THE HANDBOOK OF IMMUNOPHARMACOLOGY

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Immunopharmacology of the Gastrointestinal System

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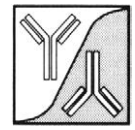

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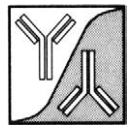


Series Preface

The consequences of diseases involving the immune system such as AIDS, and chronic inflammatory diseases such as bronchial asthma, rheumatoid arthritis and atherosclerosis, now account for a considerable economic burden to governments worldwide. In response to this, there has been a massive research effort investigating the basic mechanisms underlying such diseases, and a tremendous drive to identify novel therapeutic applications for the prevention and treatment of such diseases. Despite this effort, however, much of it within the pharmaceutical industries, this area of medical research has not gained the prominence of cardiovascular pharmacology or neuropharmacology. Over the last decade there has been a plethora of research papers and publications on immunology, but comparatively little written about the implications of such research for drug development. There is also no focal information source for pharmacologists with an interest in diseases affecting the immune system or the inflammatory response to consult, whether as a teaching aid or as a research reference. The main impetus behind the creation of this series was to provide such a source by commissioning a comprehensive collection of volumes on all aspects of immunopharmacology. It has been a deliberate policy to seek editors for each volume who are not only active in their respective areas of expertise, but who also have a distinctly *pharmacological* bias to their research. My hope is that *The Handbook of Immunopharmacology* will become indispensable to researchers and teachers for many years to come, with volumes being regularly updated.

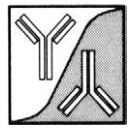
The series follows three main themes, each theme represented by volumes on individual component topics. The first covers each of the major cell types and classes of inflammatory mediators. The second covers each of the major organ systems and the diseases involving the immune and inflammatory responses that can affect them. The series will thus include clinical aspects along with basic science. The third covers different classes of drugs that are currently being used to treat inflammatory disease or diseases involving the immune system, as well as novel classes of drugs under development for the treatment of such diseases.

To enhance the usefulness of the series as a reference and teaching aid, a standardised artwork policy has been adopted. A particular cell type, for instance, is represented identically throughout the series. An appendix of these standard drawings is published in each volume. Likewise, a standardised system of abbreviations of terms has been implemented and will be developed by the editors involved in individual volumes as the series grows. A glossary of abbreviated terms is also published in each volume. This should facilitate cross-referencing between volumes. In time, it is hoped that the glossary will be regarded as a source of standard terms.

While the series has been developed to be an integrated whole, each volume is complete in itself and may be used as an authoritative review of its designated topic.

I am extremely grateful to the officers of Academic Press, and in particular to Dr Carey Chapman, for their vision in agreeing to collaborate on such a venture, and greatly hope that the series does indeed prove to be invaluable to the medical and scientific community.

C.P. Page



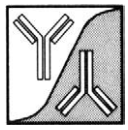
Preface

Diseases of the digestive system account for the occupancy of more hospital beds than any other group of disorders. There is a growing body of evidence that the immune system participates in the pathogenesis of a wide range of digestive diseases, including peptic ulcer disease, inflammatory bowel disease, and the gastropathy induced by NSAIDs. The gastrointestinal tract is also a major target for pathologies associated with HIV infection. For these reasons, efforts to develop novel therapies for digestive diseases are increasingly focused on the immune system.

In this volume, the immunopharmacology of the gastrointestinal tract is reviewed at four distinct levels. The first three chapters discuss immunomodulation at a cellular level, reviewing interactions of the mucosal immune system with neural, epithelial and muscular components of the gastrointestinal tract. Chapters 4 to 6 deal with cellular targets for immunomodulating drugs, including the neutrophil, the vascular endothelium and the mast cell. Chapters 7 through 10 focus on specific classes of inflammatory mediators, their roles in diseases of the gastrointestinal tract and the pharmacological agents available for modifying their synthesis and/or actions. The final chapter reviews the utility and mechanisms of action of glucocorticoids in the treatment of diseases of the gastrointestinal tract.

Each of these chapters covers not only the basic science aspects of immunopharmacology of the digestive system, but also the clinical targets for immunomodulatory therapy. The contributors have attempted to focus on these subjects from a pharmacological perspective. I am grateful to Dr. Clive Page and Dr. Carey Chapman for their assistance in assembling this volume, and to each of the contributors for their considerable efforts in putting together a comprehensive review of a rapidly expanding subject area.

John L. Wallace



1. Neuromodulation of Gastrointestinal Immune and Inflammatory Responses

A. Dean Befus

Introduction

The maintenance of the interior milieu and overall body integrity demands rapid and highly efficient systems for the detection of environmental changes and any potentially noxious agent, interpretation of the signals received and the elaboration of appropriate responses to avoid or otherwise deal with the challenge. Limiting factors in these systems can involve the sensitivity, specificity and speed of communication that orchestrates the transduction of the initial signal to the appropriate response. The nervous system is the pre-eminent communication network in the body which senses, communicates and integrates information and which then initiates suitable responses (Fig. 1.1A). It has elaborate detection, signalling and response pathways with optional circuitry to facilitate programmed responses in particular pathways.

A number of other systems which have been arbitrarily classified as distinct from the nervous system, interact with the nervous system in body homeostasis. Perhaps the best known of these is the endocrine system which interconnects glandular and other tissues. In the last few years another system, namely the immune system, has had a rebirth as an important component of the detection, integration and response circuitry of the body.

The concept of neuroimmunology has a long history as evidenced by writings from ancient cultures which record beliefs of a relationship between the mind and body in defences against infectious or other diseases. Unfortunately, until recently this field has had relatively little scientific credibility because of the lack of well-defined biochemical pathways and the complexity of the phenomena studied. However, in the last 10–15 years significant advances have been made in careful documentation and experimental manipulation of the interactions between the hitherto arbitrarily separated immune and nervous systems. Indeed, as we document the analogies in their overall actions and configurations (Fig. 1.1B), and commonality of mediators and signalling pathways (e.g. Weigent and Blalock, 1989; Jankovic, 1989, see

