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Integrative neuroimmunology of the digestive tract

V Theodorou ¹*, J Fioramonti ², L Bueno ²

¹ *École supérieure d'agriculture de Purpan, 75, voie du Toec, 31076 Toulouse;*

² *Laboratoire de pharmacologie-toxicologie, Inra, 180, chemin de Tournefeuille, BP 3, 31931 Toulouse, France*

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Summary — The epithelium of the gastrointestinal tract is continuously exposed to the external environment containing food antigens, microbes and other pathogens. Immunologic and nonimmunologic mechanisms contribute to the neutralization and elimination of these foreign antigens. The immune system of the intestine is the most extensive in the organism and involves diffuse populations of immune cells, lymphoid aggregates and intraepithelial lymphocytes. On the other hand, the functions of the digestive tract contribute to the overall host defense (mucus secretion, gastric acid secretion, water and electrolyte secretion and peristalsis). These functions are regulated by intrinsic and extrinsic nervous systems. It is currently recognized that the physiological and pathological responses of the intestine require an integrate neuroimmune network. Such neuroimmune regulation is based on anatomical and biochemical supports. Indeed, there are membrane-to-membrane contacts between axonal varicosities and the immune cells. Specific receptors for neurotransmitters such as substance P, vasoactive intestinal polypeptide and somatostatin have been identified in many immune cells. Nerve profile change has been described under pathological conditions such as parasitic infections and acute phase of inflammation. In addition to supporting the growth and survival of several populations of nerves the classical nerve growth factor (NGF) has been shown to affect an immune cell population by inducing mast cell hyperplasia. Furthermore the NGF can induce mast cell degranulation, acting directly on mast cell membrane NGF receptors or indirectly by NGF-mediated release of substance P by peripheral extrinsic or intrinsic nerves. Moreover, non-immune cells such as epithelial and smooth muscle cells can produce immunologic messengers under pathological conditions such as infectious diseases or inflammation. Besides the local regulation of gut functions, neuroimmune control can be exerted at extra-intestinal sites. During physiological and pathological conditions, gastrointestinal secretions and motor events are strongly regulated by the central nervous system. Moreover, infectious agents can induce cytokine and particularly interleukin-1 release by the brain astrocytes and microglial cells which have been shown to play a pivotal role in fever induction and modifications of the gastrointestinal functions. Visceral afferent fibers play a pivotal role in 'cross-communication' between central sites and immune response. Recent studies evoke, more specifically, the role of vagus as a key modulatory participant in the close relationship between the extraintestinal nerves and the immune sys-

* Correspondence and reprints

tem. Future work in this field will clarify the role of the different participants in the intimate communication between the gastrointestinal tract, immune system and central nervous system.

neuroimmunology / digestive tract / cytokine / mast cell / vagus

Résumé — Neuro-immunologie intégrée du tractus digestif. *L'épithélium digestif est une barrière naturelle vis-à-vis d'agents étrangers introduits par voie orale, tels qu'antigènes d'origine alimentaire, xénobiotiques ou microorganismes. Le système digestif possède deux mécanismes de défense vis-à-vis de ces agents : un mécanisme d'origine immunitaire et un autre non immunitaire. Le tractus digestif est l'organe immunocompétent le plus développé de l'organisme. Il possède des éléments immunitaires individualisés ou des cellules immunocompétentes dispersées au niveau de la muqueuse et de la sous-muqueuse. Par ailleurs, il exerce ses fonctions de défense grâce à des mécanismes non immunitaires tels que sécrétion acide gastrique, sécrétion de mucus, sécrétion hydroélectrolytique intestinale et péristaltisme. En outre, ces fonctions sont régulées par les systèmes nerveux intrinsèque et extrinsèque. À l'heure actuelle l'étude des fonctions digestives tient compte des relations étroites entre système immunitaire et système nerveux. Ces relations sont supportées par des bases anatomiques et biochimiques. Des récepteurs à de nombreux neuromédiateurs (substance P, polypeptide intestinal vasoactif, somatostatine...) ont été identifiés au niveau des cellules immunocompétentes. Le profil anatomique des terminaisons nerveuses est modifié dans des conditions pathologiques telles que les parasitoses ou phase aigue de l'inflammation. Ces modifications sont régulées principalement par le facteur de croissance des nerfs qui par ailleurs a une influence sur les populations cellulaires immunocompétentes. En effet le facteur de croissance des nerfs induit une hyperplasie mastocytaire et peut aussi dégranuler les mastocytes soit en agissant directement sur les récepteurs présents sur la membrane soit indirectement en provoquant une libération de substance P par l'innervation intrinsèque ou extrinsèque. Par ailleurs, dans des conditions pathologiques, les cellules épithéliales et/ou musculaires lisses peuvent exprimer des messages de nature immunitaire. À coté du contrôle neuro-immunitaire local des fonctions digestives, il existe également une interaction entre système nerveux et immunitaire au niveau de sites extra-intestinaux. Le contrôle des fonctions digestives par le système nerveux central est bien établi. De plus des agents infectieux provoquent la libération de cytokines et en particulier d'interleukine-1 par les astrocytes et les cellules gliales au niveau du cerveau. Ces cytokines sont responsables de l'induction de la fièvre et des modifications des fonctions digestives. Parmi les acteurs intervenant dans la communication neuro-immunitaire, les afférences viscérales jouent un rôle important. Des travaux récents attribuent un rôle clé aux nerfs vagues comme agents privilégiés de la communication complexe et étroite entre les systèmes immunitaires et nerveux. De futurs travaux permettront d'élucider le rôle des différents participants à ces relations multifactorielles entre les systèmes digestif, nerveux et immunitaire.*

neuro-immunologie / tractus digestif / cytokine / mastocyte / nerfs vagues

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INTRODUCTION

The epithelial lining of the mammalian gastrointestinal tract is continuously exposed to the external environment containing food antigens, microbes and various pathogens. Two types of mechanism permit the neutralization and elimination of the foreign antigens, one immunologic and the other non-immunologic.

The immune system of the intestine is of major importance in the organism and consists of defined lymphoid aggregates, a diffuse population of immune effector cells and a population of intraepithelial lymphocytes (Stead et al, 1987). The lymphoid aggregates contain mainly differentiated B lymphocytes (plasma cells) secreting IgA and a smaller number of T cells. The lamina propria contains B lymphocytes, helper/inducer (CD4+) and suppressor/cytotoxic (CD8+) T cells, macrophages, eosinophils and mast cells. The intraepithelial compartment consists of mainly T cells of the suppressor/cytotoxic phenotype (Elson, 1988). The degradation and elimination of the noxious agents is facilitated by several mechanisms: gastric acid and proteolytic enzymes, increased secretion of water, electrolytes and mucopolysaccharides, and stimulation of peristalsis. These events depend upon the activity of approximately 10^8 gastrointestinal neurons, a number comparable to that found in the spinal cord. These neurons contain classic neurotransmitters such as acetylcholine or norepinephrine but also about 20 other identified neuropeptides. Moreover, up to five messenger molecules have been identified in a single neuron.

