Mast Cell-Nerve Interactions

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Mutual associations between nerves and mast cells have been observed in normal conditions and in pathological ones such as human irritable bowel syndrome, atopic dermatitis, interstitial cystitis, and more. Here we review the recent literature in this field, putting emphasis on the enteric, skin, and urinary systems, and the pathophysiologival implications of this interaction in them.

Mast cells (MCs) are ubiquitous in the body, mostly perivascularly and often close to neurons. They originate from the bone marrow as undifferentiated cells that enter specific tissues and mature under microenvironmental influences, such as those of the well-characterized stem cell factor (or c-kit ligand) and nerve growth factor (NGF) or those of interleukin (IL)-3, IL-4, IL-6, and IL-9. There are two primary MC subtypes that differ markedly in their histochemical properties, types of granule-associated proteoglycans, content of neural proteases (MCPs), and cytokine profile. These subtypes are the typical connective tissue MC (CTMC), which is mostly implicated in the pathology of the skin and lungs, and the atypical mucosal MC (MMC), which is found mostly in the bladder and the gastrointestinal tract. A third less-common subtype, the brain MC, displays the staining characteristics of CTMC but the ultrastructural appearance and secretory pattern of MMC. In this context, it is important to note that our present understanding of MC subtyping through molecular markers may have to be revised in light of recent evidence that both their proteoglycan and protease profiles can be altered over a short period of time and in a precisely regulated manner. Though the reasoning for this remarkable phenomenon remains to be elucidated, it is likely to serve important functional purposes. Friend and colleagues (6) have recently demonstrated a restricted time- and strata-dependent expression of multiple members of MCPs (both subfamilies) in jejunal MCs from Trichinella spiralis-infected BALB/c mice. Since each of these expressed MCPs exhibited specificity to a particular target proteoglycan, this group suggested that the ability of the MC to change its granule protease might have a functional role in inflammatory processes. Consequently, it is only reasonable to speculate that this protease-related phenomenon might have significant indirect implications in MC-nerve interactions as well.

MCs are best known for their involvement in type I hypersensitivity, allergic or anaphylactic reactions, in which IgE attaches to the fragment constant of ε receptor I on the MC surface and, upon bridging by specific antigen or other molecules, triggers a cascade of reactions that lead to their degranulation. MCs secrete numerous vasoactive, nociceptive, and proinflammatory molecules (mediators) in response to this and many other immunomolecules (e.g., anaphylatoxins, leukocyte-derived factors, and cytokines) as well as to various stimuli such as neuropeptides, drugs, lectins, radiation, toxins, and certain viruses. These mediators are grouped into two functional subgroups: 1) granule-stored preformed molecules such as histamine, heparin, chemotactic substances, cytokines, kinins, proteases, and peptides and 2) short-lived molecules that are synthesized de novo upon trigger perturbation of the cell membrane, such as leukotrienes, prostaglandins, and platelet-activating factor. MCs do not necessarily secrete all of their mediators by the massive degranulation and expulsion of granule contents (exocytosis) typically seen in anaphylactic reactions but can also secrete them by a more subtle process of intragranular changes. This largely obscure process then leads to the differential or selective release of distinct secreted molecules. This mode of secretion has been reported in various human disorders, for example interstitial cystitis (IC) of the urinary bladder, irritable bowel syndrome (IBS), migraines, and multiple sclerosis (15).

MCs are also able to take up, store, and release a variety of biogenic amines through which it is hypothesized they participate in inflammatory reactions. Furthermore, MC mediators can sensitize sensory neurons, which further activate the MC by releasing neurotransmitters or neuropeptides (e.g. neuropeptides, somatostatin, substance P, and acetylcholine). For instance, Nechushtan and colleagues (13) demonstrated the expression of several acetylcholinesterase isoforms, forms of an essential action-terminating enzyme in the cholinergic cleft, in both mouse and human MCs, revealing another probable mode of molecular communication between MCs and neurons. They postulated that this enzyme is part of an interrelated regulatory system and is responsible for the degradation of the prodegranulatory nerve-derived acetylcholine. This hypothesis is in analogy with other known nonneuronal acetylcholinesterase-synthesizing cellular models. In general, MC-nerve associations have been found in the myocardium (7), diaphragm (2), brain (15), gallbladder, ileum, mesentery, and skin of a variety of animals at both the anatomic and molecular levels, pointing to their fundamental interdependence.
Functionally, these interactions make a major contribution to the neurogenic inflammation process, mainly through MC-derived vasodilatation, leukocyte infiltration, and protease tissue damage. These findings, together with the noted higher incidence of affective disorders in atopic individuals, suggest that activated MCs play a central role in various syndromes having immune, neural, and apparently endocrine components that are augmented under stress.

