MINIREVIEW

MAST CELLS: THE IMMUNE GATE TO THE BRAIN

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Summary

Mast cells were originally considered wandering histiocytes, but are now known to derive from the bone marrow and enter the tissues as immature or precursor cells which then differentiate under micro-environmental influences such as interleukin-3. At least three types of mature mast cells have been identified as serosal (lung, peritoneal, skin), mucosal (nasal, gastrointestinal) and brain (dural, perivascular, parenchymal) with their own distinct biochemical, morphological and functional characteristics. Mast cells are necessary for immediate hypersensitivity reactions where they release numerous biologically powerful mediators in response to immunoglobulin E (IgE) and antigen (Ag), and appear to be required for delayed reactions. Anaphylatoxins, basic peptides and drugs, as well as certain neuropeptides and hormones, can also trigger mast cell secretion. Recent evidence indicates that mast cells are found in close proximity to neurons, an association which may be regulated by nerve growth factor. Moreover, mast cells may be capable of selective release of mediators which could, in turn, regulate further secretion. This information suggests that mast cells may serve as a link between the immune, endocrine and nervous systems and could have an important role in the access of lymphocytes and pathogens to the brain. The possible role of such interactions in the pathophysiology of specific neuroinflammatory conditions is also discussed.

A probable functional relationship between the immune and nervous systems has recently acquired explosive interest even though an association between mind and health has been prevalent in many, especially Eastern, philosophies. Early investigations by Selye of the effect stress has on health and disease popularized the notion that the neuroendocrine system may regulate susceptibility to disease (1). As a result, there have been many reviews (2,3,4,5,6,7) and symposia (8,9,10) on neuroimmune modulation, as well as the appearance of specialized periodicals such as the Journal of Neuroimmunology and Psychoneuroendocrine-immune (PNEI) Perspectives, among others.

Aside of the theoretical interest in the association between the immune and nervous systems, there are specific disease entities which have eluded successful therapy and may be better understood in light of such an association. Recent evidence indicates that many such conditions are associated with an increased number of mast cells: psychosenic asthma (11,12), interstitial cystitis (13), irritable bowel disease (14,15), migraines (16), multiple sclerosis (17,18), neurofibromatosis (19,20), scleroderma (21), urticaria (22) and certain tumors (23). Moreover, mast cells and neuropeptides which trigger them to secrete powerful mediators, have been implicated in a variety of other inflammatory conditions (21,22,23,24,25,26).

Mast cells were first identified in connective tissues by Paul Ehrlich in 1887 because of the specific metachromasia exhibited by their granules when stained with toluidine blue (27). Since then, they have clearly been implicated in anaphylaxis, a term first used in 1901, because of evidence that they contain histamine which causes vessel dilation, erythema and hypotension. Mast cells were later shown to have specific receptors for IgE and to secrete many mediators when IgE bound specific Ag (28,29).
<table>
<thead>
<tr>
<th></th>
<th>Toluidine Blue Metachromasia</th>
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<th>Response to IgE + Ag Peptides</th>
<th>Degranulation</th>
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<tr>
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<tr>
<td>Tongue</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>++</td>
<td>31,52</td>
</tr>
</tbody>
</table>

1Depends on the content of heparin
2Alcian blue counter-stained with safranin
3Only to substance P
4Not determined
5Secretion appears to be selective, without compound exocytosis
Origin and distribution

Until recently, mast cells were thought to derive from peripheral tissues and were often considered to be histiocytes or tissue basophils, to indicate their apparent similarity to circulating basophils (30,31). Mast cells are now known to be derived from the bone marrow and to migrate into the tissues as precursor (P) cells which show only few of the characteristics of "mature" mast cells (32). Under the influence of microenvironmental conditions, these P cells undergo "maturation", which is often defined in terms of the secretory granule content in sulfated proteoglycans, such as heparin. Factors involved in this maturation include interleukin-3 (32), which is a fairly general hemopoietic cell growth factor (33). More recently, nerve growth factor was shown to trigger mast cells and increase their numbers (34). Bone marrow-derived mast cells appear to be similar to basophil leukemia (RBL) cells which have been shown to be identical to mucosal mast cells (35). However, the phenotypic expression of mast cells does not appear to be fixed since the proteoglycan content of mucosal mast cells can switch to that of serosal when the former are grown on a bed of fibroblasts (36).

