Theoharis C. Theoharides
Duraisamy Kempuraj
Michael Tagen
Pio Conti
Dimitris Kalogeromitros

Differential release of mast cell mediators and the pathogenesis of inflammation

Authors' addresses
Theoharis C. Theoharides[1,2,3,4], *Duraisamy Kempuraj*[1], *Michael Tagen*[1], *Pio Conti*[5], *Dimitris Kalogeromitros*[4]
[1]Laboratory of Molecular Immunopharmacology and Drug Discovery, Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine and Tufts – New England Medical Center, Boston, MA, USA.
[2]Department of Biochemistry, Tufts University School of Medicine and Tufts – New England Medical Center, Boston, MA, USA.
[3]Department of Internal Medicine, Tufts University School of Medicine and Tufts – New England Medical Center, Boston, MA, USA.
[4]Allergy Section, Attikon Hospital, Athens, Medical School, Athens, Greece.
[5]Immunology Division, Department of Cancer and Neuroscience, Chieti University Medical School, Chieti, Italy.

Correspondence to:
Theoharis C. Theoharides, PhD, MD
Department of Pharmacology and Experimental Therapeutics Tufts University School of Medicine
136 Harrison Avenue
Boston, MA 02111, USA
Tel.: (617) 636 6866
Fax: (617) 636 2456
E-mail: theoharis.theoharides@tufts.edu

Acknowledgements
Aspects of the work discussed were supported in part by grants awarded to T. C. T. from the Multiple Sclerosis Society (RG-1961-A-1), NIH (NS38326, AR47652, DK62861, DK42409, DK 44816, DK and NS55681), DOD (BC024430), and Theta Biomedical Consulting and Development Co., Inc. (Brookline, MA, USA). We thank Ms Jessica Christian for her patience and word processing skills. T. C. T. discloses that he has been awarded US patent No. 5,250,529; No. 6,020,305; No. 5,648,350; No. 5,855,884; No. 5,821,259; No. 5,994,357; No. 6,624,148; No. 6,689,748; No. 6,984,667; No. 7,115,278 and pending US patent application No. 10/811,826, as well as EPO patent No. 1365777, covering the role of mast cells in the diseases discussed herein.

Immunological Reviews 2007
Vol. 217: 65–78

Immunological Reviews
0105-2896

Summary: Mast cells are well known for their involvement in allergic and anaphylactic reactions, during which immunoglobulin E (IgE) receptor (FcεRI) aggregation leads to exocytosis of the content of secretory granules (1000 nm), commonly known as degranulation, and secretion of multiple mediators. Recent findings implicate mast cells also in inflammatory diseases, such as multiple sclerosis, where mast cells appear to be intact by light microscopy. Mast cells can be activated by bacterial or viral antigens, cytokines, growth factors, and hormones, leading to differential release of distinct mediators without degranulation. This process appears to involve *de novo* synthesis of mediators, such as interleukin-6 and vascular endothelial growth factor, with release through secretory vesicles (50 nm), similar to those in synaptic transmission. Moreover, the signal transduction steps necessary for this process appear to be largely distinct from those known in FcεRI-dependent degranulation. How these differential mast cell responses are controlled is still unresolved. No clinically available pharmacological agents can inhibit either degranulation or mast cell mediator release. Understanding this process could help develop mast cell inhibitors of selective mediator release with novel therapeutic applications.

Keywords: *brain, inflammation, mast cells, multiple sclerosis, stress, vascular permeability*

Introduction

Mast cells derive from a distinct precursor in the bone marrow (1, 2) and mature under the influence of stem cell factor (SCF) and various cytokines (3). Depending on their location or stage of maturation, mast cells express different amounts of surface antigens, some of which are involved in activation and others in cell recognition (4). Mast cells also express numerous chemokine receptors that do not induce degranulation but could render them susceptible to human immunodeficiency virus infection (5). SCF or c-kit ligand also acts as a mast cell chemoattractant, in addition to nerve growth factor (NGF) (6), monocyte chemoattractant protein-1 (MCP-1), and a molecule called 'regulated upon activation, normal T-cell expressed and secreted' (RANTES) (7). Mast cells are necessary for the development of allergic reactions, through cross-linking of their high-affinity surface receptors for immunoglobulin (Ig) E (FcεRI) (8), leading to degranulation and the release of vasoactive, proinflammatory, and nociceptive mediators; these include histamine, interleukin (IL)-6, IL-8, IL-13, prostaglandin D_2, leukotriene C_4 (LTC_4), tumor necrosis factor-α (TNF-α),

tryptase, and vascular endothelial growth factor (VEGF) (3, 9, 10) (*Table 1*). SCF enhances FcεRI-induced degranulation and cytokine production, although it does not induce degranulation on its own (11).

