The Mast Cell-Nerve Functional Unit: A Key Component of Physiologic and Pathophysiologic Responses

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Abstract
A key characteristic of mast cells appears to be an ability to span the division between nervous and immune system. Indeed, much of our understanding of the bi-directional relationship between the nervous and immune systems has come from the study of mast cell-nerve interaction. Although differences in species have been reported, morphologic as well as functional associations between mast cell and nerves are found in most tissues in many mammalian species, including humans. These interactions are involved in the regulation of physiologic homeostatic processes as well as in disease mechanisms. Here we discuss the influence of cholinergic and sensory neurons on mast cells as well as the importance of mast cell nerve interactions at specific tissue sites, including the brain.

Mast Cells

Mast cells are immunocytes with secretory functions that act locally to maintain tissue integrity, local hemodynamics and tissue homeostatic mechanisms. Mast cells are heterogeneous and exhibit site-specific adaptations induced by micro-environmental signals that lead to selective expression of potential mast cell characteristics. This flexibility of phenotype has important functional implications and allows these cells to adapt to organ or tissue specific roles [1].

While best known for their role in allergic inflammation through the ability of allergens to cross-link antigen-specific IgE bound to the high affinity IgE receptor (FceRI) expressed on the cell surface [2] mast cells have been identified as having diverse physiologic roles. These roles range from providing innate defense against bacteria [3] and protection from the venom of bees and snakes [4] to participating multiple aspects of
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adaptive immune responses such as antigen presentation [5] and lymphocyte recruitment to draining lymph nodes [6], as well as downregulation of immune responses [7]. In recent years, the pathogenic roles of mast cells have been extended to include not only allergic diseases and helminth infection, but also autoimmune diseases such as experimental allergic encephalomyelitis [8], rheumatoid arthritis [9], allograft tolerance [7], angiogenesis in tissue repair [10], and carcinogenesis [11].

A key characteristic of mast cells appears to be an ability to span the division between nervous and immune system with the cells exhibiting variably functional aspects of both systems [12]. Indeed, much of our understanding of the bi-directional relationship between the nervous and immune systems has come from the study of mast cell-nerve interaction, often considered as the archetype of neuroimmune communication. Mast cells can be activated by a range of neurotransmitters and reciprocally a variety of molecules, including histamine and serotonin, are synthesized and released by mast cells to influence neuronal activity [13] while mast cell-derived cytokines, including TNF, and growth factors, such as NGF, lower the threshold for activation of local neurons and promote nerve fiber growth [14–16].

While mast cells are distributed widely throughout the body in connective tissue and at mucosal surfaces they are concentrated at interfaces with the external environment, near blood vessels, lymphatic vessels, and nerve fibers [1]. Positioned at these strategic locations, mast cells act as sentinels of the immune system, protecting against invading microbes and signaling environmental changes or immune challenges to other cells involved in physiological and immunological responses.

There is anatomical evidence for mast cell associations with peripheral myelinated and unmyelinated nerves [17–19]. Close apposition of mast cells and neurons containing substance P, CGRP or both have been described in the rat and human gastrointestinal tract, the rat trachea and peripheral lung, the urinary bladder and several other tissues [20–22]. These interactions underlie the classical inflammatory axon reflex where antigen or noxious stimuli causes stimulation of sensory c-fibers that in turn, through collateral axons, provide an efferent route for the lateral spread of inflammatory signals [23].

While exocytosis is the most obvious event associated with secretion of the mediator molecules contained in granules the function of mast cells in health and disease often involves more subtle activities and these cells have been increasingly implicated in inflammatory processes in which degranulation is generally not observed. Mast cells can undergo ultrastructural alterations of their electron-dense granular core that are indicative of secretion but without degranulation, a process termed piece-meal degranulation, in which even molecules stored within the same granule can be secreted in a discriminatory pattern [24].