The contribution of mast cells to histamine content of various tissues was examined in special lines of mice which are grossly deficient in mature mast cells, as determined by the lack of toluidine blue staining and lack of immediate hypersensitivity responses (37). In such W/W* mice, the histamine content was substantially reduced in most tissues, except the brain where it remained at about 43% of normal controls (38). In addition, the high amount of histamine in the brains of W/W* mice was shown to be both non-mast cell and non-neuronal, implying the existence of another distinct source (39). A number of studies have shown that mast cell-deficient mice can still develop delayed hypersensitivity reactions (40,41) which are known to depend on early mast cell involvement (42,43), implying that some cells with mast cell properties must still be functional in these deficient mice. Moreover, other studies indicate that "mast cell-deficient mice" contain as many bone marrow-derived mast cell precursors as their normal controls (44). Finally, evidence from our laboratory indicates that during the development of rodent brains and in W/W* mice, there exist cells which do not stain with toluidine blue, but contain developing granules, as seen with electron microscopy (45). These findings may explain the ability of brains from W/W* mice to secrete amines in response to the mast cell secretagogue, compound 48/80 (C48/80), which was erroneously considered non-specific (46).

The morphology and staining characteristics of mature mast cells also varies from organ to organ. For instance, serosal mast cells stain metachromatically violet with toluidine blue and red when they are counter-stained with safranin following alcian blue (35). Mucosal mast cells need to be fixed properly (47), and then appear only blue whether stained with toluidine blue or alcian blue counter-stained with safranin, mostly due to their different content of sulfated proteoglycans (35). Mast cells in the brain stain both metachromatically and red with safranin, as long as they have been allowed to differentiate past the fifteenth post-natal day (48).

The ultrastructural appearance of mast cells which have been stimulated to secrete also varies considerably. For instance, peritoneal (serosal) mast cells respond to C48/80 by compound exocytosis where the electron-dense granules swell, lose their density and their cores are expelled from the cell periphery, or are found in large pits which communicate with the cell exterior (49,50,51,52). The same is true for skin and tongue cells (31,52). Mucosal mast cells, however, do not seem to degranulate (52), and neither do RBL cells (53). Moreover, brain mast cells undergo granular changes which are indicative of granular core rearrangement and partial release, but without degranulation (48). Finally, human basophils (54) and mast cells (55) are often seen to undergo "partial" or "piece-meal" secretion, but this mechanism has not been elucidated.

Such findings, coupled with the wide tissue distribution of mast cells, has led to the realization that mast cells can mature in response to local tissue requirements and can be differentiated based on their particular characteristics. Table 1 attempts to summarize some morphological and functional characteristics of mast cells in various tissues of the rat.

Stimuli of secretion

A great number of reports have shown that, in addition to IgE and Ag, mast cells secrete in response to a variety of other molecules (56), especially peptides (57,58), of which neurotensin (59), somatostatin (60,61) and substance P (62) are the most potent. However, all mast cells do not share the same secretory responsiveness to neuroptides, since mucosal mast
cells do not respond to any peptide except slightly to substance P (63) and RBL cells do not respond to any neuropeptides (62). It has been generally thought that serosal mast cells contain a low affinity peptide receptor which can be activated by the classic mast cell secretagogue, C48/80 and cationic peptides. However, cationic charge alone is not sufficient to trigger secretion since somatostatin is the most active peptide even though it contains only two net basic charges (64). Moreover, the three-dimensional structures of somatostatin and the C48/80 hexamer are such that cationic charges of each could be oriented similarly and may indicate that specific configuration is more important that absolute cationic charge (65). Additional peptides have also been shown to induce mast cell secretion; these include kinins (56,57), parathyroid hormone (66), nerve growth factor (34,67,68), adrenocorticotropic hormone (69), endorphins and opioids (70,71), as well as chymotrypsin and trypsin (72,73).

The presumed non-specific nature of the mast cell peptidergic receptors raised the question as to whether mast cells may be localized in the vicinity of cells capable of secreting neuropeptides. Recent evidence from a variety of laboratories indicates that mast cells are found in close proximity with peptidergic neurons in the gut, the skin, the diaphragm and the brain vasculature (34,74,75,76,77). Moreover, co-culture of purified mast cells with neonatal murine superior cervical ganglia showed that neuritic contact could be established between the two (78).

**Secretory mediators**

Since the discovery of histamine in mast cell granules, a significant number of other biologically active molecules have also been identified (79,80,81). Some are stored in secretory granules and include heparin, kinins, proteolytic enzymes, phospholipases and biogenic amines such as histamine, as well as dopamine and serotonin (in rodents, 82,83,84). Moreover, mast cells have the ability to take up and release such amines provided exogenously (82,83,84). Preliminary reports have also indicated the possible existence of certain peptides, such as somatostatin (85), vasoactive intestinal peptide (VIP,86) and endorphins (87). Finally, there are a number of de novo synthesized products such as prostaglandins, leukotrienes, lipoxins, as well as platelet activating factor and a variety of chemotactic moieties (80,81). In humans, the secretory granules contain various amounts of two proteolytic enzymes, chymase and tryptase (81). The relative content of these can not only differentiate them from any other immune cell, but also distinguishes various subclasses since those mast cells containing both chymase and tryptase (CT) are found in human bowel submucosa and skin and are identified as serosal, while those containing mostly tryptase (T) are considered mucosal (81). Finally, the ultrastructural appearance of human mast cells is different from that of rat mast cells since the granules of the former have distinct patterns indicating crystalline or other material (31,55).