Perhaps the best known example of all elementary MC-nerve molecular interactions is presented by NGF. Addition of this neuropeptide to a suboptimal dose of IL-3 affects in vitro cultured spleen-derived MC proliferation, differentiation (into CTMC-like form), and histamine release through a NGF receptor found on the MC surface. To tackle the molecular aspects of these NGF effects on MC, Jippo and colleagues (9) investigated the observed poor response of W/Wv mouse-derived cultured cells to NGF, manifested both by their very low numbers and phenotypic abnormalities. Having found an extremely low 125I-NGF binding capacity of these cells, they investigated the levels of p75 mRNA, one of the two low-affinity NGF receptor peptide constituents. These were found to be significantly lower than in normal CTMC-like cells, thus explaining the low level of proliferation and the abnormal phenotype of these cells. In a recent correlative work, this group elegantly confirmed their former hypothesis by showing that p75 was directly transcribed by the MC microphthalmia transcription factor (mi) and that overexpression of mi cDNA in these mutated cells completely normalized both p75 mRNA expression and the poor response to NGF. Thus it seems likely that there is a crucial in vivo dependence of MCs on NGF with important implications for both proliferation and differentiation.

Ingestion and inhalation represent the major routes of antigen intake into the body. Thus it should come as no surprise that, with a complement of plasma cells numbering ~10^10 cells/m^2, the intestine is found to be the largest lymphoid organ in the body. Given the corresponding enormity of the enteric nervous system, which comprises ~10^9 cell bodies, containing a plethora of “classic” and putative peptidergic neurotransmitters, there is ample opportunity for immunocytes and neurons to interact. Indeed, this is found to be the case. The innervation of gut-associated lymphoid tissue, as well as MCs in various mammalian intestines, have been investigated using histochemistry and immunocytochemistry, resulting in concrete anatomic and molecular evidence for their mutual interactions. For example, preliminary studies with the W/Wv MC-deficient mouse have provided data to support this hypothesis. These mice have demonstrated a 50% decrease in enteric nerve stimulation compared with normal (MC-containing) mice, and this response was normalized by MC reconstitution with bone marrow precursor cells (12).

Moreover, the response of normal mice to the stimulation of their enteric nerves was shown to be partially blocked by pretreatment with antagonists of MC mediators (e.g., antihistamines). These results, like many others, suggest that this interaction has an important homeostatic role in the regulation of gut physiology and its response to antigen. According to this concept (12), enteric MCs actually act as antigen or sensory receptors that can communicate microenvironmental information to the surrounding peripheral nerves. This transfer of information can then result in either 1) a change in the nerve function and induction of an axonal reflex in response to antigen, 2) feedback modification of MC function, or 3) relay of these data to the central nervous system. Several recent papers have explicitly demonstrated such relations in the enteric system, thereby corroborating this hypothesis.

Spatial associations in biological systems are often indicative of functional interactions. We will now describe the results of three anatomic studies showing such spatial associations in gastroenteric pathology. In both IBS and inflammatory bowel disease, the number of MCs is known to be much higher than in the normal colon and almost as high as in systemic mastocytosis. Interestingly, patients having IBS often also suffer from urological dysfunction, and IBS occurs quite frequently in patients with IC (see below). In both of these cases, MC-nerve correlation was given solid pathological basis by Theocharides (15), who histochemically detected many close MC-neuronal associations [mucosal and submucosal substance P (SP)-positive nerve fibers in juxtaposition to MCs] in the colon and the bladder of a female patient having both IC and IBS.