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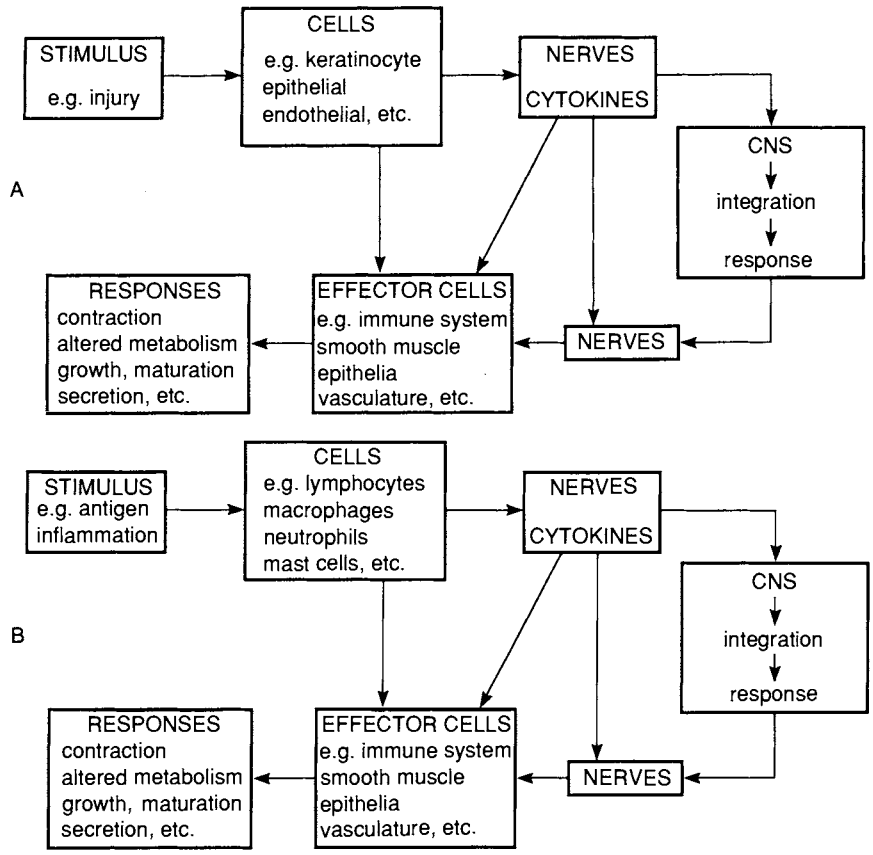

Figure 1.1 Sensory, communication and responses pathways of the nervous system: A, general; B, neuroimmunologic.

below), it is increasingly recognized that distinctions made between these systems are artificial. As outlined in Fig. 1.1B, in neuroimmune pathways the stimuli that are detected, the cells involved and the responses elicited may not fit with more classical definitions of the information gathering, interpretation and actions through the nervous system, but the rebirth of "neuroimmunology" can only serve to further our understanding of the exquisite complexities and capabilities of the mind–body relationship.

Recent studies in neuroimmunology have focused in selected areas, including: documentation that immune responses can undergo classical Pavlovian conditioning (e.g. Goetzl, 1985; MacQueen *et al.*, 1989) and that precise lesions in the central nervous system modulate immune functions (Cross, 1990); identification that cells of the immune system make mediators classically thought to be solely of nervous system origin, and the reciprocal, i.e. the nervous system makes interleukins and cytokines classically thought to be of immune system origin (e.g. Weigent and Blalock, 1989); mapping the seemingly endless array of effects of neuropeptides and other mediators classically thought to be derived from the nervous system on cells of the immune system (e.g. O'Dorisio, 1990; Roszman and Brooks, 1990); mapping the effects of mediators classically thought to be derived from the immune system on cells from the nervous system (e.g. Bateman *et al.*, 1989; Weigent and Blalock, 1989; Shibata, 1990); and investigation of immunopathology of neurologic disease (e.g. Goetzl *et al.*, 1988; Jankovic, 1989).

1.1 NEUROGENIC INFLAMMATION

Additionally, involvement of sensory neurons in vascular permeability in sites such as the skin (e.g. Kowalski and Kaliner, 1988), eye (e.g. Sullivan, 1990), synovial joints (Coderre *et al.*, 1989) and respiratory tract (Brokaw and McDonald, 1988; Kowalski *et al.*, 1989) has received increased attention as an important component of inflammatory responses. The mechanisms underlying this "neurogenic inflammation" involve release of neuropeptides such as substance P, and corresponding sensitivity to capsaicin-induced depletion of this neuropeptide, as well as an associated, but not always essential degranulation of local mast cell populations (e.g. Kiernan, 1975; Bani-Sacchi *et al.*, 1986, Coderre *et al.*, 1989; Kowalski and Kaliner, 1988; Baraniuk *et al.*, 1990) and mediator action on the vascular bed.

The magnitude and duration of the neurogenic inflammatory response is influenced by pre-existing infection and the related alterations in levels of neutral endopeptidase, an endogenous peptidase that degrades substance P and other peptides (Borson *et al.*, 1989). Proteases derived from mast cells, and presumably from many other inflammatory cells, may also regulate neurogenic components of inflammation by a similar degradation of selected neuropeptides (Brain and Williams, 1988; Caughey, 1990).

In contrast to other sites, neurogenic inflammation in the gastrointestinal tract has received relatively little study, although parasympathetic stimulation of ileal histamine release and mast cell degranulation has been documented (Bani-Sacchi *et al.*, 1986) and an abundance of evidence is available concerning neurogenic involvement in altered responses during acute inflammatory events in the intestine (e.g. Bern *et al.*, 1989; Castro, 1989; Crowe *et al.*, 1990).

1.2 NEUROIMMUNOLOGY OF THE GASTROINTESTINAL TRACT

The specialized study of neuroimmunology of the gastrointestinal tract has arisen within the last few years and has included studies of neuroregulation of mucosal immune responses (see Croitoru *et al.*, 1990; Freier and Lebenthal, 1990; Stead *et al.*, 1991a), as well as a comprehensive analysis of interactions among the cornucopia of mediators generated by the immune system, and the enteric nervous system, in the physiologic responses of target tissues such as the epithelium (Powell, 1991). Because of the unique anatomical properties of the mucosal immune system and the distribution and abundance of its components in the gastrointestinal tract (e.g. McGhee *et al.*, 1989; Targan and Shanahan, 1990), study of gastrointestinal neuroimmunology cannot be based solely upon extrapolations from studies of neuroimmunology of parenteral sites. This is further complicated by the highly specialized nature of the enteric nervous system (Cooke, 1986).

For the purposes of this discourse on the neuromodulation of gastrointestinal immune and inflammatory responses, the unusual characteristics of the mucosal immune system will be reviewed. Significant interchange between the mucosal and systemic immune and inflammatory systems occurs. A précis of the enteric nervous system and its integration with the other components of the autonomic nervous system will be presented to help place aspects of gastrointestinal neuroimmunology in appropriate perspective. Recent information on alterations in structure, distribution and abundance of enteric nerves, and in the distribution and abundance of neuropeptides during intestinal inflammatory processes will be reviewed.

Cells of the mucosal immune system must be under the continual influence of the plethora of locally and systemically derived neuroendocrine and neuropeptide signals in the microenvironment of the mucosa and its underlying connective tissue and musculature. Some examples of these types of regulatory pathways will be presented. Finally, an attempt will be made to provide a more holistic overview of the integration of the myriad of neuroimmunomodulatory responses during mucosal immune and inflammatory events. Areas where critical gaps in knowledge exist will be identified so that experimentation may be encouraged. It is hoped that from this analysis there may arise new understanding and innovative therapeutic, diagnostic or prognostic tools to improve gastrointestinal health.