The types of cytokines produced are not fixed. For instance, human umbilical cord-blood-derived mast cells (hCBMCs) primed with IL-5 released fivefold higher levels of TNF-α, IL-5, macrophage inflammatory protein-1α (MIP-1α), and granulocyte–macrophage colony-stimulating factor (GM-CSF); unlike IL-4, IL-5 did not enhance FcεRI-dependent histamine release (12). IL-4 enhances SCF-dependent mast cell proliferation and shifts IgE-dependent cytokine production in mature human mast cells to increased release of T-helper 2 cell (Th2) cytokines such as IL-3, IL-5, and IL-13 but not IL-6 expression (13). Mast cells in the presence of SCF produce predominantly proinflammatory cytokines, whereas in the presence of SCF and IL-4, also produce Th2 cytokines (14).

In addition to allergic triggers, mast cells can be activated by adenosine, anaphylatoxins, antibody light chains, bacterial and viral antigens, cytokines, endothelin, and neuropeptides (15).

***Table 1.* Mast cell mediators**

Mediators	Major pathophysiologic effects
Prestored	
Biogenic amines*	
Histamine	Vasodilation, angiogenesis, mitogenesis, suppressor T-cell activation
5-Hydroxytryptamine (5-HT, serotonin)	Leukocyte regulation, vasoconstriction, pain
Chemokines	
IL-8, MCP-1, MCP-3, MCP-4, RANTES	Chemoattraction and tissue infiltration of leukocytes
Enzymes	
Arylsulfatases	Lipid/proteoglycan hydrolysis
Carboxypeptidase A	Peptide processing
Pro-caspase 3, 4	Peptide processing
Chymase	Tissue damage, pain, angiotensin II synthesis
β-Hexosaminidase	Carbohydrate processing
Kinogenases	Synthesis of kinins, pain
Metalloproteinases	Tissue damage
Nitric oxide synthase	NO production
Peroxidases	Free oxygen radical production
Phospholipases	Arachidonic acid generation, inflammation
Tryptase	Activation of PAR, inflammation, pain, tissue damage, degradation of antigens and peptides
Polypeptides	
CRH	Inflammation, vasodilation, mast cell VEGF release
Endorphins	Analgesia, modulation of leukocyte activity
Endothelin	Sepsis
Kinins (bradykinin)	Inflammation, pain, vasodilation, mast cell trigger
Somatostatin (SRIF)	Anti-inflammatory (?), mast cell trigger
Substance P (SP)	Inflammation, pain, mast cell trigger
Urocortin (Ucn)	Inflammation, vasodilation, mast cell activation
VEGF	Neovascularization, vasodilation
Vasoactive intestinal peptide	Vasodilation, mast cell trigger
Proteoglycans	
Chondroitin sulfate	Connective tissue component, anti-inflammatory, mast cell inhibitor
Heparin	Angiogenesis, NGF stabilization, mast cell inhibitor
Hyaluronic acid	Connective tissue, component
De novo synthesized	
Cytokines	
IL-1, -3, -4, -5, -6, -9, -10, -13, -16	Inflammation, leukocyte migration, pain
IFN-γ, MIF, TNF-α	Inflammation, leukocyte proliferation/activation
Growth Factors	
SCF, GM-CSF, GnRH-I b-FGF, NGF, VEGF	Growth of a variety of cells, mast cell proliferation
Phospholipid metabolites	
LTB_4	Leukocyte chemotaxis
LTC_4	Vasoconstriction, pain
PAF	Platelet activation, vasodilation, inflammation
PGD_2	Bronchoconstriction, pain
NO	Vasodilation, neuromodulation

β-FGF, fibroblast growth factor; GnRH, gonadotropin-releasing hormone-I; LTB_4, leukotriene B_4; MIF, macrophage inflammatory factor; NO, nitric oxide; PAF, platelet-activating factor; PGD_2, prostaglandin D_2; SRIF, somatomedin release inhibitory factor, somatostatin; TGF-β, transforming growth factor-β.
*Mast cell can take up biogenic amines, store them, and secrete them.