Stead and colleagues (12) illustrated over a decade ago that ~67% of MCs in the jejunum of nematode (Nippostrongylus brasiliensis)-parasitized rats were nonrandomly juxtaposed to SP and/ or calcitonin gene-related peptide (CGRP) nerve fibers, with an additional 20% of MCs located within 2 µm of the nerves. Moreover, in 4–8% of these associations, actual membrane-to-membrane contacts were observed in which the nerve segment contained dense core vesicles typical of peptidergic nerves. Similarly, a later work by this group found such a MC-nerve anatomic association in the human gastrointestinal context as well. With the use of electron microscopy, 47–78% of human MCs examined were found to be associated with nerve fibers, with the highest levels documented in the appendix.

The specificity of MC-nerve spatial relations has been demonstrated in vitro as well, using rat basophilic leukemia (RBL) cells (analogous to MMCs) in coculture with murine superior cervical ganglia. After 18 h of coculture, neurites extending from the ganglia had formed intimate associations with the RBL cells. These associations ranged in nature from juxtaposition to almost complete engulfment of the nerve within the RBL cell. Moreover, when nerves were replaced with fibroblasts or glial cells, no associations between RBL cells and the other cell types were recognized, demonstrating the specificity of the association. These three studies are just three examples among many such studies presenting similar anatomic associations.
In contrast to the above studies, Atwood and colleagues (3) found far fewer MC-capsaicin-sensitive nerve associations than blood-nerve associations in the guinea pig ileal submucosa. Two intriguing explanations were suggested for this opposing finding. First, in contrast to the inflammatory models in which mastocytosis took place, there was no increase in the number of MCs in this work. This would suggest that the association between MCs and nerves occurs primarily when there is a significant increase in the number of MCs. The second explanation given was that in the inflammatory milieu capsaicin-sensitive nerves were altered in some way, such as an increase in the release of neuropeptides, or that the properties of the MCs were altered, resulting in a functional connection between these two cellular pathways.

We will now describe the molecular evidence supporting MC-nerve interaction in the enteric system. Histamine, one of the main mediators in MCs, was shown to have dual contradictory effects on the enteric nerves of the guinea pig (12). When applied to the myenteric and submucosal plexuses, histamine acted on the soma of the nerves to produce long-lived excitations (>4.5 h). However, when introduced to their nicotinic synapses it had the opposite effect, in that it significantly suppressed information transfer between nerves. It is now largely accepted that these findings are due to histamine binding to two different receptors, H2 and H3, propagating opposite signals that are localized in spatially distinct areas on the nerve membrane. It has been suggested that the inhibitory response, which is mediated by H3 receptors, may represent a braking mechanism to prevent prolonged excitation due to H2 activation. Mechanistically, it is now thought that histamine released from MCs suppresses the normal intestinal digestive and transit program in the gut. Moreover, it appears to selectively upregulate the microcircuits of an “alarm” pattern, leading to increased water content and propulsive force in an effort to create a “washer/sweeper” mechanism aimed at the eradication of antigen from the gut lumen and epithelial surface.

Kreis and colleagues (10) have recently described intestinal afferent nerve sensitivity to histamine in the rat. They elegantly demonstrated that histamine administration evoked a powerful and, in most cases, biphasic increase in discharge frequency. Furthermore, this response was accompanied by a decrease in arterial blood pressure and by a general (mostly) biphasic increase in intestinal pressure. These effects were completely abolished by pyrilamine, a histamine H1 receptor antagonist, whereas the administration of ranitidine and thioperamide, H2 and H3 receptor antagonists, respectively, had no effect. It is important to note that this selective histamine action on H1 receptors in the rat enteric system is in contrast to the findings of both H1 and H2 involvement previously reported in the cat, indicating a species-specific mode of action.

Histamine is not the only MC-derived mediator taking part in the interaction between MCs and the enteric nervous system. As mentioned above, a whole array of cytokines are produced by the MC. These cytokines play a central role in this interaction, although data are only beginning to accumulate on their specific abilities to affect neuronal function. For instance, it has been found that administration of IL-1 leads to increased norepinephrine metabolism in the rat hypothalamus, enhanced neuronal survival in cell culture, and the promotion of mRNA transcription of both NGF and its receptor in the nerve cell. Numerous other cytokines [e.g., tumor...
necrosis factor (TNF-α), IL-6, and leukemia inhibitory factor] have also been shown to act on enteric nerve cells in various ways, but we are still far from a thorough understanding of this particular field. Once these cytokine-nerve interactions are fully described on the molecular level, the potential of immune cells to influence nerve growth, development, and function will most certainly be understood.

