologique intestinale et maladie intestinale inflammatoire

**RÉSUMÉ:** Bien que divers aspects de l'immunorégulation soient gérés par des mécanismes autonomes du système immunitaire, des interactions régulatrices complexes et puissantes lient entre eux les systèmes nerveux et immunitaires chez les animaux sains. La muqueuse intestinale est un organe hautement intégré où agissent de concert le système nerveux et le système immunitaire; il s'y produit donc des interactions neuro-immunitaires spécialisées. Ces interactions surviennent surtout grâce à la capacité des cellules lymphoïdes de la muqueuse à reconnaître les signaux neuropeptidiques et à y répondre, qu'il s'agisse de peptide intestinal vasoactif ou de substance P libérée par les fibres nerveuses locales. Ce rapport se penche sur les mécanismes par lesquels la régulation neurophysiologique de l'activité locale et de l'accumulation localisée de cellules lymphoïdes peuvent se produire dans la muqueuse intestinale et explore les implications physiopathologiques des lésions des fibres nerveuses locales au niveau de la muqueuse des patients atteints de maladie intestinale inflammatoire.

**I**N THE PAST 10 YEARS, SUBSTANTIAL evidence has emerged to support the general concept that a complex network of interactions occur between the central nervous system (CNS) and the immune system in mammals (1-3). The purposes of this report are twofold: first, to examine the concept that the intestinal mucosa is a specialized venue for interactions between the nervous and immune systems; and second, to explore the relevance of this to the pathophysiology of inflammatory bowel disease (IBD).

Although other neuropeptides also are likely to be involved (4), emphasis will be placed on the vasoactive intestinal peptide (VIP) and substance P, two enteric neuropeptides which are normally found in great abundance in the enteric nervous system (ENS). Three special features of the intestine warrant attention. First, lymphoid cells in the intestine function in microenvironments in which they are exposed to the neuropeptides VIP and substance P. Second, lymphoid cells express receptors for these signals. Third, interaction with these peptides modulates the behaviour of lymphoid cells *in vitro* and *in vivo*.

## THE INTESTINAL MUCOSA AS A NEURAL-IMMUNE VENUE

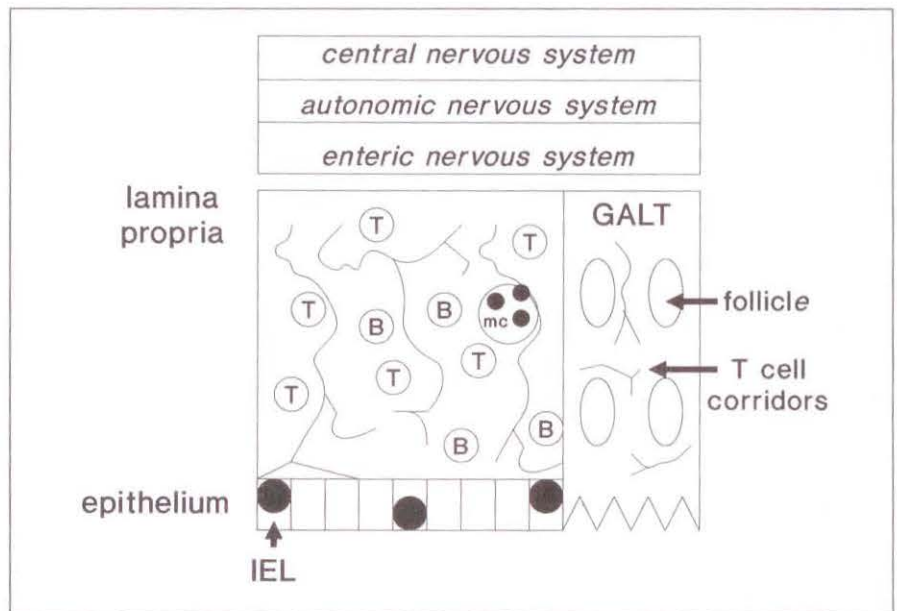
The intestine contains specialized subdivisions of both the immune and nervous systems (Figure 1). There are three major lymphoid compartments in

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the intestine: aggregated gut-associated lymphoid tissues (GALT) such as Peyer's patches, appendix and tonsils; the lamina propria, and the epithelium. Each of these contains different lymphoid cell populations and has different opportunities to contact neuropeptides.