In most studies, immune and neuronal systems have been investigated separately, but it is clear that the intestinal response to external and internal environmental changes requires an integrated neuroimmune network. Such a neuroimmune regulation is based on anatomical and biochemical supports. There are membrane-to-membrane contacts between axonal varicosities and the various immune cells. Specific receptors for neurotransmitters such as substance P (SP), vasoactive intestinal polypeptide (VIP) and somatostatin have been identified on many immune cells. Moreover, there is now evidence that nonimmune cells such as epithelial or smooth muscle cells can produce immunologic messengers under pathological conditions. However, direct measurement of the immune influence on the physiological behaviour of nerves, and particularly enteric nerves, remains difficult in complex mammalian organisms. Because of this difficulty, alternative experimental models have been used. The mollusk *Aplysia californica* has been employed as a model for studying immune-nerve interactions (Clathworthy et al, 1992; Walters et al, 1991). Immune responses in mollusks are carried out by hemocytes that have some features in common with the immune cells of mammals (Lo Verde et al, 1982; Stefano et al, 1989). Moreover, the hemolymph of mollusks contains cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF). The responses of mollusk immunocytes to mammalian IL-1 and TNF are similar to the responses of human granulocytes to these cytokines (Hughes et al, 1990). Common neuro-immune interactions between mammals and mollusks may also

exist. Indeed enhanced excitation of neurons in *Aplysia* has been observed when the axons interacted with stimulated immunocytes (Wood, 1991).

Besides the local regulation of gut functions, neuroimmune control can be exerted at extraintestinal sites. Under physiological conditions, gastrointestinal secretions and motor events are strongly regulated by the central nervous system. During pathological conditions, the neuroimmune regulation of digestive functions can occur at the level of the central nervous system. One example of this regulation are the changes in gastrointestinal motility and secretion that are induced at the central level by immune cell mediators such as IL-1.

LOCAL NEUROIMMUNE INTERACTIONS

Immune cells as transducers

Mast cells

Mast cells play a major role among the immune effector cells involved in neuroimmune communication. The gastrointestinal mucosa contains a large number of mast cells, which differ from the classical connective tissue mast cells in terms of their morphological, histochemical and functional characteristics (Befus et al, 1986a). Mucosal mast cells are of particular importance in detecting foreign antigens since they are located at the interface between the external environment and the host. They play a role in anaphylactic reactions via IgE and IgG antibodies binding to specific Fc receptors on their surface (Baird et al, 1987). Histological studies reveal an intimate association between mast cells (as well as other immune cells) and neurons in both the peripheral and central nervous system (Dimitriadou et al, 1990; Stead et al, 1989). Because of this proximity, mast cells appear to act as bidirectional information carriers

between the nervous and immune systems. Indeed, regulatory molecules from both neurons and mast cells (SP, IL-1, IL-2, IL-3, IL-6, granulocyte-monocyte colony stimulating factor, leukaemia inhibitory factor and nerve growth factor) may initiate alterations in neural plasticity which can affect gastrointestinal function. Nerve profiles change during the course of parasitic infections (*Nippostrongylus brasiliensis* infection) and nerve endings are remodeled due to the degeneration at the acute phase of inflammation and regeneration during the recovery stage (Stead, 1992). In addition to supporting the growth and survival of several populations of nerves, classical nerve growth factor (NGF) has been shown to cause intestinal mast cell hyperplasia (Aloe and Levi-Montalcini, 1977; Marshall et al, 1990). The plethora of such regulatory molecules, which must permeate the activated intestinal mucosa, could be involved in inflammation-induced nerve modeling, just as the nerve-derived molecules may affect the different immune cell populations.

Several studies support the concept that neurotransmitter receptors on mast cells cause degranulation leading to the release of inflammatory mediators (eg, 5-HT, histamine, PAF, eicosanoids and cytokines) (fig 1). Moreover, neuropeptides such as SP can induce mast cell degranulation through a nonreceptor-mediated mechanism. It has been suggested that SP is attracted to membranes containing anionic lipids, and there directs its carboxyl terminus into the hydrophobic compartment of these membranes, directly activating G proteins to induce histamine release (Stead et al, 1987). A recent study suggests that degranulation may be due to either a direct peptide effect, in particular that of NGF on mast cell NGF receptors, or to an indirect effect involving NGF-mediated SP release from the peripheral intrinsic or extrinsic sensory nerves where SP subsequently induces mast cell degranulation (Dray and Bevan,

1993). NGF regulates the development and function of SP neurons and increased levels of NGF significantly stimulate the production of SP in sensory ganglia (Kacchi et al, 1993). Furthermore mast cells generate NGF (Leon et al, 1994) and a closed morphological association between mast cells and sensory neurites containing SP/CGRP (calcitonin gene-related peptide) has been shown (Theoharides, 1990; Naukkarinen et al, 1993; Stead et al, 1987). SP, in turn, can stimulate mast cell degranulation and the liberated mediators, particularly histamine,

are able to activate primary sensory terminals to release further SP (Cacabelos, 1991). Evidence is beginning to accumulate that the SP from extrinsic or intrinsic afferent neurons is released at neuroepithelial junctions by neural stimulation (Mantyh, 1991). A local feedback cycle involving NGF, SP and mast cells may therefore be an important neuroimmune link during pathophysiological conditions, such as neurogenic inflammation (Mantyh, 1991; Nadel, 1991) (fig 2). Indeed mast cell numbers have been reported to be elevated in tissues from patients with inflammatory bowel diseases (IBD) (McAuley and Sommers, 1961; Dvorak et al, 1992; King et al, 1992). In addition, mast cells isolated from intestine-tissue specimens from IBD patients had markedly increased rates of spontaneous mediator release and synthesis (Eliakim et al, 1986; Fox et al, 1990). Drugs used in the treatment of IBD have been shown to be able to inhibit intestinal mast cell mediator release (Fox et al, 1991). However some authors have failed to find increased mast cell numbers in IBD tissues (Thompson and Buchmann, 1979) or have reported a decrease (Sanderson et al, 1986). This controversy may be due to the heterogeneity of tissue specimens used, in terms of differing disease severity or different stages in the disease process or in the variety of treatments used by the patients. Recently it has been reported that chronic colonic inflammation induced by intrarectal administration of trinitrobenzene sulfonic acid (TNB/ethanol) in rats is accompanied by a regional mast cell hyperplasia or influx to the area of inflammation (Gelbmann and Barrett, 1995). In this study, tissue histamine levels presumed to reflect mast cell population levels were increased following the induction of colitis, following a delayed time course that became significantly elevated two weeks after treatment and continued to increase over four weeks. These findings suggest that the mast cell hyperplasia or influx that is reflected by an increase in tis-

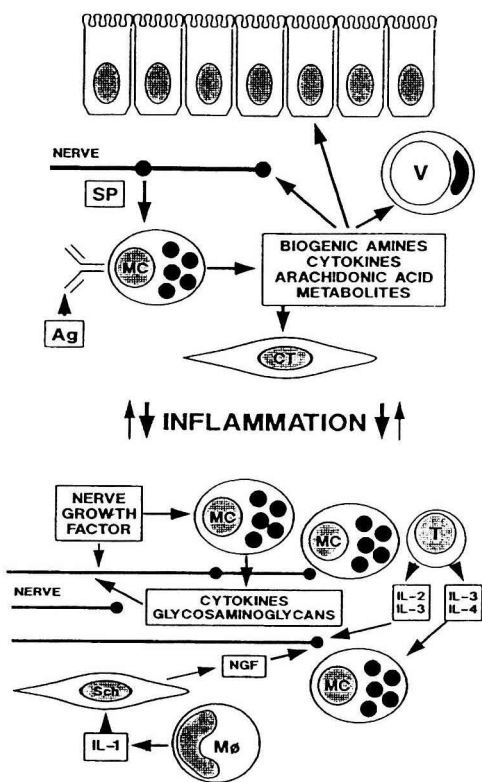


Fig 1. Schematic representation of the potential for increased communication between mast cells and nerves in the inflamed intestinal mucosa. Ag: antigen, CT: connective tissue cell, IL: interleukin, Mo: monocyte/macrophage, MC: mast cell, NGF: nerve growth factor, Sch: Schwann cell, SP: substance P, T: T lymphocyte, V: vasculature (from Stead, 1992).