2. *The Intestinal Immune System*

2.1 GUT-ASSOCIATED LYMPHOID TISSUE GALT

In the gastrointestinal mucosa and at other mucosal surfaces such as the respiratory tract, there are specialized lymphoid aggregates. In the gastrointestinal tract these include the tonsils and adenoids, Peyer's patches, appendix, solitary lymphoid nodules, an ileo-caecal lymphoid aggregate called the sacculus rotundus (in some species) and the bursa of Fabricius. These aggregates share a relatively standard morphology (Fig. 1.2) and are generally considered to be major inductive sites for mucosal immune responses (McGhee *et al.*, 1989). Most are considered to be *secondary* lymphoid tissues which are responsive to antigens and generate immune responses throughout life.

By contrast, the avian bursa of Fabricius is a *primary* lymphoid tissue, analogous for the B cell arm of the immune system to the thymus for the T cell arm of the system. In mammals the bursal equivalent appears to be dispersed in adult bone marrow and, at least for species such as the sheep (Reynolds and Kirk, 1989), in a large, unique Peyer's patch in the terminal ileum. These primary lymphoid tissues arise early in life and appear to be largely antigen-independent and responsible for the development of lymphocyte populations and the repertoire of antigenic specificities they recognize.

GALT have a specialized lymphoepithelial layer lining the lumenal surface, which because of its unusual cellular composition, particularly the microfold or M cell, is capable of continual sampling of the antigenic make-up of the intestinal contents and the transport of these food and microbial antigens to components of the underlying immune apparatus. Under this lymphoepithelium there are large follicular structures enriched in rapidly proliferating B lymphocytes, many of which undergo negative selection and programmed cell death (Motyka and Reynolds, 1991), presumably as part of a mechanism for the development of a rich repertoire of antigenic specificities

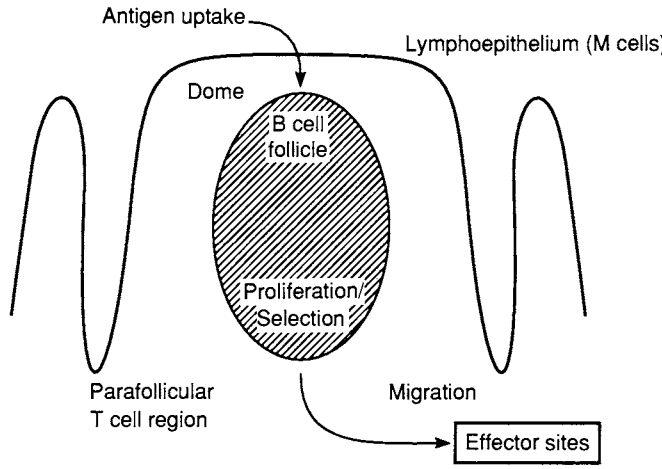

Figure 1.2 GALT inductive sites responsible for antigen uptake, sensitization of the immune system and development of effector functions such as IgA antibodies at mucosal sites.

in the B cell or immunoglobulin-producing arm of the immune system, but with tolerance to self. Adjacent to the follicles there are parafollicullar regions which are enriched for T lymphocytes and which possess specialized high endothelial postcapillary venules, particularly prominent sites of lymphocyte migration from the circulation.

GALT are sites of the induction of mucosal immune responses (Brown and Kloppel, 1990; Fiocchi, 1990) and also of the generation of specific hyporesponsiveness to mucosal antigens, a response called oral tolerance (e.g. Bruce and Elson, 1990). Mucosal antibody responses include a striking IgA response which is a result of the abundance of IgA precursor cells in GALT and their local induction by mechanisms which remain to be fully understood (McGhee *et al.*, 1989). Antibodies of other isotopes are also produced locally in the mucosa, or are derived from the circulation by mechanisms which enhance the local leakage of large plasma proteins into tissue microenvironments.

Both T_H (CD_4) and $T_{C/S}$ (CD_8) subsets of T cells are found in GALT. Of the CD_4 + cells, T_{H1} and T_{H2} helper subsets are present. It has been postulated that given the inductive nature of the GALT environment, the activity of T_{H1} cells, with their lymphokine repertoire including IL-2 and γ_{IFN}, is predominant in this site, whereas T_{H2} cells with their repertoire of IL-4, IL-5 and IL-6 are predominant in the mucosal lamina propria where effector functions are foremost (Husband and Dunkley, 1990). The CD_8 +, $T_{C/S}$ cells from GALT can generate classical cytotoxic T effector cells (London *et al.*, 1987), as well as suppressor populations apparently involved in systemic tolerance to orally-derived antigens (oral tolerance; McGhee *et al.*, 1989; Bruce and Elson, 1990).

Effector cells whose differentiation and maturation are initiated in GALT, such as IgA B cells and cytotoxic T lymphocytes, do not complete their maturation in these sites, but migrate through the lymphatics to the draining mesenteric lymph nodes and subsequently into the thoracic duct and circulation. From the circulation, many of them can selectively enter and are retained in the intestinal lamina propria where they may proliferate and complete their maturation to fully functional IgA producing plasma cells or other effector cells (Ottaway, 1990). In the circulation, cells of the mucosal immune system may exchange information with cells derived from systemic lymphoid sites. Cells derived from the GALT, and normally to a much lower extent, cells derived from systemic lymphoid sites, may also localize at other mucosal surfaces, such as in the bronchial mucosa or urogenital tract. This is a reciprocal association, as cells which have undergone induction at these other mucosal sites may also localize in the intestinal mucosal. The reciprocity not only involves the migrations of IgA B cells and other lymphocyte populations, but also IgA antibodies themselves which are selectively transported from the circulation into secretions at all mucosal sites by the receptor–transporter secretory component, produced by mucosal epithelial cells.

From observations such as these, the concept of a common mucosal immune system developed (Bienenstock and Befus, 1983); a system integrated by cell and molecular exchange among all mucosal surfaces throughout the body. Within the bases of this common mucosal system there lies a tremendous, but hitherto untapped, potential for oral vaccination to protect such diverse sites as the eyes and respiratory and urogenital tracts (see McGhee and Mestecky, 1990). How the nervous system modulates this mucosal immune network, or the detailed workings of any of its members, has been largely unexplored.