Mast cells in close proximity to unmyelinated nerve fibers have been observed to contain granules showing ultrastructural features of activation or piecemeal degranulation that have been associated with differential secretion. Histamine content of intestinal tissue increased flowing vagal stimulation without notable degranulation of...
mast cells [25, 26]. Selective secretion of IL-6 from mast cells appears to be distinct from degranulation and may contribute to the development of inflammation [27]. Serotonin can be released independently from histamine [28] and differential synthesis and release of arachidonic acid metabolites prostaglandins and leukotrienes have also been reported [29].

Mast cells are derived from progenitor cells that translocate from the bone marrow to tissue sites where they locally undergo differentiation into mature forms [30]. Studies have identified the remarkable facility of mast cell populations to respond to changes in the environment by significant alterations in multiple aspects of their phenotype, including morphology, mediator content, degranulation pattern and proliferative potential [1]. Consequently, mast cells can be divided into various subpopulations with distinct phenotypes. In rodents, mast cells are often classified broadly, based on tissue location, as mucosal mast cells (MMC) or connective-tissue type mast cells (CTMC) [31, 32]. However, mast cells possess a remarkable degree of plasticity and even apparently fully differentiated CTMC will transform their phenotype to that of MMC if transplanted into a mucosal environment [33]. Alternatively, and most often in human tissue, mast cells can be classified based on the protease content of secretory granules that differ between tissues, i.e. MCt for cells containing only tryptases and MCtcp for those containing tryptase and chymase [30]. Tryptase is present in all mast cell subtypes and can activate cells through cleavage of protease-activated receptors (PAR) [34]. Proteases regulate neurons and glia in the central nervous system by cleaving PAR [35]. Furthermore, tryptase has been shown to cleave PAR2 on primary spinal afferent neurons, which causes the release of substance P, and CGRP and sensitization of co-expressed TRP channels that together cause plasma extravasation, amplification of inflammation and thermal and mechanical hyperalgesia [36]. Purified tryptase stimulates calcium mobilization in myenteric neurons [37] presumably through PAR2 because activation of PAR2 with trypsin or peptide agonists strongly desensitizes the response to tryptase. Mast cell proteases have also been demonstrated to degrade nerve products by enzymatic cleavage and thus may act to limit the effects of neurogenic signals [38]. It is proposed that IgE-mediated mast cell activation results in the release of tryptase from intracellular secretory granules, and the tryptase activates PAR2 on sensory neurons to stimulate the release of substance P and CGRP [39]. These neuropeptides induce the further activation of mast cells as well as inflammatory response such as arteriolar vasodilation and increased blood flow.

**Molecules Involved in Mast Cell Nerve Attachments**

Co-culture systems of mast cells and neurons have been particularly informative regarding the molecules involved in the formation of a neuroimmune synapse.

The adhesion molecule, cell adhesion molecule 1 (CADM1) is localized on both sides of most synapses in the brain and functions as a homophilic adhesion molecule.
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spanning the synaptic cleft in the nervous system. CADM1 was also found to mediate mast cell/fibroblasts adhesion [40] and was subsequently demonstrated to be critical to mast cell-nerve interactions [41].

CADM1 is highly localized at the contact site between mast cells and neurites and the attachment of mast cells is dose-dependently reduced in the presence of an anti-CADM1 blocking antibody. Mast cells lacking CADM1 attach poorly to neurites, while attachment is significantly enhanced with ectopic expression of this adhesion molecule [41, 42].

The neuron-induced activation of CADM1-deficient mast cells is also markedly reduced while the response rate of CADM1+ mast cells is decreased dose-dependently in the presence of an anti-CADM1 blocking antibody. Furthermore antagonism of the substance P receptor, NK-1, attenuated the CADM-1 deficient mast cell response to neurite activation at much lower concentrations than in wild type mast cells [41, 42].

These studies indicate that CADM1 is involved not only in the physical association between mast cells and nerves but also contributes to the functional relationship enhancing mast cells sensitivity to neuronal signals. Thus, it appears that CADM1 is involved not only in the physical association between mast cells and nerves but it also contributes to the functional relationship and to the development of a microenvironment that enhances mast cells sensitivity to neuronal signals.