The aggregated GALT tissues form the principal immunological effector compartment of the intestine and act as antigen detectors and processors. Structurally, they have many similarities to lymph nodes, but they are at the gut surface and have an overlying epithelium which is specialized for the sampling of intestinal lumen contents. Beneath this microfold-bearing epithelium, macrophages and dendritic cells are found that permit antigen presentation to lymphocytes (5). B cells are organized in the follicles that surround the T cell and vascular corridors of these lymphoid compartments. GALT structures are the major sites at which the activation and programming of immunoglobulin (Ig) A-producing B cells and mucosally directed T effector cells are initiated, and the immune responses that occur in these compartments generate the precursors of the effector cells that migrate via the central circulation (6,7) to populate the other two lymphoid compartments of the intestinal mucosa.

There is regional variation in the innervation of GALT tissues. For example, tonsils are innervated via the IX cranial nerve and branches of the pharyngeal and palatine nerves (8). The appendix of the rabbit is innervated by fibres derived from the mesenteric ganglia, and finely varicose nerve fibres branch throughout the internodular T cell dependent areas and surround the vasculature (9,10). Similar innervation is found in rabbit Peyer's patches (11), and the density and pattern of innervation in these GALT tissues is similar to that found in regional lymph nodes (10) where most of the innervation also is restricted to T cell corridors of the lymphoid organs. Neuropeptide-containing nerves have been identified in these organs, although they comprise only a minority



**Figure 1** A schema of the immune compartments of the intestine and their innervation. The lamina propria consists of approximately 50% lymphoid cells. T cells, B cells and mucosal mast cells (mc) are represented. This compartment is innervated densely with peptidergic fibres arising from the submucosal plexus of the enteric nervous system. The epithelial layer contains intraepithelial lymphocytes (IEL). The organized gut-associated lymphoid tissues (GALT) contain recognizable B cell follicles and T cell corridors similar to lymph nodes. The GALT is less densely innervated with peptidergic fibres than is the lamina propria and is served by the enteric and/or autonomic nervous system depending upon the region of the gut tract where they are situated.

of the nerve population. In human tonsils, both substance P-containing and VIP-containing nerve fibres have been identified along the vasculature, scattered throughout the T cell corridors and extending to beneath the epithelium (12). Within T cell regions, areas have been identified in which T cells appear to be in close proximity to substance P-containing and VIP-containing fibres (12); these nerves have been identified in mesenteric lymph nodes of a number of species (13-15); and in murine Peyer's patches, nerve fibres enter the tissue in concert with the vasculature (a substantial number of these are VIP-containing nerve fibres) (16). Although less dense than those in adjacent portions of lamina propria, VIP-ergic nerves are prominent in the T cell corridors and some VIP-containing nerves come into close proximity to the specialized high endothelial post capillary venules through which B and T cells migrate into this tissue from the bloodstream (6,16).

Large numbers of affector T and B

lymphocytes are found within the lamina propria. Approximately 50% of this compartment consists of lymphoid cells (17). The lamina propria of the intestine receives a dense supply of nerve fibres, most of which arise from the intrinsic neurons of the ENS and much of which arises locally from the submucosal plexus (18). Although only a small proportion of the extrinsic nerves which serve the gut use neuropeptides (19), a significant minority of the neurons of the myenteric plexus (and most of those in the submucosal plexus) are peptidergic (20,21). VIP-containing nerve fibres are the most prevalent nerves in the lamina propria. VIP nerves form dense networks of fibres around the epithelial crypts, and ramify throughout the tissue (21,22). Substance P-using fibres share a similar, though less dense, pattern in the lamina propria (20-22).