Mast cells are endowed with more mediators destined for secretion than any other secretory cell, yet they are primarily known to secrete them in allergic reactions which represent an extreme case of pathology and where some mediators released have opposing results (eg, heparin and platelet activating factor). The ability of mast cells to release mediators differentially or selectively could have great physiological significance and recent evidence indicates that mast cells may be able to release at least some preformed mediators differentially (42,88,89,90) and without degranulation (90,91,92). Moreover, different secretagogues appear to favor the release of either prestored or de novo synthesized mediators (93).

Mast cells also express receptors for histamine (94) and benzodiazepines (95), although the evidence available, however, indicates that many of the mast cell secretory products could modulate mast cell secretion (Table 2). For instance, prostaglandins E, and E, (70,90), histamine (94,96), heparin (97), leukotriene D, (98,99) and lipoxin B (99) inhibit secretion, while kinins (56,57,61), leukotriene C (98,99) and prostaglandin D (100) stimulate secretion. In addition, many of these mediators could be secreted by other cell types, such as endothelial cells, leukocytes and neurons, and could regulate mast cell secretion.

**Brain localization and possible function**

Mast cells have been identified in the brain, especially in the leptomeninges, around the third ventricle, the thalamus and hypothalamus (101,102,103,104,105,106,107). These mast cells are found around the intraparenchymal vessels and their staining characteristics are similar to serosal mast cells in that they show violet metachromasia with toluidine blue (48,105), while they appear red when counter-stained with safranin following alcian blue (48, Fig. 1). However, the brain mast cells also stain with Sudan black (48), which is indicative of the
presence of lipids, thus differentiating them from both the serosal and mucosal mast cells, which do not contain lipids. These findings further suggest that the distinction between type I brain mast cells (serosal-like) and type II, found mostly in the rabbit brain (105) with lipid-like material, but without metachromasia, is not correct. Until specific molecular probes are used, functional characteristics may be required to complement morphological studies in order to better differentiate mast cells in various tissues.

TABLE 2

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Effect on Secretion</th>
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<tbody>
<tr>
<td>Endorphins</td>
<td>↑</td>
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<tr>
<td>Histamine</td>
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<td>94,96</td>
</tr>
<tr>
<td>Heparin</td>
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<tr>
<td>Kinins</td>
<td>↑</td>
<td>56,57,61</td>
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<tr>
<td>Leukotriene C4</td>
<td>↑</td>
<td>98,99</td>
</tr>
<tr>
<td>Leukotriene D4</td>
<td>↓</td>
<td>98,99</td>
</tr>
<tr>
<td>Lipoxin A</td>
<td>-</td>
<td>99</td>
</tr>
<tr>
<td>Lipoxin B</td>
<td>↓</td>
<td>99</td>
</tr>
<tr>
<td>Prostaglandin D2</td>
<td>↓</td>
<td>100</td>
</tr>
<tr>
<td>Prostaglandin E1</td>
<td>↓</td>
<td>70</td>
</tr>
<tr>
<td>Prostaglandin E2</td>
<td>↓</td>
<td>90</td>
</tr>
<tr>
<td>Serotonin</td>
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<tr>
<td>Somatostatin</td>
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<td>Trypsin</td>
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</tr>
<tr>
<td>Vasoactive Intestinal Peptide</td>
<td>↑</td>
<td>57,61,93</td>
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An interaction between mast cells and neurons would obviously be very important in areas where these cells abound, such as on cerebral microvasculature (Fig. 1). There, mast cell vasoactive molecules, such as histamine (107,108,109,110,111) could alter the integrity of vessels of the blood-brain barrier (112,113,114) and not only allow access to blood lymphocytes, but also attract them to the site by releasing chemotactic substances (79,80,81). The involvement of mast cells in vascular permeability (115) and the initiation of inflammatory responses (116) was recently documented beyond doubt. Secretion of histamine and leukotrienes could also act as neurotransmitters, especially at the hypothalamic-pituitary axis, where they have been implicated in prolactin and antidiuretic hormone release (117,118).