2.2 SPECIALIZED CELLS OF THE MUCOSAL IMMUNE SYSTEM

There are multiple cellular components in the mucosal

immune system and they will not all be reviewed in detail here. Many of these cells are not unique to the mucosal immune system or appear to be specially adapted to the mucosal environment. They circulate widely and can be recruited when germane signals are generated by inflammatory or other stimuli. It is presumed that to a major extent, the neuromodulation of their recruitment and subsequent actions will involve mechanisms similar to those acting with other cell types in the gastrointestinal tract or to basic neuromodulatory mechanisms shared by sites throughout the body.

In addition, the mucosal immune system encompasses cellular components (e.g. cells of the IgA cycle, intraepithelial leucocytes, and mucosal mast cells) that appear to be unique to the mucosal immune system and/or highly adapted for development and function in mucosal environments, especially the gastrointestinal tract.

2.2.1 IgA Development

The fundamental principles underlying the differentiation and maturation of IgA producing cells have been outlined above (see also McGhee *et al.*, 1989). The developmental cycle of this cell lineage has been central to the evolution of our understanding of the unique characteristics of the mucosal immune system, ranging from the inductive phase and cell differentiation, to selective localization and effector functions. Although the role of the nervous system in the IgA cell cycle and in IgA secretion has received relatively little attention, some information is available (see below) which provides a useful schema for comparable investigations of the role of the nervous system in the ontogeny and functions of other components of the mucosal immune system. Unfortunately, there is no information about the reciprocal action, i.e. the role of IgA or cells in different phases of the IgA cell cycle on the development, maintenance or function of the enteric or systemic nervous systems.

2.2.2 Intraepithelial Leucocytes

Within the normal intestinal epithelium there is a large (10–15%) population of leucocytes (IEL). Most of these, at least under normal conditions, are lymphocyte-like in their morphology and 20–70% of these possess electron-dense cytoplasmic granules with a proteoglycan core and a number of stored mediators (Cerf-Bensussan and Guy-Grand, 1991; Befus, 1992). IEL encompass a highly heterogeneous population of cells; in the mouse the majority of which (about 95%) express the T lymphocyte marker, CD_3. In addition, about 90% of these are CD_8 + and the remainder are CD_4 +. Thy 1, previously thought to be a pan-T cell marker, in the mouse is expressed on only 50% of IEL. Normal young adult laboratory-reared mice express the TCR α/β heterodimeric form of the T cell receptor for antigens on about 70% of their IEL, and the TCR γ/δ form on about 27% of IEL. The marked abundance of CD_8 + cells and TCR γ/δ expression and the low proportion of Thy 1+ cells are prominent distinctions of IEL; to date, no other leucocyte population has been discovered with these characteristics. Moreover, in enlightening experiments, Guy-Grand *et al.* (1991) established that of the CD_8+ IEL, 63% express the homodimeric α/α form of CD_8, whereas about 37% express the heterodimeric CD_8 α/β form.

It can be concluded that there are two major populations of IEL, one which is Thy 1 +, TCR α/β+ and heterodimeric CD_8 α/β+ and is derived from antigen-driven proliferating T cells in GALT. The other major population is largely Thy 1−, TCR γ/δ+ and homodimeric CD_8 α/α + and appears to develop independently of the thymus and of antigenic stimulation (analogous to cell development in a primary lymphoid tissue). Guy-Grand *et al.* (1991) proposed that in addition to possessing thymus-dependent, antigen-responsive T cells, "... the gut epithelium ... has an inductive property, attracting progenitors of bone marrow origin, and triggering their TCR rearrangement and α/α CD_8 chains expression, thus giving rise to a T cell population that appears to belong to the same lineage as γ/δ thymocytes and to recognize an antigenic repertoire different from that of α/β CD_8 + IEL".

The spectrum of functions of IEL is incompletely known, but many of the cells are able to express potent cytotoxic activity (Cerf-Bensussan and Guy-Grand, 1991). In addition, IEL contain mast cell, natural killer cell and T cell precursors and can produce γIFN, IL-2 and IL-5 (see Befus, 1992). It is believed that when activated, part of their functional activities are to modulate the development and function of absorptive and secretory epithelial cells themselves. Their possible reciprocal interactions with the nervous system have been unexplored.

2.2.3 Gastrointestinal Mast Cell Populations

Mast cells contain a plethora of potent pro-inflammatory mediators with the capability of modulating the ontogeny, differentiation and function of epithelial, endothelial, smooth muscle, connective tissue, myeloid and neuronal cells throughout the gastrointestinal tract. Moreover, within the gastrointestinal mucosa of rodents there is a unique mast cell population, the IMMC; with ontogenetic, biochemical and functional characteristics which distinguish it from the other well-recognized mast cell population which is widely scattered in the body, namely the CTMC. Although both mast cell populations are present in the gastrointestinal tract, IMMC are most abundant in the lamina propria, whereas CTMC are numerically dominant in the muscle layers and serosa. In rodents it is possible to study IMMC and CTMC in relative purity and isolation, but unfortunately, with human mast cells it has been impossible to physically separate these two populations for independent study.

The developmental biology and nature of mast cell

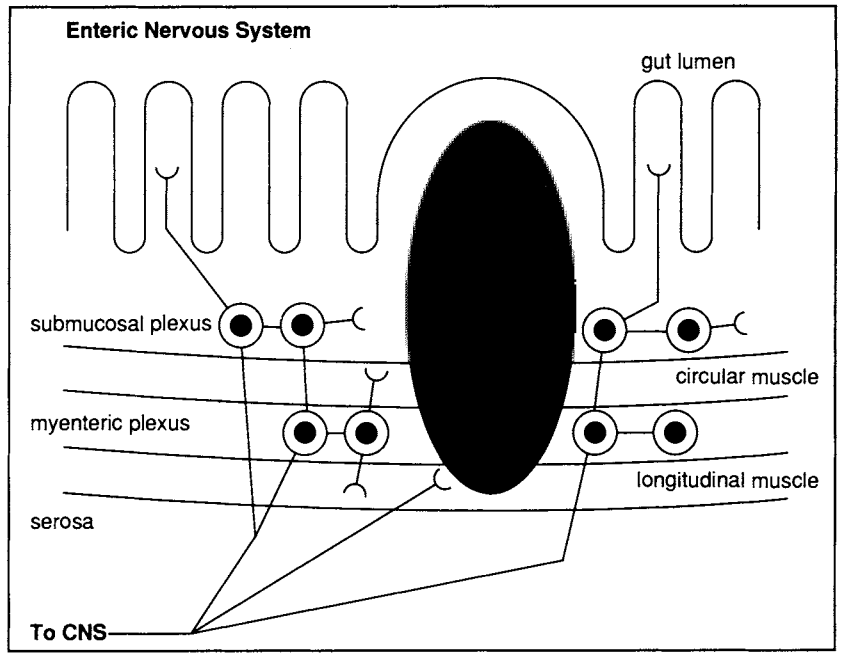

Figure 1.3 The enteric nervous system.

heterogeneity has been reviewed recently (e.g. Galli, 1990; Bissonnette and Befus, this volume). These distinct mast cell populations arise from a common progenitor cell and appear to be able to "transdifferentiate" from one to the other. Although they share a number of critical characteristics which identify them as mast cells, they differ in their thymic dependency, mediator content and responsiveness to selected secretagogues and anti-allergic drugs.