Conceptually it is important to consider how close a lymphoid cell must be to a nerve for a neuropeptide to affect the response of the lymphocyte. Direct lymphoid cell-nerve contacts may

**TABLE 1**  
**Some effects of substance P and vasoactive intestinal peptide on lymphoid cell functions in vitro**

Modulates	Substance P	Vasoactive intestinal peptide
<b>Monocytes:</b>		
Chemotaxis	yes	yes
Interleukin-1 production	yes	?
Respiratory burst	yes	yes
Lipid mediator	yes	?
<b>Lymphocytes:</b>		
Proliferation to:		
T cell mitogens	yes	yes
Allogeneic stimulus	?	yes
Production of:		
Immunoglobulin	yes	yes
Interleukin-2	?	yes
Interleukin-5	?	yes
Cytotoxicity	yes	yes
<b>Mast cells (MC):</b>		
Stimulation of secretion:		
Peritoneal MC	yes	yes
Lamina propria MC	yes	no

occur under some circumstances, but it has also been argued that 'en passant' release of neuropeptides from the varicosities of nerve fibres in the mucosa may permit signalling of target cells over short distances (23). The opportunity for lymphoid cells to approach neuropeptides using nerves probably varies in different regions of the mucosa. For example, the densely innervated regions surrounding the base of crypts is the place at which capillaries form larger diameter post capillary venules, the vessels through which lymphocytes leave the bloodstream to enter the mucosa (24,25). Lymphoid cells reaching the mucosa must migrate through this region to reach other sites and may have the opportunity to interact with VIP or substance P at this stage. The average linear density of mucosal nerves in human bowel is of the order of 2 m/mm<sup>3</sup> of tissue (26,27). It has been calculated that the mean radial distance between lymphoid cells is approximately 6 µm and that the distance between a lymphoid cell and a VIP-containing nerve is approximately 4 µm (27). Mast cells may have a special predilection for achieving close proximity with nerves. Studies of

mucosal mast cells in rat jejunum show that about 5% of the mast cells have direct nerve contacts, approximately 30% of the mast cells are within 1 µm, and 90% are within 2 µm of nerve fibres (28).

The lymphoid cells in the intraepithelial compartment are a specialized effector cell population that supports cytotoxic activity and expresses a variety of T cell determinants in humans, mice and rats (29). Terminal portions of the VIP- and substance P-containing nerve fibres can be identified just beneath the basement membrane of the epithelium (20,22) and may provide for local signalling of epithelial and intraepithelial cells.

#### INTERACTIONS OF NEUROPEPTIDES WITH LYMPHOID CELLS IN VITRO

Specific receptors for the neuropeptides substance P and VIP have been demonstrated on lymphoid cells of humans and experimental animals. Human circulating mononuclear cell preparations show specific binding of substance P and VIP (30-35), while lymphocytes isolated from the lamina propria of human colon demonstrate specific binding of VIP (36). An impor-

tant feature that emerges from these studies is that the ability to bind neuropeptides varies within the major lymphocyte subsets. For example, the binding properties for VIP vary within different phenotypically and functionally defined subpopulations of lymphocytes, and only minority populations of CD4 T cells, CD8 T cells, B cells and large granular lymphocytes express the ability to bind this neuropeptide (33). Subset restriction also occurs with the binding of substance P to human T cell subpopulations (35). Some lymphocyte subpopulations, therefore, might be able to recognize only one of these neuropeptides, while some lymphocytes might not recognize either peptide, and there may be some cells that are able to recognize both substance P and VIP at the same time.

In mice, suspensions of lymphocytes from abdominal lymphoid tissues (spleen, mesenteric lymph nodes and Peyer's patches) have been shown to bind VIP (37) and substance P (38) specifically, and VIP receptors have been identified on lymphocytes from the murine intestinal mucosa (39). A feature that emerges from these studies is that there are regional tissue differences in lymphocyte binding properties for the neuropeptides, but the extent to which these tissue variations represent selective accumulation of lymphoid cells with particular receptor expressions or a response of cell receptors to signals from the local environment is unknown.

Both substance P and VIP have the ability to regulate a variety of important immunological functions in vitro (Table 1). Although a complete picture is not yet available, the observations certainly support the notion that these neuropeptides are potent modulators of major immunoregulatory pathways.