One case in point may be that of multiple sclerosis (MS), a neurologic condition with still unknown etiology, the pathophysiology of which involves infiltration of white matter by lymphocytes and macrophages leading to demyelination (119). A number of studies suggest that mast cells may participate in the pathophysiology of this disease (17,18) because mast cells: a) secrete vasoactive molecules which can alter the blood-brain barrier (114) and attract lymphocytes in the brain parenchyma; b) contain phospholipases which could degrade myelin in vitro (120,121), and result in demyelination of brain slices (18,122); c) secrete not only in response to neuropeptides (123,124), but also to partially purified myelin basic protein (121,125); d) are inhibited from secreting by such drugs as nedocromil (126) and amitriptyline (127), which have been shown to block mast cell-associated peripheral neuritis and experimentally-induced encephalomyelitis (EAE), respectively; e) can be depleted of their secretory biogenic amines with subsequent prevention of the development of EAE (128); f) mast cell-deficient mice are resistant to sinobis virus encephalitis (129).
FIG. 1

Histochemical and ultrastructural appearance of brain mast cells. (A) Thalamic perivascular (arrows) mast cells (curved arrow) showing metachromasia after acidified toluidine blue staining (Bar = 6.6 μm). (B) Electron micrograph of an ultrathin section showing a thalamic microvessel (L = lumen) where an endothelial cell (E) is encircled by a mast cell (M). Note the numerous electron-dense, homogeneous cytoplasmic granules (g) and the abundance of rough endoplasmic reticulum (RER) present in this 31 day old rat; the older the animal the less the RER present (Bar = 1.5 μm).

The pathophysiology of MS has been linked with a possible early viral infection and it is of interest that mast cells have been shown to secrete in response to viruses (130,131), as well as in response to lymphokines released from activated T cells (23). It is also of interest to note that there is an increased incidence of allergies and of migraine headaches in patients with MS (132), and both of these conditions have been associated with mast cells. In fact, mast cells have been proposed as central in the pathophysiology of common migraines (16) and cluster headaches (133). Moreover, the mast cell stabilizing drug disodium cromoglycate (134) has been shown to alleviate the headaches in a population of patients with suspected food allergy (135) which has increasingly been considered a critical factor in migraines (136). Migraines (137) and MS (138) occur more frequently in women and are known to be associated with the menstrual cycle and birth control pills. It should, therefore, be noted that secretion from mast cells has recently been shown to be augmented by estradiol or triggered by progesterone (139).

Identification of mast cells in the brain has rightly been dependent on their morphology, especially staining characteristics. However, some erroneous conclusions could be reached if mast cells are immature and, therefore, unable to stain metachromatically. For instance, W/Wr
mice have been extensively used as a model for an animal deficient in mast cells (37). Yet, these mice still have delayed hypersensitivity reactions (40,41) which are considered to be dependent on early mast cell secretion (42,43). In addition, brains of W/W~ mice contain a lot of "non-mast cell" and "non-neuronal" histamine (39) which can still be depleted with the mast cell secretagogue C48/80 (46). Moreover, such deficient mice have numerous mast cell precursors (44) which, by electron microscopy appear to contain electron-dense granules at various stages of development (45). Such cells, which are also present in normal fetal rats, do not stain with typical mast cell stains (45,48), yet they can synthesize histamine (140) and can take up and release serotonin in response to C48/80 (124). Consequently, functional studies must complement ultrastructural observations in order to provide explanations to phenomena which have so far appeared contradictory or confusing.

Functional studies have already shown that histamine can be released from hypothalamic slices, rich in mast cells, by C48/80 (141,142). Other studies have shown that thalamic/hypothalamic mast cells secrete in response to somatostatin and substance P (124,143), and this secretion was inhibited by certain tricyclic psychotropic drugs (144). The ability of brain mast cells to secrete in response to substance P acquired special significance recently since MS plaques were shown to be infiltrated by substance P immunoreactive astrocytes (145) and astrocytes synthesize the mast cell growth factor interleukin-3 (146). This evidence, along with the ability of substance P and myelin basic protein to stimulate brain mast cell secretion (147,148), led to the testing of a particular tricyclic drug in the prevention of EAE (149). A randomized double-blind study with this drug is now in progress for remitting-relapsing MS at the Massachusetts General Hospital.

FIG. 2
Interplay between mast cells, neurons and vessels in the brain. Mast cells could be triggered to release a number of mediators in response to endogenous and exogenous stimuli, some of which are listed. These mediators could then affect local vasculature, neurons and mast cells leading to a cyclic reaction.
Conclusion

Mast cells have been found in critical areas of the brain, especially in the thalamus and hypothalamus, where they are in association with vessels and neurons (Fig. 2). There, they may secrete in response to either endogenous stimuli such as kinins, myelin basic protein, neuropeptides or neurotransmitters, as well as exogenous agents such as antigens, cold, trauma or viruses. Secretion of mast cell mediators could then trigger a cyclic reaction by affecting all local organs involved, as well as mast cell secretion itself (Fig. 2).

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References


111. W.I. ROSENBLUM, Brain Res. 49 75-82 (1973).