In their bidirectional interactions with the nervous system, mucosal mast cells are extensively studied cells (see below). Their development and the stimulation and regulation of their secretory function have been investigated. Moreover, mast cell products modulate neuronal growth and neurotransmission.

3. *Innervation of the Gastrointestinal Tract*

The innervation of the gastrointestinal mucosa encompasses extrinsic parasympathetic and sympathetic divisions of the autonomic nervous system, as well as the intrinsic, enteric division (see Cooke, 1986). The enteric nervous system (Fig. 1.3) includes the myenteric plexus, found between the outer longitudinal muscle layers and the inner circular muscle. This plexus has a high proportion of neurons which innervate the muscle layers and probably play a major role in the integration of intestinal motility. A second plexus is located in the submucosa and has fibres which innervate mucosal and submucosal tissues and probably manage a large component of the neurogenic control of epithelial function, blood flow and other mucosal and submucosal functions. The myenteric and submucosal plexuses contain large numbers of ganglia. Fibres from both, as well as from the extrinsic innervation of the gut, ramify throughout the layers of the intestinal wall. The entire system is integrated into a finely tuned unit with multiple internal controls and significant exchange from the periphery and the central nervous system. This complexity represents a tremendous challenge for the gastrointestinal neurophysiologist, and much remains to be established about the circuitry and the chemical and electrical signalling involved. Little progress has been made in defining the innervation of the mucosal immune system.

Felten and his colleagues (1990) reviewed the innervation of primary and secondary lymphoid tissues throughout the body and described the limited knowledge of the innervation of GALT. Noradrenergic fibres derived from the sympathetic mesenteric ganglia pass through T cell zones of GALT and enter the lamina propria where they may be found in close association with a number of cell types. Parasympathetic innervation of mucosal lymphoid tissues is poorly known.

Neuropeptide-containing fibres have also been described in GALT and throughout the mucosa and muscle layers, but their precise origin and pathways are inadequately understood. Substance P and CGRP-containing fibres are in close proximity to mucosal mast cells and other cell types (Stead *et al.*, 1987; Arizono *et al.*, 1990; see below). Ottaway *et al.* (1987) established that VIP-containing fibres run adjacent to the postcapil-

lary venules, a prominent site of immigration of lymphocytes expressing VIP receptors (Ottaway, 1984). Detailed mapping of the extrinsic and intrinsic innervation of the gastrointestinal tract, particularly as it relates to GALT and the mucosal immune system remains an important challenge.

4. *Inflammation and Gastrointestinal Neuroplasticity*

Degenerative and proliferative changes in enteric nerves are consistent features of the pathology of the IBDs, Crohn's disease and ulcerative colitis (see Koch *et al.*, 1991). Dvorak (1991) recently reviewed some of these pathological changes in IBD and described both hyperplasia and hypertrophy, as well as various phases of cell injury and necrosis, including swelling and damage to neurotubules, neurofilaments, dense core vesicles and other axonal components. There are changes in the structure of adjacent smooth muscle cells also, and activation of infiltrating eosinophils and local mast cells.

Koch *et al.* (1991) also described changes in colonic smooth muscle function in Crohn's colitis, notably a reduction in inhibitory junctional potentials evoked by field stimulation. Such abnormalities could explain the high amplitude contractions generated in response to rectal saline infusions in colitis patients and their rectal hypomotility. In studies of abnormalities in motility that might arise from neuronal damage, a number of workers have assessed changes in the distribution and abundance of various neuropeptides in involved and uninvolved regions of the intestine in patients with IBD. Koch *et al.* (1991) briefly reviewed this literature and observations from their laboratory. In the mucosal-submucosal tissue layer in Crohn's colitis they observed a reduction in concentrations of VIP, PHM, NPY, peptide YY and somatostatin. The results for ulcerative colitis specimens were similar, with the exceptions of elevated levels of NPY and substance P. These changes were not distributed in a consistent pattern throughout anatomical compartments of the intestinal walls, as evidenced by analyses of neuropeptides in the external muscle layer.

In a detailed morphometric analysis of VIP and S-100 immunoreactive fibres in the lamina propria and submucosal of colonic specimens resected from patients with Crohn's disease or ulcerative colitis, Kubota *et al.* (1992) established that there was a significant decrease in immunoreactive fibres in the lamina propria as the severity of inflammation increased. However, there was no association with the type of IBD. The authors postulated that this loss of neuropeptide innervation of the colonic mucosa was a result of nonspecific inflammatory damage to enteric nerve fibres and that this may contribute to malfunction of mucosal immunoregulation.

By contrast, Koch *et al.* (1991) postulated that the damage to the enteric nervous system in IBD reflects a primary abnormality and that patients with this neuronal injury experience deficits in the normal functions of enteric nerves, including in functions of absorption and secretion, motility, blood and lymph flow and immunity. Following this loss of neuroimmune regulation, inflammation may become more pronounced, in turn exacerbating neuronal and other tissue damage. This is an interesting hypothesis, but given the present evidence, it is equally probable that neuronal damage is not a primary lesion, but, as suggested by Kubota *et al.* (1992), is secondary to ongoing and relatively nondiscriminating inflammatory events. Indeed, Dvorak (1991) noted the association with activated eosinophils and mast cells and neuronal injury, and Stead *et al.* (1989, 1990a) noted the close association between nerves and mast cells in the human intestinal mucosa and fibrotic appendix.

Unfortunately, such cross-sectional investigations of resected samples from IBD patients present many problems. The tissues are often badly inflamed and/or fibrotic, and frequently these injury and repair processes have been chronic. Moreover, there have often been aggressive but highly variable attempts with drug intervention. Obviously, data based upon such samples presents difficulties in interpretation and in elucidating the primary or most relevant lesions.

Animal models of intestinal inflammation and repair present more controlled tools to investigate some of the underlying neuroimmune phenomena and the time course of these and other events. Using the rat–nematode (*Nippostrongylus brasiliensis*) model, Stead and coworkers (Stead *et al.*, 1987) established that 35 days after infection, about 3 weeks after most of the worms were expelled from the intestine and the inflammation has largely subsided, but when there was still a pronounced hyperplasia of IMMC, up to 90% of all the mast cells in the lamina propria were closely apposed to subepithelial nerves. Furthermore, these nerves were shown to contain substance P and CGRP. The authors proposed that these observations provided anatomical evidence for communication between the immune and nervous systems in the gastrointestinal mucosa. Other authors have provided evidence that this physical proximity is not restricted to mast cells, but involves other cells of the immune system as well (Arizono *et al.*, 1990).