Substance P is a potent stimulus of human monocyte chemotaxis (40), affecting the production of interleukin (IL) 1 in response to endotoxin (41), and regulating the production of IL-6 and tumour necrosis factor (42). Substance P enhances the cellular respiratory burst of guinea pig macrophages, and their production of

oxygen-free radicals and arachidonic acid metabolites (43). VIP also is a potent stimulator of the chemotaxis of human monocytes (44), but unlike substance P, it inhibits the respiratory burst of stimulated peripheral blood monocytes (45).

The proliferative response of both human and murine lymphocytes to mitogenic stimulation is enhanced by substance P (34,46). In concanavalin A-stimulated murine cultures, substance P augments Ig production and preferentially increases IgA production by cultures of Peyer's patch and spleen lymphocytes (46). With spleen cells from mice infected with *Schistosoma mansoni*, however, substance P specifically decreases Ig secretion by spontaneously activated individual B cells (47).

In mouse lymphocyte cultures, VIP inhibits the proliferative response of T cells to mitogens (37,46,48) and inhibits the proliferative response of mouse lymphocytes in mixed lymphocyte cultures (49). VIP suppresses the activation of CD4 T cells (50) and inhibits their production of IL-2 in concanavalin A-stimulated cultures (50). In such cultures, VIP also inhibits the production of IgA by Peyer's patch lymphocytes, but enhances IgA production by cultures of spleen or mesenteric node lymphocytes (46). In cultures of T cells from the intestinal mucosa of mice, VIP induces the release of IL-5 (39), a cytokine which promotes B cell development and differentiation.

Substance P stimulates histamine secretion by both peritoneal and lamina propria rat mast cells (51). In contrast, VIP is a secretagogue for peritoneal, but not intestinal, mucosa mast cells (51).

VIP inhibits the cytotoxic activity of peripheral blood lymphocytes in humans and mice (52-54). VIP also inhibits the cytotoxic capacity of lymphocytes from mouse spleen and mesenteric lymph nodes (54), but appears unable to modulate the cytotoxic activity of intraepithelial preparations (55). In contrast, the cytotoxic activity expressed by murine intraepithelial suspensions is enhanced in the

presence of substance P, whereas that of spleen cells is not affected (56).

### EFFECTS OF NEUROPEPTIDES ON LYMPHOID CELLS IN VIVO

In vitro observations suggest that substance P and VIP can influence the integrity of mucosal immune reactions in two principal ways: by direct stimulation or inhibition of the activity of individual lymphoid cells, and by modulation of the production or release of regulatory cytokines that control subsequent immune events. Another feature that contributes to the continued integrity of intestinal immunity is the ongoing replacement and remodelling of the cellular constituents of the intestinal lymphoid compartments which are sustained by lymphocyte migration. There are a number of mechanisms by which the nervous system can affect lymphocyte migration (57), and several studies support the concept that neuropeptides substance P and VIP influence the outcome of mucosal reactions by altering the distribution and retention of migrating lymphoid cells in particular compartments in vivo.

Infusion of substance P into mice by means of subcutaneous osmotic pumps has been shown to increase production of IgA by lymphocytes from Peyer's patches (58) and to alter the cytotoxic activity of IEL retrieved from the epithelium (55). Although only a small proportion of the infused substance P in these experiments would be expected to reach the CNS of the animals (59), infusion of substance P directly into the intracerebral ventricles of rats increases the available number of T cells circulating in the bloodstream (60). Thus, a component of the effects of substance P infusion on the activity of lymphocytes in the intestinal compartments may be mediated by the redistribution of migrating cells.

In sheep, acute infusion of substance P into the afferent lymphatics of lymph nodes enhances the output of lymphocytes in the efferent nodal lymph (61), while in rats, regional intra-arterial injection of substance P in the mesenteric vasculature increased the total output of lymphocytes in the abdominal duct

lymph (62). In each of these studies, subset selectivity appears to be involved. In sheep, T cell output from the node particularly appeared to be enhanced (61), while in the rat studies, there was a marked increase in B cells in the lymph (62). Similar studies (61, 63) with VIP have shown that infusion into the afferent lymphatic of sheep lymph nodes decreases the efferent output of lymphocytes and, in particular, the output of CD4 T cells from the node, whereas administration of VIP into small branches of the mesenteric artery serving the intestine in rats decreases the output of CD4 T cells in the lymph draining the intestine (64).