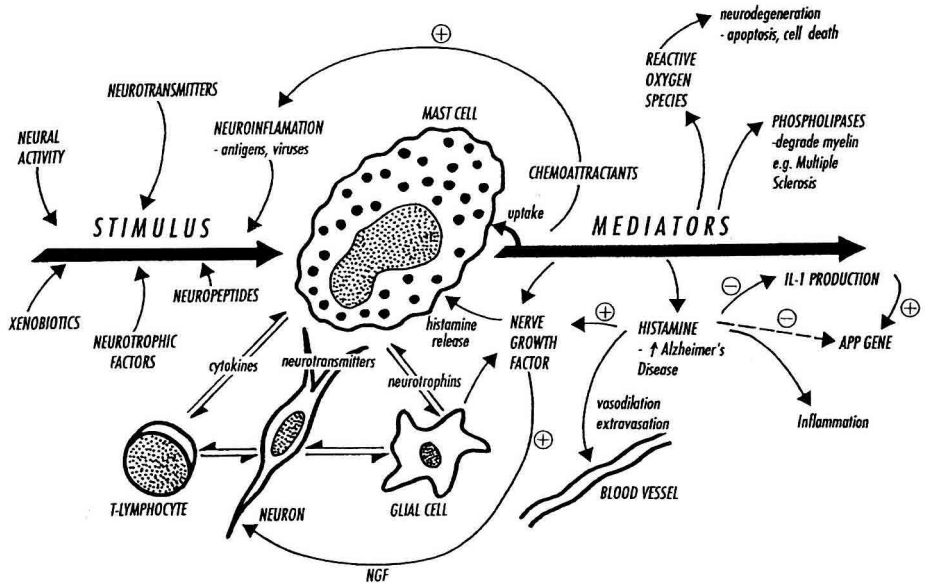


Fig 2. Mast cells and the neuroimmune axis. Mast cells are intimately involved in the 'cross-communication' between the nervous and immune systems and thereby contribute to neurophysiological and neuropathological processes, for example by affecting the integrity of the blood-brain barrier and participating in neuroinflammation (from Purcell and Atterwill, 1995).

sue histamine levels is localized to the area of ongoing inflammation, and that the stimulus for the mast cell increase is probably a microenvironmental factor regulating mast cell proliferation and development such as stem cell factor (SCF) (Gelbmann and Barrett, 1995).

Neurotransmitters may also trigger immune responses acting on neuropeptide receptors located on other immune cells such as T-cells, B-cells and macrophages, suggesting that local neural signals in the lymphoid follicles and lamina propria regulate different aspects of the immune response and thereby alter intestinal function (Ottaway, 1991; Stead et al, 1987, 1989). Moreover, several studies on antigen challenge-induced intestinal anaphylaxis have revealed the involvement of cholinergic neurons releasing acetylcholine at muscarinic receptors in the regulation of epithelial secre-

tory responses (Javed et al, 1994; Wang et al, 1993; Baird et al, 1987).

Other immune cells

Granulocytic leukocytes have been found to be implicated in the regulation of intestinal functions. These phagocytes are activated by bacterial products and release potent chemicals such as reactive oxidants, proteases and arachidonic acid metabolites. These cells also synthesize and react to various cytokines. Ongoing activation of phagocytic cells during chronic diseases may account for significant intestinal pathology. Bacterial endotoxins (lipopolysaccharides, LPS) are a general activator of phagocytes. In Crohn's disease, monocytes are primed by LPS to increase their release of toxic oxygen metabolites (Baldassano et al, 1993).

Neutrophils isolated from human blood can be stimulated to cross monolayers of the T84 human colonic cell line in response to gradients of chemotactic bacterial peptides such as FMet-Leu-Phe (Nash et al, 1991). This is associated with a dramatic decrease in transepithelial resistance and increased fluxes of inert probes as well as protein antigens. Treatment with interferon- γ (IFN- γ) elicits enhanced neutrophil transepithelial migration, particularly in the luminal-to-serosal direction (Colgan et al, 1993). Infiltration and activation of neutrophils reflect inflammatory states (Morris et al, 1989; Krawisz et al, 1984). The quantification of the amount of neutrophil infiltration in the inflammatory areas is assessed through the measurement of the activity of an enzyme called myeloperoxidase (MPO) found in cells of myeloid origin such as neutrophils (Bradley et al, 1982). Activated neutrophils in the intestinal lumen can evoke Cl^- secretion from the epithelium through the release of a molecule originally termed a neutrophil-derived secretagogue and later identified as cAMP (Madara and Stafford, 1989). Neutrophil-induced permeability changes across the monolayers of the human Caco-2 epithelial cell line are inhibited by antioxidant compounds or protease inhibitors (Main et al, 1994). A recent study reports that during pulmonary inflammation, primary afferent nerves, through the release of SP, regulate neutrophil accumulation and function by facilitating the actions of inflammatory agents such as leukotriens B4 and platelet activating factor on neutrophil adhesion migration and biochemical reactivity (Mathison et al, 1993).

Eosinophils have been shown to accumulate in the intestinal mucosa of rats infected with nematode parasites (Befus et al, 1986b). This is probably a late consequence of mast cell activation since mast cell products include chemotactic factors and/or cytokines (IL-5) for eosinophils (Gordon et al, 1990). Similarly to neutrophils,

eosinophils contain stored proteolytic enzymes and, following activation, can synthesize arachidonic acid metabolites and cytokines. In addition, the release of eosinophil cationic protein has been observed in response to allergens in food allergic patients (Knutson et al, 1993) and in response to gliadin in patients with celiac disease (Lavö et al, 1989). Moreover, eosinophils have been shown to contribute to the inflammatory response in neurogenic inflammation. Human eosinophil granules contain major basic proteins and synthetic cationic proteins which can increase airway responsiveness in vivo (Gundel et al, 1991; Coyle et al, 1993). It has been shown that eosinophil-derived cationic proteins can activate sensory C-fibers in the airways releasing primarily SP, and can participate in the development of airway hyperresponsiveness and plasma extravasation (Coyle et al, 1994). These proteins may therefore function as an important link between inflammatory cell accumulation and sensory nerve activation.

Macrophages have been found to be numerous during various experimentally-induced inflammatory states. Indeed their number increased during the acute phase of experimental colitis induced by intracolonic administration of 30% ethanol in rats, while lymphocytes play a pivotal role during the chronic stage in this model (Palmen et al, 1995), and in a model of orally-induced colitis by 3% dextran sulfate sodium (DSS) in guinea pigs (Iwanaga et al, 1994). Their presence in the inflammatory areas is closely associated with their phagocytic properties, as has been shown in a DSS-induced colitis in mice where morphological studies reveal a partial phagocytosis of DSS by macrophages. Moreover macrophages are an important source of inflammatory mediators and cytokines, particularly IL-1. They have the ability to present antigens and the epithelial-macrophage association is suggested to be

due to active recruitment by the enterocyte (Perdue and McKay, 1994). In addition, macrophages synthesize and release SP which can up-regulate IL-1 production suggesting autocrine regulation (Pascual and Bost, 1990). Moreover macrophages produce nitric oxide (NO) when they are exposed to LPS or cytokines (IL-1, IFN- γ , TNF- α). NO is known to increase epithelial permeability by modulating mast cell activity (Kanwar et al, 1992). In high concentrations, NO has cytotoxic and/or cytostatic properties that are important for the elimination of microorganisms but that may also injure intestinal cells.