Indeed, variable splicing of CADM1 in neurons may be one of the molecular mechanisms that fine tune the nerve-mast cell interaction. Recently, BMMC were found to adhere to neurites expressing CADM1d splice variant more firmly than those expressing the CADM1b variant [43]. Furthermore, neuritis attached to CADM1c expressing mast cells via CADM1d were more responsive to stimulation by histamine than neurites binding with other CADM1 isoforms [43]. Therefore, it appears that CADM1d is a specific neuronal isoform that enhances nerve-mast cell interaction. Interestingly CNS neurons increase expression of CADM1d during maturation and this may influence the changes in mast cell distribution and perhaps function during CNS development.

Neurons and bone marrow-derived mast cells also express N- and E-cadherins. When in monoculture these adhesion molecules are concentrated primarily in the cytoplasm of BMMC [44, 45], but in the presence of neurons N-cadherin but not E-cadherin localizes to sites of interaction at the plasma membrane [45]. Similarly, β-catenin, a protein that associates with cadherins in the cytoplasm, also accumulates at the plasma membrane of neurite attached BMMC suggesting that N-cadherin is also involved in the contact formation between nerves and mast cells.

Nerve Growth Factor

Nerve growth factor (NGF) receptors are found on mast cells and act as autoreceptors regulating NGF synthesis and release by the cell [15, 46]. The NGF produced by
mast cells can act on neurons to induce the expression of neuropeptides and lower the threshold of firing [47]. NGF has also been shown to induce degranulation and histamine release from mast cells [48], while in vivo administration of NGF in neonatal rats causes a marked increase in size and number of mast cells in peripheral tissue [49]. Indeed, mast cell proliferation in response to NGF is partially mediated by mast cell degranulation [49].

Interestingly, NGF can have anti-inflammatory as well as proinflammatory effects depending on the situation and the concentration of the growth factor. Nasal treatment of mice with NGF was shown to induce airway hyperresponsiveness as measured by electrical field stimulation [50] and nasal treatment with anti-NGF prevented the development of allergen induced airway inflammation and hyperresponsiveness [51]. Conversely, there is evidence that the increased production of NGF in the central nervous system during brain disease such as multiple sclerosis can suppress inflammation by switching to an anti-inflammatory immune response [51]. Significantly, the injection of CD4+ lymphocytes transfected with the NGF gene, either before or after the induction of allergic encephalomyelitis, inhibited the onset of demyelination [52]. Furthermore, studies support a role for interaction between nerves and NGF secreted by mast cells in the intestinal lamina propria or epithelium. NGF is enhanced in IBD [53] as is the expression of the high-affinity NGF receptor, TrkA, gene [53] and overall it appears that NGF has a protective role in colitis [54].

Sensory Neuropeptides

Peripheral sensory nerves that are involved in pain, touch and temperature perception regulate inflammation locally through the release of neuropeptides. Peripheral neuropeptides that are known to regulate inflammation include substance P, calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP). The stimulation of peripheral nerves results in the characteristic features of local inflammation, including vasodilation, vascular leakiness, edema and pain.

In clinical dermatology and gastroenterology, it is acknowledged that peptide mediators released from cutaneous c-fibers and the enteric nervous system respectively give rise to neurogenic inflammation and underlie a range of conditions, including dermatitis, psoriasis, eczema, Crohn’s disease and colitis [55–58]. In asthma research there is also great interest in the role these peptides play in airway inflammation, mast cell activation, cytokine secretion, bronchial hyperresponsiveness, and mucoid secretion [59, 60]. In particular, great attention has been paid to the role of tachykinins such as substance P.

Substance P and the Functional Relationship between Neurons and Mast Cells

Substance P is an 11 amino acid peptide that acts principally at the neurokinin-1 (NK-1) G-protein coupled receptor. Substance P is generally regarded as pro-
inflammatory, stimulates secretion of TNF, IL-1, IL-2 and IL-6 from macrophages and T lymphocytes in vitro [61].