Interference with the ability of lymphocytes to recognize VIP also affects migratory properties. A decrease in the expression of VIP receptors by murine T cells, brought about by in vitro exposure of the lymphocytes to the neuropeptide, decreases the efficiency with which T cells subsequently migrate from the blood into Peyer's patches and mesenteric lymph nodes in intact recipients (65,66). These abdominal lymphoid tissues have VIP-containing nerves associated with the specialized endothelium of the post capillary venules across which blood-borne lymphocytes must migrate to reach these compartments (16).

The secretion of mucosal mast cells can be triggered through neural signalling in vivo in an intriguing way. In rats immunized to produce an IgE-dependent hypersensitivity state and subjected to repeated exposure to the antigen in the context of an audiovisual conditioning stimulus, the subsequent re-exposure of the animals to the cognitive signal provokes the release of mucosal mast cell products in the intact animal (67); this reaction may be mediated through the interaction of the CNS with substance P-containing nerves within the mucosa (which the mast cells can associate with) (28,55).

### RELEVANCE TO IBD

A unifying hypothesis consistent with current information is that a number of pivotal cellular events involved in the integrity of intestinal mucosal immunity can be regulated by the

neurophysiological signals substance P and VIP. It is well established that the enteric nervous system is disrupted in both Crohn's disease and ulcerative colitis, and that these disruptions affect both VIP- and substance P-using nerves (27, 68-72).

Until recently, most of the studies of nerves in IBD-affected tissues have been restricted to qualitative observations suggesting nerve damage, necrosis, and regeneration. A systematic analysis that quantified Schwann cell markers and VIP-immunostaining, however, has demonstrated a profound reduction of the density of nerves in the bowel in Crohn's disease and ulcerative colitis (27). That study emphasizes the marked decrease in the nerve density of the lamina propria in IBD affected mucosa and its correlation with inflammation. In the presence of severe inflammation, the density of VIP-using nerves in the lamina propria was reduced by 90%, and even in the absence of inflammation VIP, nerve density was only about 50% of that in control specimens (27).

It also has been reported that increased plasma concentrations of VIP may be a marker of disease activity and/or the progress of disease in patients with IBD (73). The damage to VIP-containing nerves in the lamina propria (and perhaps the increased

concentrations of circulating VIP in patients with active disease) is probably a consequence of the inflammatory response in the mucosa and likely affects substance P-using nerves (69,71,72). Under these circumstances, one would expect that the immunoregulatory effects normally exerted by substance P and VIP in the mucosa would be compromised severely, and that the loss of these neuropeptide-mediated influences on local lymphoid cell activity would play a role in perpetuating the immunological aspects of the pathophysiological response in the tissue. If alternate ways could be found of providing these local regulatory influences or ways in which to promote regeneration or protection of the enteric nerves, however, new modes of therapy for IBD might be possible. For example, the primary structure of the receptors for both substance P and VIP have been identified (74,75). The receptor molecules are both members of the heptahelical neurotransmitter family of proteins. The development of agents that could function in vivo as selective agonists or antagonists of these receptors on lymphoid cells in the intestine may offer, in the future, means by which aspects of local immunoregulatory balance could be restored in the disease-affected bowels of IBD patients.

## CONCLUSIONS

The intestinal mucosa is likely a specialized venue for neural-immune interactions in intact animals including humans. The three functional lymphoid compartments of the intestine contain networks of nerves, many of which use the neuropeptides substance P and VIP; and subpopulations of T cells, B cells, monocytic cells and mast cells can recognize and respond to these neurophysiological signals. Interaction of substance P and VIP with lymphoid cells has been implicated in the accumulation of lymphoid cells in particular compartments, the activation and responsiveness of the cells, and the production of immunoglobulins and interleukins. The diverse effects exerted by these neuropeptides on immune responses suggest that the ENS plays an important local role in maintaining the integrity of the intestinal immune system, and that disruption of this regulation may influence the pathophysiology of both Crohn's disease and ulcerative colitis. Further exploration of these processes is needed before the contribution of peptide-mediated neural-immune interactions to the natural history and fundamental biology of IBD can be fully understood, but future developments may offer new means of intervening therapeutically in these diseases.

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