Analyses of the time course of the development of this close physical relationship between mast cells and enteric nerves following infection with *N. brasiliensis* suggested that there is a remarkable plasticity of intestinal mucosal neurons during the evolution of inflammatory events (Stead *et al.*, 1991b). Whereas neurofilament subunits prominent in "mature" neurons were sparse in the normal or inflamed intestinal mucosal, NSE-containing nerves were abundant in the mucosal of normal animals. B-50 growth associated protein (GAP)-43-containing nerves, a marker of nerve growth which is abundant in regenerating nerves and in areas of synaptic plasticity,

were four times more abundant than NSE-containing nerves in these same normal animals. This phenotype of mucosal nerves suggests that there is ongoing modelling of the enteric fibres (Sharkey *et al.*, 1990).

After nematode infection there was a dilation and degeneration of enteric nerves following the initial degranulation of resident mast cells. Subsequently, and in correlation with the hyperplasia of IMMC, there was evidence for nerve regeneration. B-50-containing fibres were significantly increased in number, and the proportion of dilated degenerating fibres decreased (Stead *et al.*, 1991b). Whether or not the mediators released from degranulating IMMC are the direct cause of the initial damage to the nerves, there does appear to be a correlation between neuronal damage, IMMC activation and local inflammatory responses.

It is also possible that IMMC are the source of neurotrophic factors important in nerve regeneration. In coculture experiments, neurite growth towards and contact with an IMMC-like cell line (RBL) was pronounced (Blennerhassett *et al.*, 1991). Once in contact with a neurite, RBL ceased to divide and increased their granularity, morphological evidence of maturation. These observations, taken together with those which established that NGF causes activation of CTMC and hyperplasia of both CTMC and IMMC (Stead *et al.*, 1990b; Matsuda *et al.*, 1991) suggest that the relationship between neuronal development and mast cell activation and development requires careful investigation. Moreover, it suggests that neuroimmune regulation is an important component of the maintenance of gastrointestinal integrity and that its loss is relevant to the pathogenesis of IBD and other intestinal diseases.

5. *Neuroregulation of Cells of the Mucosal Immune System*

In recent years there has been a deluge of literature on the neuromodulation of the differentiation, maturation and functions of cells of the immune system and its mucosal compartment. The list of neuropeptides and neuroendocrine factors that have been studied is seemingly endless, for example, substance P, VIP, CGRP, somatostatin, norepinephrine, adrenalin, ACh, neurotensin, opioids, PHM, ACTH, CRF, prolactin, α-melanocyte stimulating hormone. The spectrum of cellular events that have been assessed following treatments with these factors has been equally large, including: proliferation, differentiation, maturation and a wealth of immune functions from immunoglobulin, mediator and cytokine synthesis and secretion, cell migration and competency in response to other factors, to endocytosis (see Table 1.1). It is difficult to summarize this literature and thus the readers may wish to consult other reviews, notably, Bateman *et al.* (1989), Berczi (1989), Jankovic (1989), Rabin *et al.* (1989), Weigent and Blalock (1989), Bar-Shavit and Goldman (1990), Croitoru *et al.* (1990), O'Dorisio (1990), Freier and Lebenthal (1990) and Stead *et al.* (1991a). Herein, a few specific examples will be described so that the potential of neuroimmune interactions can be conceptualized and placed in the context of personal research interests by each reader.

Neuroimmune regulation may arise at a local level through reflex or other localized reactions, or it may arise from a distant site in the gastrointestinal tract, periphery or from the central nervous system. Studies *in vitro* focus on what are likely to be highly localized events where a given mediator interacts with a particular cell type. Unfortunately, the physiologic concentrations of mediators in such situations are difficult to determine and it may be that many of the studies *in vitro* employ unrealistic neuropeptide concentrations (e.g. mast cell studies). Studies *in vivo* have more potential to address some difficult questions of peripheral or CNS control of mucosal immune reactions, but such studies have been few and it is a challenge to minimize the variables involved and to isolate one for rigorous study.

5.1 LYMPHOCYTES AND THE IGA CELL CYCLE

Using dispersed populations of cells from Peyer's patches, mesenteric lymph nodes and spleen, Stanisz *et al.* (1986) demonstrated with substance P, VIP and somatostatin that there were peptide and site-specific effects on mitogen-induced proliferation of IgA, IgM and IgG levels in culture supernatants. Substance P stimulated Con A-induced proliferation in lymphocytes from all three sites, whereas VIP and somatostatin inhibited proliferation (Table 1.1). Furthermore, substance P enhanced the levels of IgA in culture supernatants, and this was especially marked (300%) with Peyer's patch cells. By contrast, VIP significantly inhibited (70% reduction) the levels of IgA in similar cultures. To evaluate the potential relevance of these observations to immune responses *in vivo*, Scicchitano *et al.* (1988a) used miniosmotic infusion pumps to elevate circulating levels of substance P. They confirmed that this neuropeptide could enhance lymphocyte proliferation and immunoglobulin synthesis, particularly IgA, *in vivo*. The mechanisms underlying these observations remain unclear because of the mixture of B and T cells in various phases of development in each tissue studied and because of the complexity of possible regulatory pathways both in cultures and *in vivo*. However, some of the pathways involved have begun to be dissected.

For example, receptors for substance P and somatostatin have been found on both T and B lymphocytes, and T lymphocytes also express VIP receptors (see Croitoru *et al.*, 1990; O'Dorisio, 1990). Sixty-eight percent of cells in the IgA plasmacytoma, MOPC-315, express about 41 000 somatostatin binding sites per cell

Table 1.1 Examples of neuroregulation of mucosal immune responses

	Neuroregulation	
Immune response	*Effect*	*Mediator*
Lymphocyte proliferation	enhanced	substance P
	depressed	VIP, somatostatin
mRNA levels		
IgA α chain	enhanced	substance P
IgM μ chain	enhanced	substance P, ACTH
Levels in cultures		
IgA	enhanced	substance P
	depressed	VIP, somatostatin
IgM	enhanced	substance P, ACTH
	depressed	somatostatin
Secretion *in vivo*		
IgA (gut)	enhanced	cholinergic stimulation
		hypnotic suggestion
IgG (gut)	enhanced	cholinergic stimulation
Secretory component (lacrimal gland)	depressed	cholinergic stimulation

with a K_d of 1.6 nM (Scicchitano *et al.*, 1988b). Somatostatin inhibits proliferation of these cells and at concentrations of 10 nM to 1 μM it also inhibits the levels of IgA in culture supernatants (Table 1.1).