Intestinal epithelial cells, smooth muscle and immunity

A more recent revelation is that immune cell products as well as bacterial products may induce epithelial and smooth muscle cells to express an 'activated' phenotype with an important position in the mucosal immune cascade. Furthermore there is increasing evidence that information transfer between these cells and immune cells is bidirectional. Epithelial expression of MHC class II antigens occurs predominantly on the basolateral epithelial membrane in rodents (Mayrhofer et al, 1983) and on the apical and basolateral surface of epithelial cells in humans (Brandzaeg et al, 1992). MHC antigens on the luminal aspect of enterocytes are thought to function as receptors for antigens processed during digestion. The expression of MHC II antigens by enterocytes in healthy humans as well as in germ-free animals is low. During inflammation, however, this expression is enhanced (Bland and Kambarage, 1991; Masson and Perdue, 1990). In vitro studies support the hypothesis that epithelial MHC II expression is a function of cytokine secretion and particularly the IFN- γ released from T-cells (MacDonald et al, 1988). However other

studies have reported that in athymic rats, intestinal enterocytes can express MHC II antigens. This suggests that the release of IFN- γ or other non-T-cell-derived cytokines or factors by intraepithelial leukocytes can evoke this epithelial response (Mayrhofer et al, 1983). The physiological meaning of such responses is that orally-ingested antigens usually do not stimulate the inflammatory process; rather they result in a down-regulation of systemic responses generating 'oral tolerance'. Increased MHC II expression during inflammation may be part of an adaptive response to limit inflammation following capture and presentation of luminal antigens that have penetrated the gut via damaged areas. Furthermore epithelial cells can also express intracellular adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1), and lymphocyte function-associated antigen-3 (CD58) has been described on HT-29 and CaCo-2 epithelial cells (Kvale et al, 1992). The expression of these molecules was induced by cytokines, primarily IFN- γ , IL-1, TNF- α and IL-6. However the expression of these molecules has not yet been observed in situ. Nevertheless these data suggest that activated epithelial cells may, in this manner, attract macrophages and polynuclear granulocytes to a site of inflammation.

Several in vitro studies have provided evidence that the epithelium itself is a source of cytokines. Immunostaining has revealed the presence of IL-6 in human enterocytes (Hyams et al, 1993; Mayer et al, 1992). Rat IEC-6 epithelial cells can synthesize IL-6 when stimulated by IL-1 β , TGF β (McGee et al, 1993a), TNF- α and cholera toxin (McGee et al, 1993b). Epithelial cells can also synthesize and release IL-8 when stimulated by IFN- γ , TNF- α , IL-6 and after culture with *Escherichia coli* LPS (Eckman et al, 1993). Appreciable amounts of TNF- α mRNA have been localized in Paneth cells (Keshav et al, 1990). IL-8 attracts neutrophils into the site of inflammation and induces histamine

release by human basophils (Krieger et al, 1992). In addition an IL-6 receptor has been described on human epithelium (Shirota et al, 1990) and functional IL-2 receptors have been found on rat IEC-6 epithelial cells (Ciacci et al, 1992). The synthesis and release of these cytokines, however, requires the confirmation of their relevance *in vivo*.

Intestinal smooth muscle is also able to express ICAM-1 and MHC II antigens (Hogaboam et al, 1994; Dzwonkowski et al, 1991). This expression is induced by intraluminal antigens and cytokines (particularly IFN- γ). These proteins can be recognized by TcR receptors localized on T helper-cells inducing T-cell activation and clone expansion. Moreover longitudinal intestinal smooth muscle cells can express cytokines. In healthy rats, IL-1 β stimulation of longitudinal muscle cells evoked a time- and concentration-dependent increase in the expression of IL-6 mRNA, and this was accompanied by the secretion of IL-6 protein into the medium (Khan et al, 1995). Bortolami et al (1993) provided direct evidence for the presence of IL-1 receptors on smooth muscle cells. IL-6 exerts effects on vascular muscles through prostaglandin synthesis by the muscle cells (Ohkawa et al, 1994) and on enteric nerves by potentiating the action of IL-1 β in suppressing the release of noradrenaline from the myenteric nerves (Rühl et al, 1995).

EXTRAIESTINAL NEUROIMMUNE INTERACTIONS

Interesting results from research in psychoneuroimmunology have revealed that lymphoid tissues are innervated by the extrinsic nervous system (Felten and Felten, 1991) and that lymphocytes not only express cell surface receptors for hormones, neuropeptides and neurotransmitters (Carr, 1992; Weigent et al, 1994) but can themselves synthesize and secrete a variety of

classic hormones (Blalock, 1992). Moreover centrally- or peripherally-produced cytokines and particularly IL-1, can signal receptors in the central nervous system (CNS) that participate in the activation of the hypothalamic-pituitary-adrenal axis (Besedovsky et al, 1977; Hughes and Chin, 1994) and control digestive immunity.

A striking example highlighting the importance of the CNS regulation of immunity is a model of conditioned antigen-evoked immunity. In this model, an immune response is generated by applying a stimulus to animals that have been conditioned by pairing the stimulus with an antigen administration able to elicit an immune response unconditionally. The immune response obtained in this model involves conditioned histamine or protease II release from the mast cells of rodents sensitized to the antigen used (Russet et al, 1984; Dark et al, 1987; MacQueen et al, 1989).

The presence of specific saturable and high affinity binding sites for cytokines and particularly IL-1 β has been demonstrated in the CNS of normal rat brains (Katsuura et al, 1988). Autoradiographic studies revealed a high receptor density in astrocytes rich in IL-1 immunoreactivity present in the hippocampus, cerebral cortex, olfactory bulb, hypothalamus and choroid plexus in rodents (Farrar et al, 1987). There is also evidence that distinct receptor subtypes for IL-1 exist. The structure of IL-1 receptor in the brain has been shown to be similar to the IL-1 type I receptor described on T cells and fibroblasts (Farrar et al, 1987). In contrast, little is known of the intracellular mechanisms of IL-1 action in the brain. Growing evidence indicates that the activation of IL-1 receptors in the brain can be modulated by the IL-1 receptor antagonist, IL-1ra (Arend, 1991). This IL-1 receptor antagonist recognizes the 80-kDa IL-1 type I receptors with a higher affinity than for the 60-kDa IL-1 type II receptors characterized on B cells/macrophages (Arend, 1991; Dinarello and Thompson,

1991) and is expressed in the brain at a location corresponding to the presence of IL-1 immunoreactivity and IL-1 receptors (Licinio et al, 1991). Moreover recent findings suggest that infectious agents or cytokines such as IL-1 β , administered peripherally, increase an immediate-early *c-fos* gene protein product (Fos-LI) and IL-1 mRNA (Wan et al, 1994; Laye et al, 1994) in the brain and particularly in the the medullary nucleus tractus solitarius (NTS), the predominant termination area of visceral vagal afferents (Ritter et al, 1992). Subdiaphragmatic vagotomy blocks this effect, reinforcing the hypothesis of a close communication between peripheral and central neural elements and the immune system.

More recent studies reveal the role of the CNS in the control of mast cell-mediated reactions. Indeed, cholinergic vagal afferents are involved in the disturbances induced by antigen challenges and trigger a stimulated intestinal motility, since capsaicin, a toxin that induces the degeneration of C and A δ afferent fibers (Fargeas et al, 1993a; Castex et al, 1994), attenuates this motor effect when applied perivagally. The vagal implication could be expressed through a signal transmission to the CNS. This hypothesis is supported by immunohistochemical studies, which monitor the expression of the immediate early *c-fos* gene in order to map postsynaptic activation in the intact nervous system. Following antigen challenge *c-fos* expression has been obtained in the PVN at the forebrain level, the LPB at the postmesencephalic junction and the NTS at the medulla oblongata level. This *c-fos* expression was associated with intestinal anaphylaxis-induced alterations of intestinal motility, and the destruction of vagal afferences simultaneously reduced brain *c-fos* expression and intestinal motility disorders (Castex et al, 1995).