Substance P is perhaps the best-known and most studied neurotransmitter in relation to mast cell activation. In vitro, high (micromolar) concentrations of substance P induce mast cell degranulation while in co-culture system substance P from activated neurites induces Ca$^{2+}$ mobilization and degranulation of mast cells [62]. In addition to degranulation substance P also promotes production of lipid mediators such as prostaglandin D$_2$ and leukotriene C$_4$ and proinflammatory cytokines including TNF and IL-6 [63, 64].

Interestingly, low (picomolar or nanomolar) concentrations of substance P, that do not induce mediator release can increase cellular responsiveness to subsequent stimulus and so ‘prime’ the cell to degranulate with reapplication of a subthreshold dose [65]. This effect has great physiological significance as it likely underlies the ability substance P to activate mast cells at concentrations occurring in situ.

As described previously, the heterogeneity of mast cells is well reported and studies have described large variations in the susceptibility of mast cells to activation on challenge with neuropeptides in different tissues as well as within the same tissues in different species. Indeed, not all mast cells are activated by substance P and expression of functional NK-1 receptors appears to be dependent on microenvironmental factor. Bone marrow derived mast cells BMMC co-cultured with fibroblasts for several weeks can respond to substance P [66] and BMMCs cultured in the presence of IL-4 and stem cell factor gain sensitivity to substance P by expression of functional NK-1 receptors [67]. Bischoff and colleagues determined that human intestinal mast cells do not constitutively express NK1, NK2 or NK3 receptors [68]. However, when stimulated by IgE receptor crosslinking these mast cells expressed NK1 but not NK2 or NK3 receptors, again suggesting that specific tissue conditions such as allergic inflammation may lead to mast cell expression of NK1 [68].

Electron-microscopic studies of the rat basophilic/mast cell line, RBL-2H3, cultured with SCG neurons have indicated that bradykinin-induced activation of the neurons leads to marked morphological changes in the mast cells, including membrane ruffling, associated with cell activation and exocytosis of intracellular granules and are similar to that seen following antigen stimulation of these cells [69]. Membrane ruffling and concentration of CD63, a protein located on the granule and plasma membranes of basophils and mast cells, was observed on pseudopodial extensions of the mast cell in contact with activated neurites, but not on noncontacting pseudopodia [69]. Importantly, these morphological changes were dependent on NK-1 receptor activation indicating that neuron-derived substance P induces degranulation in associated mast cells. Furthermore, association of mast cells to neurons for several days can change phenotypic and functional characteristics of the mast cell, increasing granule contents and FcεRI expression [70, 71]. This functional relationship between mast cells and substance P containing sensory nerves is thought to play a critical role in the stress induced exacerbation of a number of inflammatory conditions [72, 73].
In rats, acute immobilization stress triggered mast cell activation and degranulation via substance P released from primary afferent sensory nerve fibers in the skin [72] and the bladder [21]. Joachim et al. [73–75] utilized a mouse model of allergic airway inflammation in a series of studies which provide compelling evidence that SP is key to stress-induced enhancement of antigen-induced airway inflammation. In this model, acoustic stress increased SP expression in lung tissue and mouse airway nerves as well as increased airway inflammation in an NK-1 receptor-dependent manner [75]. In addition, many of the immunological effects of stress in this model could be mimicked by the addition of exogenous SP [76].

This pro-inflammatory neuroimmunomodulatory role for substance P is also apparent in stress-induced exacerbation of atopic dermatitis. Acoustic stress was demonstrated to enhance the number of SP-positive nerve fibers in the skin of mice [77]. These nerve fibers were more frequently in contact with mast cells and this was associated with a significant increase in the number of degranulated mast cells [77]. Correspondingly, in a mouse model of atopic dermatitis stress enhanced eosinophil infiltration, epidermal thickness accompanied by increased neurogenic inflammation which included mast cell degranulation [78]. As with the model of stress-induced asthma, the exacerbation was significantly reduced in mice deficient in the NK1 receptor [78].