Substance P receptors (about 600/cell: K_d 0.69 nM) have been found on the murine B lymphomas, 4F10 (IgA producing) and 5F5 (IgM producing) (Pascual *et al.*, 1991). Substance P enhances LPS-induced IgA and IgM production by a mechanism that cannot be fully explained, but which involves increases in mRNA for the respective immunoglobulin. Thus, at least part of the action of these neuropeptides on IgA and other immunoglobulin production appears to be through direct action on the B cells.

The role of neuropeptide receptors on T lymphocytes has been less well studied, although modulation of VIP receptors on T cells by preincubation of the cells with VIP decreased the rate of entry of cells into Peyer's patches and mesenteric lymph nodes *in vivo* in a tissue-selective manner (Ottaway, 1984). The expression of neuropeptide receptors on T cell subpopulations and the alterations in cell function induced by receptor occupancy are important subjects for study.

Receptors for ACTH (Clarke and Bost, 1989), β_2-adrenergic agents (e.g. Fuchs *et al.*, 1988) and opioids (Carr *et al.*, 1988) have also been demonstrated on T and B lymphocyte populations and their numbers appear to be modulated by various stimuli. Presumably their expression varies among subsets of these cells and perhaps through different phases of the cell cycle. Unfortunately, there appears to have been no studies of their expression on cells derived from the mucosal immune system. However, for ACTH receptors on the B cell lymphoma, 5F5, occupancy augments IgM secretion and μ heavy chain mRNA expression (Bost *et al.*, 1990; Table 1.1). The observation that ACTH enhances immunoglobulin production and yet glucocorticoids, whose synthesis and secretion is stimulated by ACTH, generally depress immune function seems paradoxical. Perhaps extra-pituitary sources of ACTH such as lymphocytes (Weigent and Blalock, 1989) provide for microenvironmental regulation of B lymphocyte function independent of levels of the circulating hormone (Bost *et al.*, 1990).

Using rats with isolated intestinal loops, Wilson *et al.* (1982) and Freier *et al.* (1987) established that IgA release into lumenal perfusates is under autonomic control (Table 1.1). Atropine, the muscarinic antagonist depresses the levels of IgA in perfusates, whereas cholinergic agonists enhanced the levels of IgA. By contrast, Kelleher *et al.* (1991) established that output of secretory component by acinar cells of the lacrimal gland was inhibited by cholinergic agonist, carbachol. Interestingly, the release of IgG into intestinal perfusates was also enhanced, and the molecular size of IgA was heterogeneous, suggesting that this immunoglobulin transport into the intestinal lumen was not solely by a secretory component-mediated mechanism, but by a more general pathway of protein transfer into the lumen (see Freier and Lebenthal, 1990).

There is obviously a wealth of potential local and/or centrally derived cholinergic and adrenergic mechanisms that might be involved in immunoglobulin transport and other aspects of intestinal immune function. Although many immune responses can undergo classical Pavlovian conditioning (e.g. MacQueen *et al.*, 1989, see below), there is no information on conditioning mucosal lymphocyte functions. However, it is interesting that children taught self-hypnosis with specific suggestions for control of immunoglobulin levels in saliva significantly increased salivary IgA levels during the experimental period (Olness *et al.*, 1989; Table 1.1). Perhaps in the future it may be possible to manipulate the pathways

involved for use in therapy or maintenance of good health.

5.2 INTRAEPITHELIAL LEUCOCYTES

Despite the innervation of the mucosa and its epithelial layer, and the unusual nature of IEL and compelling suggestions from their abundance, location and novelty that IEL populations must be significant in mucosal homeostasis, defences and pathogenesis, there are few studies of the neuroimmunology of IEL. One of the best recognized functions of IEL is their ability to express cytotoxic activity against a range of cell targets. Croitoru *et al.* (1990) suggested that substance P enhanced the natural killer activity of murine IEL both *in vivo* and *in vitro*. It is obvious that there must be an array of local and central neuroregulatory events which influence IEL and remain to be studied.

5.3 MAST CELL POPULATIONS

The innervation of mast cells has been reviewed in detail recently (Stead *et al.*, 1990b). However, mast cells have been the subject of considerable investigation with regard to bidirectional interactions with the nervous system, and thus a short summary is appropriate at this point.

The ontogeny of mast cells is influenced by NGF, an important factor in the growth and differentiation of components of the sympathetic nervous system. When administered to neonatal animals, NGF enhanced the numbers of IMMC and CTMC. In IL-3-driven cultures of murine bone marrow-derived mast cells, Matsuda *et al.* (1991) established that NGF facilitated the differentiation of the cells towards a CTMC-like phenotype. The authors postulated that bone marrow-derived mast cells cultured in IL-3 may induce the production of NGF by fibroblasts, which in turn selects for the expression of CTMC-like phenotypic characteristics in the proliferating mast cells. What role this mast cell–IL-3–fibroblast interaction might have in the development of the mast cell–nerve functional unit described by Stead *et al.* (1987; 1990b) and supported by the *in vitro* co-culture experiments of Blennerhassett *et al.* (1991) remains to be assessed.

There is little information on the action of other neuronal-related factors on mast cell development. Levine *et al.* (1990) evaluated the hypothesis that the density of synovial mast cells is dependent upon local innervation. Capsaicin treatments to deplete unmyelinated primary afferent nerves, as well as chemical lesions in sympathetic postganglionic neurons, induced a significant decrease in the number of synovial mast cells. Furthermore, spontaneously hypertensive rats with exaggerated sympathetic activity had an increased number of synovial mast cells. The authors concluded that primary afferents and sympathetic efferents exert a trophic effect on mast cell density. Whether similar effects exist on the number of mast cells in the gastrointestinal mucosa remains to be studied.

Many neuropeptides induce the secretion of histamine from human skin mast cells (e.g. Benyon *et al.*, 1989) and rat peritoneal mast cells (e.g. Shanahan *et al.*, 1985), but these same neuropeptides are impotent or at least much less active against other human mast cell populations or rat IMMC. In comparison to IgE-dependent stimuli, neuropeptides are much less potent in stimulation of arachidonic acid metabolites (e.g. Benyon *et al.*, 1989). Furthermore, the concentrations of these neuropeptides used to induce histamine secretion are often in micromolar or greater range and their physiological relevance has been questioned. Despite numerous efforts, there is no unequivocal evidence that classic receptor binding sites for neuropeptides exist on mast cells. Indeed, it has been argued that mast cell activation involves the interaction of cationic residues on the peptide with complementary charged structures on the mast cell surface (Foreman and Piotrowski, 1984) and that mast cell populations differ in this surface structure(s).