This close relationship between extra-intestinal nerves and immune response is also observed during infection diseases.

Endotoxins, which are lipopolysaccharides (LPS) present in the outer membrane of Gram-negative bacteria, play a pivotal role in inducing host responses to infection. Among these responses, fever appears to be the most significant. Additionally, LPS administered intravenously to experimental animals induces the inhibition of forestomach and abomasal contractions in sheep and intestinal motor disturbances in rats and rabbits (Duranton and Bueno, 1984; Pons et al, 1989; Fioramonti et al, 1984). Moreover microbial pathogens evoke cytokine release (Dinarello and Thompson, 1991). IL-1 released by the brain astrocytes and microglial cells after LPS injection seems to have a pivotal role in the endotoxin-induced fever (Rothwell, 1991; Kluger, 1991) and the modifications of the gastrointestinal functions (Taché and Saperas, 1993; Fargeas et al, 1993b).

In sheep, fever and gastroduodenal hypomotility induced by peripheral LPS administration involves central IL-1 release, since central but not peripheral administration of recombinant human IL-1 β induces effects similar to those of LPS in a dose-dependent manner and IL-1ra, an antagonist of the IL-1 receptor, is able to block the LPS-induced effects by acting at the central level (Plaza et al, 1996). These effects obtained in sheep are mediated by prostaglandins and probably PGE₂, but not corticotropin release factor (CRF). In LPS-induced fever, message transmission between IL-1 and PGs occurs in specific blood-brain barrier areas such as the organum vasculosum of the lamina terminalis (OVLT) (Watkins et al, 1995a).

The best documented centrally-mediated effects of IL-1 are found in thermogenesis, anorexia, and elevation of circulating corticosterone, insulin, and IL-6. Many of these responses are mediated through the release of CRF and/or prostaglandins providing evidence that IL-1 triggers a physiological response during the immune recognition of

an infectious agent. However, the illness responses induced by LPS and involving proinflammatory cytokines can occur in the absence of detectable blood levels of LPS or cytokines (Kluger, 1991). For example, IL-1 β and LPS are frequently undetectable in plasma during LPS-induced fever, while antibodies directed against IL-1 β , administered systemically, block the fever produced by even low doses of LPS (Kluger, 1991; Long et al, 1990). These findings suggest an additional route of cytokine-to-brain communication, namely a neural rather than a humoral route. Indeed there is increasing evidence that the activation of subdiaphragmatic vagal afferences mediates a wide range of illness responses produced by intraperitoneal LPS. Moreover the vagus contains a very large number of sensory afferent fibers in addition to the more classically-recognized parasympathetic efferent fibers (Ritter et al, 1992). Recent studies have shown that a subdiaphragmatic vagotomy blocks the fever (Watkins et al, 1995b), hypothalamic norepinephrine depletion (Fleshner et al, 1995), hyperalgesia (Watkins et al, 1994a), and social- and food-motivated behavior suppression (Laye et al, 1994; Bluthé et al, 1994) produced by IL-1 β , TNF- α and LPS. The fact that transection of the splanchnic nerve does not block illness responses (Watkins et al, 1994b) indicates that the signals are relayed to the brain via the vagus, rather than the spinal cord via the splanchnic nerve. An important role of visceral afferents has been demonstrated in other studies using experimentally-induced gastrointestinal inflammation models. Indeed systemic capsaicin treatment, which destroys primary afferent neurons, exacerbates inflammatory reactions in the stomach and colon (Pabst et al, 1993; Evangelista and Meli, 1989; Evangelista and Tramontana, 1993; Leung, 1992; Goso et al, 1993). Subdiaphragmatic vagal destruction of afferent fibers, by local perivagal capsaicin treatment, exacerbates colonic inflammation (unpublished data), revealing the cytoprotective/anti-

inflammatory role of vagal afferents in this effect. In addition it has recently been shown that increases of intestinal water secretion and MPO, which occur in rats during the inflammatory diarrhea induced by *Clostridium difficile* toxin A, are neurally mediated, involving more specifically capsaicin-sensitive sensory-afferent neurons (Castagliuolo et al, 1994). In addition, the IL-1 β -induced colonic hypersecretory effects in rats involve primary afferent fibers (Theodorou et al, 1994a,b; Eutamène et al, 1995). These findings highlight the important message-carrying role of visceral, and particularly vagal, afferents in the communication between neural and immune systems.

Exogenous IL-1 β acts on the CNS to inhibit gastric acid secretion (Saperas et al, 1990), ulcer formation and small-intestinal motility, and to stimulate colonic motor functions (Fargeas et al, 1993a). IL-1 β acts, at specific hypothalamic sites to inhibit gastric acid secretion through PGE₂ release (Saperas et al, 1990). In fed rats, the central administration of IL-1 β induces the stimulation of caeco-colonic contractions and the reappearance of the fasting motor pattern characterized by the presence of migrating myoelectric complexes (MMC). The colonic motor effect involves CRF release, whereas the intestinal motor event is mediated by PGs release (Fargeas et al, 1993a).

CONCLUSION

All these data together demonstrate the complex and intimate relationships between intestinal and extraintestinal nerves and immune responses in the digestive tract. Mast cells play a pivotal role in these relationships. Besides their close morphological association with nerve endings, they share many of the features of neurons, in terms of responsiveness to neurotrophism and neuropeptides. The 'cross-communication' between mast cells and cells of the

nervous system may be implicated in a range of diseases which have an inflammatory or autoimmune component. Another key to the digestive neuroimmune interactions is the influence of neuropeptides on the release of cytokines.

Future work in this field is required to clarify the roles of the different participants in this relationship and, more specifically, the role of the vagus as a key modulatory axis in the communication between gastrointestinal tract, immune system, and central nervous system.