**CGRP**

CGRP is a 37 amino acid neuropeptide that mediates its effects through G-protein coupled receptors and is expressed predominantly in sensory nerve fibers [68]. There appears to be great heterogeneity in the response of mast cells to CGRP. The LAD2 human mast cell line does not degranulate in response to CGRP [79] and BMMC expresses functional CGRP1 receptors and even though their activation does not induce degranulation of these cells, it does cause mobilization of Ca²⁺ from intracellular stores and piecemeal release of mMCP-1 [24]. In the rat, peritoneal mast cells are unresponsive to CGRP but the neuropeptide does induce histamine release from dura mater mast cells [80]. CGRP-induced activation of meningeal mast cells may be involved in the pathogenesis of headaches as the histamine released induces direct vasodilatation and activates a subset of nonmechanically sensitive neurons in the rat meninges [81]. Furthermore, CGRP induces greater histamine release from mast cells in bronchoalveolar lavage from patients with airway inflammation associated with chronic cough and cough variant asthma than from control subjects [82]. Parasite infection with schistosomiasis in the mouse ileum is accompanied by mastocytosis and a corresponding increase in the density of CGRP-immunoreactive extrinsic primary afferent nerve fibers in the lamina propria [83]. In this model, mucosal mast cells were found in close apposition to the dense network of extrinsic primary afferent nerve fibers that contained CGRP but not substance P [83]. This anatomical evidence taken together with evidence that CGRP can directly activate mast cells suggests that this neuropeptide has a role in the functional relationship between mast cells and neuronal networks. One such example of this interaction
may be in the repair of intestinal mucosa injury. CGRP is released from neuroeffector junctions of extrinsic afferents as early as 4 h after the induction of intestinal mucosal injury and aids protection and repair through induction of vasodilatation and modulation of mucosal blood flow [84]. In addition, it appears that CGRP may induce additional protective responses through its action on mast cells. In an in vitro model of intestinal epithelial damage, the media of CGRP-conditioned mast cells induced a significant TGFβ-dependent increase in epithelial restitution as assessed by cell proliferation and migration [85].

**Vasoactive Intestinal Peptide**

Vasoactive intestinal peptide (VIP) is a 28 amino acid peptide that exerts its action on cells through two G-protein-coupled receptors, VPAC1 and VPAC2 [86]. VIPergic signaling is involved in regulating intestinal motility, induces cardiac vasodilation and, centrally, plays an essential role in the maintenance of circadian rhythm [87]. VIP is expressed widely in the nervous system, the endocrine system and the immune system and can be considered a true neuroimmunoendocrine mediator.

The interactions between VIP and mast cells appear complex and knowledge of the functional significance is limited. Mast cells can produce VIP [88], express VIP receptors [89] and degranulate in response to the peptide [79]. However, there is also evidence that VIP can stabilize mast cells in vivo [90, 91]. Furthermore, mast cell-derived proteases degrade VIP [92, 93] and have been demonstrated to limit the toxicity associated with high concentrations of VIP [92].

Systemically administered VIP can attenuate the motor response changes, neuronal cell death, and myelin sheet loss characteristic of a rat model of Parkinson's disease by 6-OHDA administration into the corpus striatum [94]. Evidence suggests that the protective effect of VIP in this model could, at least in part, be mediated by brain mast cells. Electron-microscopic studies of mast cells in the corpus stratum demonstrated that VIP treatment changes the ultrastructural morphology of mast cells in a manner characteristic of piecemeal degranulation [94].

Human airway smooth muscle cells secrete the mast cell-chemoattracting chemokine, fractalkine (FKN) [95]. Pretreatment of mast cells with VIP increases the chemoattractant effect of FKN on mast cells [95]. Interestingly, in asthmatic patients, there is an increase in both FKN and VIP expression in airway smooth muscle [96] and a positive correlation between VIP staining and mast cell infiltration of the smooth muscle layer [95]; thus, it has been suggested that airway smooth muscle-derived FKN may contribute to mast cell recruitment in asthma.

**Cholinergic Neurons**

In recent years, the immunoregulatory role of the parasympathetic nervous system has come into focus with the cholinergic anti-inflammatory pathway. The anti-