Interestingly, field stimulation of the rat ileum in a manner designed to selectively activate parasympathetic nerve endings, induced histamine secretion and morphological evidence of intestinal mast cell degranulation (Bani-Sacchi *et al.*, 1986). As expected, these responses could be inhibited by the cholinergic antagonist, atropine, but it is not clear if the mast cells involved were localized to the mucosa or involved those in the muscle and serosal layers alone or as well. In studies of the sensitized canine lung, Leff and coworkers have established that cholinergic stimulation enhances antigen-induced histamine secretion and that sympathetic stimulation inhibits mast cell degranulation (Garrity *et al.*, 1985; White *et al.*, 1989). α-Adrenergic stimulation did not significantly alter mast cell secretion, whereas β_2-adrenergic stimulation inhibited secretion of mast cell mediators. The extent to which these observations apply to the gastrointestinal tract and to other species remains to be evaluated.

The relevance of these types of neuroregulatory pathways to the classical Pavlovian conditioning of mast cell and other cells of the immune system is unclear. However, it is evident that basophils and mucosal mast cells can be conditioned to activate in response to odours (Russell *et al.*, 1984) or light and sound clues (MacQueen *et al.*, 1989). The prevalence and physiologic and pathophysiologic consequences of conditioned mast cell responses in the gastrointestinal tract are unexplored.

Similarly, the frequency or significance of other systemic neuroendocrine regulatory events which might influence mast cell or other inflammatory cell function in the gastrointestinal tract are unknown. For example, recently we have shown that the superior cervical ganglia modulate the release of an immunomodulatory factor(s) from the submandibular glands, which in turn impacts dramatically on pulmonary and other inflammatory

events associated with life-threatening antigen challenge in a sensitized host (Ramaswamy *et al.*, 1990; Mathison *et al.*, 1992). Intestinal inflammation is a large part of the pathogenesis in this rat model, but the overall relevance of the sympathetic trunk–submandibular gland axis, and the mediators involved, in the modulation of inflammatory events is not evident. It is worth additional study because our recent observations establish that the pathogenesis of endotoxic shock is also modulated by this axis (R. Mathison, A.D. Befus and J.S. Davison, unpublished).

Finally, in the bidirectional interactions between mast cells and the nervous system, the role of mast cell products in neuronal functions in the gastrointestinal tract and elsewhere must be dissected carefully. Firstly, the relevance of studies which identified that mast cells contain VIP (Cutz *et al.*, 1978) has not been established. Observations that mast cell activation can modify local neurotransmission and that this may involve mast cell-derived PGD_2 and perhaps other mediators must be explored in greater detail (Undem and Weinreich, 1989). Furthermore, the very interesting results from Caughey and others (see Caughey, 1990) that chymase and tryptase from mast cells can degrade selected neuropeptides and influence the overall tissue response to released tachykinins and VIP, must be investigated in the context of gastrointestinal pathophysiology, especially given the mast cell subtype specific distribution of some of these enzymes.

5.4 OTHER CELLS

There is evidence that many other cell types are influenced by neuropeptides (e.g. Bar-Shavit and Goldman, 1990; O'Dorisio, 1990). Many of the studies have focused on substance P, particularly because capsaicin has been used widely to deplete substance P from primary afferent nerves and then to evaluate responses in the face of such deficits (e.g. Donnerer *et al.*, 1990). Obviously, studies *in vivo* which have used these approaches involve complicated neuroregulatory pathways and the interpretation of the results is often difficult. However, from numerous studies it is clear that levels of substance P are elevated in inflammatory sites such as arthritic joints (e.g. Marshall *et al.*, 1990) and during the course of experimentally induced exudates (Tissot *et al.*, 1988).

In addition to the activation of lymphocytes and mast cells, substance P can stimulate the production of IL-1, IL-6 and TNFα by macrophages (Lotz *et al.*, 1988) and the responsiveness of neutrophils to FMLP and C5a can be potentiated (Perianin *et al.*, 1989). Administration of substance P *in vivo* induces platelet aggregation, neutrophil margination and migration in tissues (Ohlen *et al.*, 1989). The spectrum of actions of this and other neuropeptides must be catalogued and attempts made to dissect their relevance *in vivo*. Furthermore, the cellular origins of these peptides must be explored in the context of specific inflammatory events, because in addition to their potential neurogenic origin, peptides are also found in inflammatory cells (e.g. VIP in mast cells, Cutz *et al.*, 1978; substance P in eosinophils, Weinstock and Blum, 1989).

6. *Integration of Intestinal Neuroimmunology*

In the study of the conceptually simplistic, but in practice highly complex, interactions among the immune, nervous and endocrine systems in the gastrointestinal tract, a patient reductionist approach will be important in the expansion of our knowledge base. The reciprocity of interactions is beginning to be appreciated, but to date the focus has been largely on the neuromodulation of intestinal immune and inflammatory responses (e.g. Stead *et al.*, 1991a), on the neuromodulation of immune-mediated responses such as epithelium Cl^- secretion (Castro, 1989, Powell, 1991), or the changes in the nerve–smooth muscle unit in intestinal inflammation (Collins *et al.*, 1991). Detailed mapping of the pathways and the spectrum of potential responses must occur in the coming years.

The reciprocity of interactions may arise at the local level, between the specific cells of the mucosal immune system and the enteric nervous system. Alternately, the interactions may entail central or peripheral interactions at any level within the signalling of the hypothalamic–pituitary–adrenal axis (Bateman *et al.*, 1989). For example, in studies of streptococcal cell wall-induced arthritis in Lewis rats, Sternberg *et al.* (1989) demonstrated that these susceptible animals exhibited a hypothalamic defect in synthesis and secretion of CRF. It is interesting that conditioned colonic hypermotility could be attributed to the central release of CRF and was mediated through the autonomic nervous system (Gue *et al.*, 1991). Perhaps conditioned gastrointestinal immunophysiology involves similar pathways.

In the Obese strain of chickens that develops spontaneous autoimmune thyroiditis in association with an endogenous virus, there are reduced levels of active serum corticosterone, and a relative unresponsiveness of the hypothalamic–hypophyseal axis to stressful stimuli such as antigenic challenge (Kroemer *et al.*, 1988). Whether the pathogenesis of any enteric infection or chronic inflammatory condition such as IBD might encompass abnormalities in such neuroimmune regulatory pathways remains to be determined. Clearly, the neuronal damage documented in IBD and the evidence of ongoing modelling of the enteric nervous system suggest that compromises of such pathways may be relevant in some conditions.

Just as gastrointestinal neurophysiologists are carefully

mapping the neuronal pathways of centrally mediated responses such as acid secretion, mucosal immunologists must team with neurophysiologists to map peripheral and central pathways that regulate immune and inflammatory events in the mucosa and other layers of the gastrointestinal wall. With these basic approaches, employing reductionist and more holistic experimental strategies, the next decade promises to be a period of enlightenment for those interested in the physiology of the normal and diseased gastrointestinal tract.

7. *References*

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