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REFERENCES

- Aloe L, Levi-Montalcini R (1977) Mast cells increase in tissues of neonatal rats injected with the nerve growth factor. *Brain Res* 133, 358-366
- Arend WP (1991) Interleukin receptor antagonist: a new member of the interleukin 1 family. *J Clin Invest* 88, 1445-1451
- Baird AW, Cuthbert AW, MacVinish LJ (1987) Type I hypersensitivity reactions in reconstructed tissues using syngeneic cell types. *Br J Pharmacol* 91, 857-869
- Baldassano RN, Schreiber S, Johnston RB, Fu RDJ, Muraki T, MacDermott RP (1993) Crohn's disease monocytes are primed for accentuated release of toxic oxygen metabolites. *Gastroenterology* 105, 60-66
- Befus AD, Bienenstock J, Denburg JA (1986a) *Mast Cell Differentiation and Heterogeneity*. Raven, New York
- Befus AD, Lee T, Ernst PB, Egwang T, MacElroy P, Gauldie J, Bienenstock J (1986b) Unique characteristics of local responses in host resistance to mucosal parasitic infections. *Vet Parasitol* 20, 175-194
- Besedovsky HO, Sorkin E, Felix B, Haas H (1977) Hypothalamic changes during the immune response. *Eur J Pharmacol* 7, 323-325
- Blalock JE (1992) Production of peptide hormones and neurotransmitters by the immune system. In: *Neuroimmunoendocrinology* (JE Blalock, ed), Karger, Basel, 1-24
- Bland PW, Kambarage DM (1991) Antigen handling by the epithelium and lamina propria macrophages. *Gastroenterol Clin North Am* 20, 577-596
- Bluthe RM, Walter V, Parnet P, Laye S, Lestage J, Verrier D, Poole S, Stenning BE, Kelley KW, Dantzer R (1994) Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism. *CR Acad Sci Paris* 317, 499-503
- Bortolami M, Ennes HS, McRoberts JA, Snape WJ, Cominelli F (1993) IL-1 enhances PGE2 production in rabbit colonic smooth muscle cells through a type I IL-1 receptor. *Gastroenterology* 104, A673
- Bradley PP, Priebe DA, Christensen RD, Rothstein G (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 78, 206-209
- Brandzaeg P, Halstensen TS, Huitfeldt HS, Krajci P, Kvale D, Scott H, Thane PS (1992) Epithelial expression of HLA, secretory component (Poly-Ig-receptor) and adhesion molecules in the human gastrointestinal tract. *Ann NY Acad Sci* 664, 157-179
- Cacabelos R (1991) Histaminergic system: neuroendocrine function of brain histamine. *Methods Find Exp Clin Pharmacol* 12, 341-376
- Carr DJJ (1992) Neuroendocrine peptide receptors on cells of the immune system. In: *Neuroimmunoendocrinology* (JE Blalock, ed), Karger, Basel, 84-105
- Castagliuolo I, Lamont JT, Letourneau R, Kelly C, O'Keane JC, Jaffer A, Theoharides TC (1994) Neuronal involvement in the intestinal effects of *Clostridium difficile* toxin A and *Vibrio cholerae* enterotoxin in rat ileum. *Gastroenterology* 107, 657-665
- Castex N, Fioramonti J, Fargeas MJ, Moré J, Bueno L (1994) Role of 5-HT3 receptors and afferent fibers in the effects of mast cell degranulation on colonic motility in rats. *Gastroenterology* 107, 976-984
- Castex N, Fioramonti J, Fargeas MJ, Bueno L (1995) *C-fos* expression in specific rat brain nuclei after intestinal anaphylaxis: involvement of 5-HT3 receptors and vagal afferent fibers. *Brain Res* 688, 149-160
- Ciacchi C, Mahida YR, Dignass A, Koizumi M, Podolsky DK (1992) Functional interleukin-2 receptors on intestinal epithelial cells. *J Clin Invest* 92, 527-532
- Clathworthy AL, Castro GA, Walters ET (1992) Long-term increase in action potential amplitude and soma excitability in Aplysia sensory neurons following peripheral immune reaction. *Soc Neurosci Abstr* 18, 595-612
- Colgan SP, Parkos CA, Awtrey CS, Lichtman AH, Matthews JB, Delp C, Madara JL (1993) Neutrophil migration across cultured intestinal epithelial monolayers is modulated by epithelial exposure to IFN-gamma in a highly polarized fashion. *J Cell Biol* 120, 785-798
- Coyle AJ, Ackerman SJ, Irvin CG (1993) Cationic proteins induce airway hyperresponsiveness depen-

- dent on charge interactions. *Am Rev Respir Dis* 147, 896-900
- Coyle AJ, Perretti F, Manzini S, Irvin CG (1994) Cationic protein-induced sensory nerve activation: role of substance P in airway hyperresponsiveness and plasma extravasation. *J Clin Invest* 94, 2301-2306
- Dark K, Peeke HVS, Ellman G, Salfi M (1987) Behaviorally conditioned histamine release. *Ann NY Acad Sci* 496, 583-587
- Dimitriadou V, Lambrecht-Hall M, Reichler J, Theoharides TC (1990) Histochemical and ultrastructural characteristics of rat brain perivascular mast cells stimulated with compound 48/80 and carbachol. *Neuroscience* 39, 209-224
- Dinarelo CA, Thompson RC (1991) Blocking IL-1: interleukin-1 receptor antagonist in vivo and in vitro. *Immunol Today* 12, 404-410
- Dray A, Bevan S (1993) Inflammation and hyperalgesia: highlighting the team effort. *TIPS Trends Pharmacol Sci* 14, 287-290
- Duranton A, Bueno L (1984) Central opiate mechanism involved in gastro-intestinal motor disturbances induced by *E coli* endotoxin in sheep. *Life Sci* 34, 1795-1799
- Dvorak AM, McLeod RS, Onderdonk A, Monahan-Earley RA, Cullen JB, Antonioli DA (1992) Ultrastructural evidence for piecemeal and anaphylactic degranulation of human gut mucosal mast cells in vivo. *Int Arch Allergy Appl Immunol* 99, 74-83
- Dzwonkowski P, Stead RH, Blennerhassett MG, Collins SM (1991) Induction of class II major histocompatibility complex (MHCII) in enteric smooth muscle. *Gastroenterology* 100, A577
- Eckman J, Jung HC, Schürer-Maly C, Panja A, Morzycka-Wroblewska E, Kagnoff MF (1993) Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. *Gastroenterology* 105, 1689-1697
- Eliakim R, Rachmilewitz D, Ligumsky M, Razin E (1986) Pathogenesis of ulcerative colitis: increased colonic chondroitin surface E synthesis indicates possible involvement of E-mast cells. *Gastroenterology* 90, 1404-1410
- Elson CO (1988) The immunology of inflammatory bowel disease. In: *Inflammatory Bowel Disease* (JB Kirsner, RG Shorter, eds), Lea and Febiger, Philadelphia, 299-317
- Eutamène H, Theodorou V, Fioramonti J, Bueno L (1995) Implication of NK1 and NK2 receptors in rat colonic hypersecretion induced by interleukin-1 β : role of nitric oxide. *Gastroenterology* 109, 483-489
- Evangelista S, Meli A (1989) Influence of capsaicin-sensitive fibers on experimentally-induced colitis in rats. *J Pharm Pharmacol* 41, 574-575
- Evangelista S, Tramontana M (1993) Involvement of calcitonin-gene related peptide in rat experimental colitis. *J Physiol Lond* 39, 210-213
- Fargeas MJ, Fioramonti J, Bueno L (1993a) Involvement of capsaicin-sensitive afferent nerves in the intestinal motor alterations induced by intestinal anaphylaxis in rats. *Int Arch Allergy Immunol* 101, 190-195
- Fargeas MJ, Fioramonti J, Bueno L (1993b) Central action of interleukin-1 on intestinal motility in rats: mediation by two mechanisms. *Gastroenterology* 104, 377-383
- Farrar WL, Kilian PL, Ruff MR, Hill JM, Pert CB (1987) Visualization and characterization of interleukin 1 receptors in brain. *J Immunol* 139, 459-463
- Felten SY, Felten DL (1991) Innervation of lymphoid tissue. In: *Psychoneuroimmunology* (R Ader, DL Felten, DJ Cohen, eds), Academic Press, San Diego, 27-69
- Fioramonti J, Bueno L, Du C (1984) Alterations of digestive motility by *Escherichia coli* endotoxin in rabbits mediated through central opiate receptors. In: *Gastrointestinal Motility* (C Roman, ed), MTP Press, Lancaster 549-556
- Fleshner M, Goehler LE, Hermann J, Felton JK, Maier SF, Watkins LR (1995) Interleukin-1 β induced corticosterone duration and hypothalamic NE depletion is vagally mediated. *Brain Res Bull* 37, 605-610.
- Fox CC, Lazenby AJ, Moore WC, Yardley JH, Bayless TM, Lichtenstein LM (1990) Enhancement of human intestinal mast cell mediator release in active ulcerative colitis. *Gastroenterology* 99, 119-124
- Fox CC, Moore WC, Lichtenstein LM (1991) Modulation of mediator release from human intestinal mast cells by sulfasalazine and 5-amino-salicylic acid. *Dig Dis Sci* 38, 1105-1112
- Gelbmann CM, Barrett KE (1995) Role of histamine in a rat model of colitis. *Inflam Res* 44, 386-392
- Gordon JR, Burd PR, Galli SJ (1990) Mast cells as a source of multifunctional cytokines. *Immunol Today* 11, 458-464
- Goso C, Evangelista S, Tramontana M, Manzini S, Blumberg PM, Szallasi A (1993) Topical capsaicin administration protects against trinitrobenzene sulfonic acid-induced colitis in rats. *Eur J Pharmacol* 249, 638-648
- Gundel RH, Letts LG, Gleich GJ (1991) Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 87, 1470-1473
- Hogaboam CM, Hewlett B, Stead RH, Snider DP, Collins SM (1994) Cytokine induced class II major histocompatibility antigen complex and intracellular adhesion molecule expression on murine intestinal smooth muscle cells. *Gastroenterology* 106, A700
- Hughes TK Jr, Chin R (1994) Interactions of neuropeptides and cytokines. In: *Neuropeptides and Immunoregulation* (B Scharrer, EM Smith, GB Stephano, eds), Springer, Berlin, 101-119
- Hughes TK, Smith E, Chin R (1990) Interaction of immunoreactive monokines (interleukin 1 and tumor-