Before moving to the description of other systems, it is important to note that MC-enteric nerve interaction is doubtless not a one-sided relationship and that the flow of information is bidirectional. Indeed, the consequences of nerve stimulation and mediator release on MC function have caught researchers’ interest in recent years. Investigations carried out in the rat have shown that electrical field stimulation of the ileum results in increased histamine release and concomitant MC degranulation. Furthermore, it is now known that the neuropeptides neurotensin, somatostatin, SP, and vasoactive intestinal peptide (VIP) cause CTMC degranulation and histamine release. It is both important and interesting to note that of these neuropeptides, only SP is found to evoke histamine release from MMCs. This, once again, highlights the heterogeneous nature of the MC, which shows species- and tissue-specific differences in size, mediator content, and susceptibility to activation signals. Moreover, dealing specifically with SP-MC interactions brings to the fore another fascinating key point. Since no SP (tachykinin) receptor has been found as yet on the MC membrane, it has been suggested that SP and other amphiphilic neuropeptides affect MCs by direct activation of their plasmalemma G proteins. Thus it is suggested that the effects of SP on MCs may represent an adaptive response to environmental changes that is not restricted by the need for synaptic junctions or membrane-bound receptors. Moreover, histamine and SP release from MCs and nerves, respectively, point to the probable existence of autocrine and paracrine control loops in the interplay between these two cells.

The involvement of neurotensin (NT), another important neuropeptide, in colonic inflammation has recently been investigated by Castagliuolo and colleagues (5). They used a rat toxin A-treated colonic loop (bringing about MMC activation, copious fluid secretion, and inflammation) as a model for acute colon inflammation in the rat. According to their model, the inflammatory mediator-induced NT upregulation overcomes the opposing action of NT-mediated NTR down-regulation, thus maintaining mutual interregulation. These innovative results display explicitly for the first time the central roles played by NT (and NTR) in the pathophysiology of acute colon inflammation in the rat.

We will now deal with the second major physiological system in which close contacts between MCs and nerves have been described and attributed significant functions: the skin. MC-nerve interaction in the skin has been assumed for a long time, yet both its scale and significance in normal and pathological states are only now beginning to be fully understood. It is important to reiterate that, in the dermal system, we are dealing with the connective tissue subtype CTMC, in contrast with the former gut-associated MMC.

The human dermis, like many other mammalian ones, is richly innervated by an elaborate plexus of myelinated and unmyelinated axons responsible for conduction of both sensory and autonomic impulses. Using a novel in situ histochemical method, Botchkarev and colleagues (4) showed that these nerve fibers formed close (<2 µm) contacts with MCs in mouse skin, confirming the results of many other studies using various different methods. These associations were further shown to be highly selective for distinct nerve fiber types (SP and/or CGRP), with a striking hair-cycle-dependent regulation, relating to both MC numbers and activity, as well as to nerve fiber topographical innervation. These nerve fiber endings, along with other skin cells (e.g., keratinocytes, different immunocytes, microvascular endothelial cells, and fibroblasts), are known for their ability to secrete various neuropeptide mediators into the tissue environment. These neuropeptides include tachykinins [SP, neurokinin (NK) A and B], CGRP, somatostatin, VIP, and others. They constitute the very heart of MC-nerve interaction through their direct receptor-mediated effects on surrounding MCs and their fundamental involvement in generating the subsequent neurogenic inflammation. To address the key role of these mediators in the context of this review, we will briefly highlight two of these neuropeptides: SP and CGRP. For more extensive reading, please refer to the recent study by Scholzen and colleagues (14).