- necrosis factor) in the bivalve mollusc *Mytilus edulis*. *Proc Natl Acad Sci USA* 87, 4426-4429
- Hyams JS, Fitzgerald JE, Treem WR, Nyzga N, Kreutzer DL (1993) Relationship of functional and antigenic interleukin-6 to disease activity in inflammatory bowel disease. *Gastroenterology* 104, 1285-1292
- Iwanaga T, Hoshi O, Han H, Fujita T (1994) Morphological analysis of acute ulcerative colitis experimentally induced by dextran sulfate sodium in the guinea pig: some possible mechanisms of cecal ulceration. *Gastroenterology* 29, 430-438.
- Javed NH, Barrett KE, Wang YZ, Bidinger J, Cooke HJ (1994) Enhanced tissue responsiveness in colonic ion transport of cow's milk-sensitized guinea pigs. *Agents Actions* 41, 25-31
- Kacchi K, Furulawa Y, Ikegami R, Nakamura N, Omae F, Hashimoto Y, Hayashi K, Furukawa S (1993) Pharmacological induction of physiologically active nerve growth factor in rat peripheral nervous system. *J Pharmacol Exp Ther* 264, 321-326
- Kanwar S, Wallace JL, Befus AD, Kubes P (1992) Nitric oxide modulates mucosal barrier function: a potential role for mast cell (abstr). *Gastroenterology* 104, A1044
- Katsuura G, Gottschall PE, Arimura A (1988) Identification of a high-affinity receptor for interleukin-1 beta in rat brain *Biochem Biophys Res Commun* 156, 61-67
- Keshav S, Lawson L, Chung P, Perry VH, Gordon S (1990) Tumour necrosis factor mRNA localised to Paneth cells of normal murine intestinal epithelium by in situ hybridization. *J Exp Med* 171, 327-332
- Khan I, Blennerhassett MG, Kataeva GV, Collins SM (1995) Interleukin-1 β induces the expression of interleukin-6 in rat intestinal smooth muscle cells. *Gastroenterology* 108, 1720-1728
- King T, Biddle W, Bhatia P, Moore J, Minner Jr PB (1992) Colonic mucosal mast cell distribution at line of demarcation of active ulcerative colitis. *Dig Dis Sci* 37, 490-495
- Kluger MJ (1991) Fever: role of pyrogens and cryogens. *Physiol Rev* 71, 93-127
- Knutson TW, Bengtsson U, Dannaeus A, Ahlstedt S, Stalenheim G, Hällgren R, Knutson L (1993) Intestinal reactivity in allergic and nonallergic patients: an approach to determine the complexity of the mucosal reaction. *J Allergy Clin Immunol* 91, 553-559
- Krawisz JE, Sharon P, Stenson WF (1984) Quantitative assay for acute inflammation based on myeloperoxidase activity: assessment of inflammation in rat and hamster models. *Gastroenterology* 87, 1344-1350
- Krieger M, Brunner T, Bischoff SC, von Tscharnar V, Walz A, Moser B, Bagglioni M, Dahinden CA (1992) Activation of human basophils through the IL-8 receptor. *J Immunol* 149, 2662-2667
- Kvale D, Krajci P, Brandtzaeg P (1992) Expression and regulation of adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58) in human intestinal epithelial lines. *Scand J Immunol* 35, 669-676
- Lavö B, Knutson L, Lööf L, Odling B, Venge P, Hällgren R (1989) Challenge with gliadin induces eosinophil and mast cell activation in the jejunum of patients with celiac disease. *Am J Med* 87, 655-660
- Laye S, Bluth MR, Kent S, Combe C, Medina C, Parnet P, Kelley K, Dantzer R (1994) Subdiaphragmatic vagotomy blocks the induction of IL-1 β mRNA in mice brain in response to peripheral LPS. *Am J Physiol* 268, R1327-R1331
- Leon A, Buriati A, Dal Toso R, Fabris M, Romanello S, Aloe L, Levi-Montalcini R (1994) Mast cells synthesize, store, and release nerve growth factor. *Proc Natl Acad Sci USA* 91, 3793-3743
- Leung FW (1992) Role of capsaicin-sensitive afferent nerves in mucosal injury and injury-induced hyperemia in rat colon. *Am J Physiol* 262, G332-G337
- Licinio J, Wong ML, Gold PW (1991) Localization of interleukin-1 receptor antagonist mRNA in rat brain. *Endocrinology* 129, 562-564
- Lo Verde PT, Gherson J, Richards CS (1982) Amebocyte accumulations in *Biomphalaria glabrata*: fine structure. *Dev Comp Immunol* 6, 441-449
- Long NC, Otterness I, Kunkel SL, Vander AJ, Kluger MJ (1990) Roles of interleukin-1 beta and tumor necrosis factor in lipopolysaccharide fever in rats. *Am J Physiol* 259, R724-R728
- MacDonald TT, Weinel A, Spencer J (1988) HLA-DR expression in human fetal intestinal epithelium. *Gut* 29, 1342-1348
- MacQueen GM, Marshall J, Perdue MH, Siegel S, Bienenstock J (1989) Pavlovian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. *Science* 243, 83-85
- Madara JL, Stafford J (1989) Interferon- γ directly affects barrier function of cultured intestinal epithelial monolayers. *J Clin Invest* 83, 724-727
- Main C, Brattsand R, Järkelid L, Weislander E (1994) Neutrophil mediated impairment of colonic epithelial cell (Caco-2) barrier function. Effects of protease inhibitors and antioxidants. *Gastroenterology* 106, A727
- Mantyh PN (1991) Substance P and the inflammatory and immune response. *Ann NY Acad Sci* 632, 263-271
- Marshall JS, Stead RH, McSharry C, Nielsen L, Bienenstock J (1990) The role of mast cell degranulation products in mast cell hyperplasia. I. Mechanism of action of nerve growth factor. *J Immunol* 144, 1886-1892
- Masson S, Perdue MH (1990) Changes in distribution of Ia antigen on epithelium of the jejunum and ileum of rats infected with *Nippostrongylus brasiliensis*. *Clin Immunol Immunopathol* 57, 83-95

- Mathison R, Davison JS, Befus AD (1993) Neural regulation of neutrophil involvement in pulmonary inflammation. *Comp Biochem Physiol* 106C, 39-48
- Mayer L, Panja A, Li Y, Siden E, Pizzimenti A, Gerardi F, Chandswang N (1992) Unique features of antigen presentation in the intestine. *Ann NY Acad Sci* 664, 39-46
- Mayrhofer G, Pugh CW, Barclay AN (1983) The distribution, ontogeny and origin in the rat of Ia-positive cells with dendritic morphology and Ia antigen in epithelia, with special reference to the intestine. *Eur J Immunol* 13, 112-122
- McAuley RL, Sommers SC (1961) Mast cells in non specific ulcerative colitis. *Am J Dig Dis* 6, 233-236
- McGee D W, Beagley KW, Aicher WK, McGhee JR (1993a) Transforming growth factor- β and IL-1 β act in synergy to enhance IL-6 secretion by the intestinal epithelial cell line, IEC-6. *J Immunol* 151, 970-978
- McGee DW, Elson CO, McGhee JR (1993b) Enhancing effect of cholera toxin on interleukin-6 secretion by IEC-6 intestinal epithelial cells: mode of action and augmenting effect of inflammatory cytokines. *Infect Immunity* 61, 4637-4644
- Morris GP, Beck PL, Herridge MS, Depew W, Szewczuk MR, Wallace JL (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 96, 795-803
- Nadel JA (1991) Neutral endopeptidase modulates neurogenic inflammation. *Eur Resp J* 4, 745-754
- Nash S, Parkos C, Nursat A, Delp C, Madara JL (1991) In vitro model of intestinal crypt abscess. *J Clin Invest* 87, 1474-1477
- Naukkarinen A, Harvima L, Paukkonen K, Aalto ML, Horsmanheimo M (1993) Immunohistochemical analysis of sensory nerves and neuropeptides and their contacts with mast cells in developing and mature psoriatic lesions. *Arch Dermatol Res* 285, 341-346
- Ohkawa F, Ikeda U, Kawasaki K, Kusano E, Igarashi M, Shimada K (1994) Inhibitory effect of interleukin-6 on vascular smooth muscle contraction. *Am J Physiol* 266, H898-H902
- Ottaway CA (1991) Neuroimmunomodulation in the intestinal mucosa. *Gastroenterol Clin North Am* 20, 511-529
- Pabst MA, Schöninkle E, Holzer P (1993) Ablation of capsaicin sensitive afferent nerves impairs defence but not repair of rat gastric mucosa. *Gut* 34, 897-903
- Palmen MJ, Dieleman LA, Van der Ende MB, Uyterlinde A, Pena AS, Meuwissen SG, Van Rees EP (1995) Non-lymphoid and lymphoid cells in acute, chronic and relapsing experimental colitis. *Clin Exp Immunol* 99, 226-232
- Pascual DW, Bost KL (1990) Substance P production by P388D1 macrophages: a possible autocrine function for this neuropeptide. *Immunology* 71, 52-56
- Perdue MH, McKay D (1994) Integrative immunophysiology in the intestinal mucosa. *Am J Physiol* 267, G151-G165
- Plaza MA, Fioramonti J, Bueno L (1996) Role of central interleukin-1 β in gastrointestinal motor disturbances induced by lipopolysaccharide in sheep. *Dig Dis Sci* (in press)
- Pons L, Droy-Lefaix MT, Braquet P, Bueno L (1989) Involvement of platelet-activating factor (PAF) in endotoxin-induced intestinal motor disturbances in rats. *Life Sci* 45, 533-541
- Purcell WM, Atterwill CK (1995) Mast cells in neuroimmune function: neurotoxicological and neuropharmacological perspectives. *Neurochem Res* 20, 521-532
- Ritter S, Ritter RC, Barnes CD (1992) *Neuroanatomy and Physiology of Abdominal Vagal Afferents*. CRC, Ann Arbor
- Rothwell NJ (1991) Functions and mechanisms of interleukin-1 in the brain. *Trends Pharmacol Sci* 12, 430-436
- Rühl A, Hurst S, Collins SM (1995) Synergism between interleukins 1 β and 6 on noradrenergic nerves in rat myenteric plexus. *Gastroenterology* 107, 993-1001
- Russel M, Dark KA, Cummins RW, Ellman G, Galloway E, Peeke HVS (1984) Learned histamine release. *Science* 225, 583-587
- Sanderson IR, Leung KBP, Pearce FL, Walker-Smith JA (1986) Lamina propria mast cells in biopsies from children with Crohn's disease. *J Clin Pathol* 39, 279-283
- Saperas ES, Yang H, Rivier C, Tache Y (1990) Central action of recombinant interleukin-1 to inhibit acid secretion in rats. *Gastroenterology* 99, 1599-1606
- Shirota K, Leduy L, Yuan S, Jothy S (1990) Interleukin-6 and its receptor are expressed in human intestinal epithelial cells. *Virchows Arch B Cell Pathol* 58, 303-308
- Stead RH (1992) Nerve remodelling during intestinal inflammation. In neuro-immuno-physiology of the gastrointestinal mucosa. In: *Implications for Inflammatory Diseases* (RH Stead, MH Perdue, H Cooke, DW Powell, K Barrett, eds), NY Acad Sci, New York, 443-455
- Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J (1987) Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* 84, 2975-2979
- Stead RH, Dixon MF, Bramwell NH, Riddell RH, Bienenstock J (1989) Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. *Gastroenterology* 97, 575-585
- Stefano GB, Leung MK, Zhao X, Scarrar B (1989) Evidence for the involvement of opioid neuropeptides in the adherence and migration of immuno competent

- invertebrate hemocytes. *Proc Natl Acad Sci USA* 86, 626-630
- Taché Y, Saperas E (1993) Central actions of interleukin-1 on gastrointestinal function. *Methods Neurosci* 17, 169-184
- Theodorou V, Eutamène E, Fioramonti J, Junien JL, Bueno L (1994a) Interleukin-1 induces a neurally mediated colonic secretion in rats: involvement of mast cells and prostaglandins. *Gastroenterology* 106, 1493-1500
- Theodorou V, Marche P, Fioramonti J, Bueno L (1994b) Effet d'hypersecretion de l'interleukine-1 β chez le rat in vivo: origine et implication de l'innervation sensitive. *Gastroenterol Clin Biol* 18, A48
- Theoharides TC (1990) Mast cells: the immune gate to the brain. *Life Sci* 46, 607-617
- Thompson H, Buchmann P (1979) Mast cell population in rectal biopsies from patients with Crohn's disease. In: *The Mast Cell: Its Role in Health and Disease* (J Pepys, AM Edwards, eds), Pitman Medical Publishing, Tunbridge, 697-701
- Walters ET, Alizadeh H, Castro GA (1991) Similar alterations induced by axonal injury and learning in Aplysia. *Science (Wash DC)* 253, 797-799
- Wan W, Wetmore L, Sorensen CM, Greenberg AH, Nance DM (1994) Neural and biochemical mediators of endotoxin and stress-induced *c-fos* expression in the rat brain. *Brain Res Bull* 34, 7-14
- Wang L, Stanisz AM, Perdue MH (1993) Activation of mast cells by substance P in the regulation of ion secretion in the mouse intestine. *Gastroenterology* 104, A1066
- Watkins LR, Wiertelak EP, Goehler LE, Smith KP, Martin D, Maier SF (1994a) Characterization of cytokine-induced hyperalgesia. *Brain Res* 654, 15-26
- Watkins LR, Wiertelak EP, Goehler LE, Mooney-Heiberger K, Martinez J, Furness L, Smith KP, Maier SF (1994b) Neurocircuitry of illness-induced hyperalgesia. *Brain Res* 639, 283-299
- Watkins LR, Maier SF, Goehler LE (1995a) Cytokine-to-brain communication: a review and analysis of alternative mechanisms. *Life Sci* 11, 1011-1026
- Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, Maier SF (1995b) Blockade of interleukin-1 induced fever by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 183, 27-31
- Weigent DA, Blalock JE (1994) Role of neuropeptides in the bidirectional communication between the immune and neuroendocrine system. In: *Neuropeptides and Immunoregulation* (B Scharrer, EM Smith, GB Stephano, eds), Springer, Berlin, 14-28
- Wood JD (1991) Communication between minibrain in gut and enteric immune system. *News Physiol Sci* 6, 64-69