SP, a common neurokinin ligand, is the well-characterized classic mediator of the “triple dermal response,” consisting of erythema, edema, and itching. Released by sensory neurons, it mediates its dermal action via two designated pathways. The first, MC-dependent pathway involves release of histamine and TNF-α through the NK1 receptors present on MCs, which in turn cause vasodilatation by acting on vascular smooth muscles (via H1 receptors). The second, MC-independent pathway involves either SP or NKA, which can exert their vasodilatory effects directly through NK1 receptors present on vascular endothelial cells. In addition to these dermal effects, SP is known for its ability to modulate the cytokine expression pattern of MCs (and keratinocytes) manifested by

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selective induction of TNF-α mRNA and protein synthesis in the murine MC. Like SP, CGRP is assumed to be another central coplayer in the dermal MC-nerve interaction. Often co-localized either with SP or somatostatin, CGRP is a 37-amino acid-amidated peptide that is considered to be one of the most abundant neuropeptides in the skin. Although its central involvement in MC-nerve associations remains to be demonstrated conclusively, there are currently two distinct lines of supporting evidence, one anatomic and one deductive. First, CGRP has been histochemically detected in unmyelinated sensory nerves in contact with dermal MCs in both humans and rodents. Second, on the basis of analogy with CGRP-stimulated histamine release displayed by dura mater MCs, one could deduce that dermal MCs might also be susceptible to such a CGRP stimulus.

Another general aspect of immense importance in this dermal neuropeptide-MC interaction concerns the removal of these neuropeptide mediators, thus terminating their action. Although neuropeptide reuptake via pinocytosis occurs and neuropeptides are also internalized via receptor-mediated endocytosis, the preferred and most commonly used mechanism of agonist removal seems to be their degradation by several enzymatic neuropeptide-degrading enzymes. Within this context, it is easy to understand why it is currently speculated that localized MC-derived proteolytic enzymes, such as trypsin or chymase, that are able to efficiently degrade SP, NKA, NKB, CGRP, VIP, and others play an important role in regulating the neurogenic inflammation process.

Turning to the clinically-oriented aspects of MC-nerve dermal interaction, we start with a novel and rather intriguing hypothesis involving the essential role of MCs in wound healing, a theory that is currently finding growing support (8). According to this hypothesis, neural modulation of the MC influences wound repair in practically all tissues and organs, yet it is most notable at interfaces of the organism and the environment, i.e., the skin and mucosa of the respiratory and gastrointestinal tracts. MCs are known to be degranulated by wounding trauma, either through an immediate sensory nerve-stimulated response or through their direct physical wounding trauma, which then leads (via an axon reflex) to the release of neural preformed mediators into the local connective tissue environment. The effects of these mediators at the locality of the damaged tissue surface are likely to be minimal, yet their actions on the adjacent connective tissue are apparently crucial to the recruitment and delivery of cellular and soluble effectors to the injury site. Histamine, for example, appears to have dual positive H1 receptor-mediated effects on the early phases of wound healing, i.e., vasodilatation and increased vascular permeability. Correspondingly, heparin is suspected to be involved in both the sealing of the wound’s immediate superficial layer and the maintenance of perfusion in the immediate adjacent connective tissue, where the clotting potential of the nearby vasculature would otherwise harm cell influx, nutrition, and wound healing. Central effects on latter phases of wound healing and different cell types (fibroblasts and endothelial cells), as well as actions of other MC mediators, are also suggested as parts of this appealing hypothesis. For instance, basic fibroblast growth factor, which has only recently been shown to be produced and secreted by cutaneous MCs in significant amounts, was shown to induce protease elaboration, chemotaxis, and mitogenesis on endothelial cells. Likewise, IL-4 (the “fibrogenic cytokine”) was found to increase synthesis of collagen types I and III, laminin, and fibronectin. Furthermore, and possibly most important, there are the contributions of MC-derived TNF-α and transforming growth factor-β, which in an IgE-dependent modus were shown to stimulate fibroblasts to produce collagen. This transient and marked increase of pro-1 collagen mRNA levels in murine dermal fibroblasts was found to be exclusively MC dependent and occurred within a few hours of injury, even before inflammatory cells were recruited to the wound. Moreover, MC-derived TNF-α was recently attributed another important indirect role in the development of the dermal inflammatory process. Apparently, axons within the murine epidermis, when activated chemically with capsaicin, promote neuropeptide release (e.g., SP) to the immediate vicinity of perivascular dermal MCs. This then leads to their degranulation and thus to an increased synthesis and display of endothelial adhesion molecules (E-selectin) and to subsequent stimulation of cytokine-mediated MC-endothelial interaction as an essential part of the proinflammatory cascade.

Perhaps the most tangible and documented form of dermal disorder involving MCs is atopic dermatitis (AD). On the basis of the manifestation of emotional stress-induced onset and exacerbation of AD, altered patterns of cutaneous innervation, and abnormal expression of neuropeptides observed in the lesional skin, it has been suspected for quite some time that AD comprises an additional neural constituent. This interaction between the nervous system and the immune system leads to the phenomenon of neurogenic inflammation. According to current understanding, histamine released from MCs is thought to amplify skin neurogenic inflammation. In turn, some types of neuropeptides that are released from stimulated sensory nerve endings induce chemical mediators from skin MCs, which lead to the cell-mediated inflammatory skin condition. A recent publication by Masahiko and colleagues (11) strengthens this idea of the essential involvement of dermal MC-nerve interactions in AD even further. Ultrastructural investigation of the morphological effects induced by cyclosporin A (CsA) administration on this interaction clearly demonstrated that it induces an apparent altered neural innervation and neuropeptide expression in lesional skin AD. Although the exact mechanism has not been sufficiently elucidated, CsA has been shown to exert its therapeutic effects by specific inhibition of MC degranulation and by observed alterations of the topographical relationship between MCs and cutaneous lesional skin mentioned above. Previous works had already shown CsA to inhibit MC degranulation, but this group demonstrated in addition that CsA did not inhibit
SP-induced MC histamine release, suggesting that the inhibitory effect of CsA is stimulus dependent. Moreover, it is clear from this work that, although a month of CsA administration resulted in indisputable clinical and histological improvements, MC numbers in AD lesions remain unchanged.

The urinary bladder is the third site to be discussed here in which MC-nerve interactions have been ascribed to IC, another rampant pathological disorder. This sterile condition, which occurs primarily in women (90% of 100,000 cases in the US), is characterized by frequency of urination, nocturia, suprapubic pain, and dyspareunia and is known to be exacerbated during ovulation and under stress, hence implicating neurohumoral processes. The most prevalent etiological theories to explain IC pathophysiology appear to be the somewhat disputed altered bladder lining and the unequivocal increase in numbers of activated MCs in the diseased bladder. This latter typical MC proliferation, which plays a major role in this disease, has been documented repeatedly by ultrastructural criteria and confirmed further both by increased levels of methylhistamine and the unique MC tryptase in 24-h urine of IC patients. In addition, an array of numerous cumulative in vivo and in vitro studies independently corroborated MC-nerve central involvement in this disorder. For example, bladder MCs were shown to be more responsive to a number of secretagogues, such as IgE-antigen, SP, and acetylcholine. Likewise, investigating stress effects on rat bladder MC degranulation, Theoharides and colleagues (1) have recently demonstrated that intraperitoneal administration of the NTR antagonist SR-48692 1 h before stress drastically decreased MC degranulation rates (66.5% inhibition), proving the fundamental involvement of NT. Besides these, biopsies taken from diseased bladders of IC patients suggested the general notion by which release of neuropeptide Y and CGRP, and increased anatomic interconnections between SP nerve fibers and MCs (in the submucosa). In line with these findings and many others, this group proposed the general notion by which release of neuropeptide Y and CGRP, and increased anatomic interconnections between SP nerve fibers and MCs (in the submucosa). In line with these findings and many others, this group suggested the general notion by which release of neurotransmitters under stress (like the above NT and SP) may lead to local MC secretion of vasoactive, proinflammatory, and nociceptive mediators, which could contribute to the clinical symptoms of IC. Mechanistically, it is proposed that defects at the protective glycosaminoglycan layer of the bladder could allow potential MC secretagogues (e.g. allergens, food dyes, preservatives, hormonal factors, toxins, and others) to penetrate the normally impermeable bladder lining and activate bladder MCs. Although specific MC proliferation or migration factors have not been identified in IC as yet, it is assumed that damaged urothelial cells, as documented for epithelial cells, produce cytokines, such as stem cell factor and IL-3, and thus lead to MC proliferation and IC.

The nature of the mutual interactions between MC and nerves has only recently begun to be explored. In this review, we have placed special emphasis on the complex molecular network that takes part in this association. Further study of this system at the molecular, cellular, and tissue levels will probably provide us with better understanding of the observed involvement of MCs in several severe human clinical disorders. Moreover, by learning how to modulate these interactions, we will probably be able to gain better control over these important diseases.

References