Simultaneous addition of C3a and IgG led to increased degranulation of human mast cells (16). Monomeric IgE has been shown to reduce histamine, LTC, and IL-8 and maintain histamine release from human cultured lung mast cells (17). Ig-free light chains can also elicit immediate hypersensitivity-like responses (18, 19) with subsequent T-cell-mediated immune responses (20). The anti-bacterial peptides, human β-defensins, can reunite mast cells and induce degranulation (21). Consequently, mast cells could play an important role in innate or acquired immunity (3, 22, 23) as well as limit endothelin-related toxicity during bacterial infections (24). Increasing evidence also indicates that mast cells are critical for the pathogenesis of a number of inflammatory diseases (*Table* 2), but this role could only be achieved if mast cells could release selective mediators without degranulation (*Fig.* 1) that would otherwise lead to allergic or anaphylactic reactions (15).

Mast cells are also known to infiltrate a number of tumors, but they appear intact with light microscopy and are considered to induce angiogenesis and provide an environment conducive to cancer growth (25–28). Angiogenesis in endometrial cancer increases with tumor progression, and angiogenic tryptase secreted by host mast cells cooperates in this induction (29).

Differential release of mast cell mediators

Mast cells are ubiquitous in the body, including the brain where they do not express FcεRI protein under normal conditions (30), not surprising as the brain is not known to develop allergic reactions and as IgE does not cross the blood–brain barrier (BBB). Moreover, mast cells are rarely seen to degranulate during autoimmune (31) or inflammatory processes (32). The only way to explain how this versatile cell may regulate immune responses or how it could be involved in inflammatory diseases without causing anaphylactic shock is through 'differential' or 'selective' release of mediators (33) without degranulation (34, 35).

***Table* 2. Inflammatory diseases involving and mast cell activation**

Disease	Major pathophysiologic role
Asthma	Bronchoconstriction, pulmonary inflammation
Atopic dermatitis	Skin vasodilation, T-cell recruitment, inflammation, itching
Coronary artery disease	Coronary inflammation, myocardial ischemia
Chronic fatigue syndrome	Brain inflammation, exhaustion
Chronic prostatitis	Prostate inflammation, pain
Fibromyalgia	Muscle inflammation, pain
Inflammatory bowel disease	Gastrointestinal inflammation, pain
Interstitial cystitis	Bladder mucosal damage, inflammation, pain
Migraines	Meningeal vasodilation, inflammation, pain
Multiple sclerosis	Increased BBB permeability, brain inflammation, demyelination, T-cell activation
Neurofibromatosis	Skin nerve growth, fibrosis
Osteoarthritis	Articular erosion, inflammation, pain
Psoriasis	Skin inflammation, T-cell recruitment
Rheumatoid arthritis	Joint inflammation, cartilage erosion
Rhinosinusitis	Nasal and sinus inflammation

Mast cells could release the content of individual granules (36) that may contain different mediators at different locations. A number of innate and exogenous molecules can trigger mast cells to release key mediators differentially or selectively (34, 35, 37) (*Table* 3). This process was originally reported for serotonin, which could be released without histamine (33). Serotonin could also be released without arachidonic acid metabolites (38–40). Differential release of eicosanoids was also reported without histamine (39). Others also showed differential release of IL-6 in response to bacterial lipopolysaccharide (LPS), in the presence of the phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin, or triggered by SCF (41–44). IL-1 can also stimulate human mast cells to release IL-6 selectively (45). Recently, corticotropin-releasing hormone (CRH) was shown to stimulate selective release of VEGF without degranulation and histamine or tryptase release from the human mast cell line (HMC-1) and hCBMCs (46). Prostaglandin E_2 (PGE_2) also induced selective VEGF release (47) as well as release of MCP-1 without degranulation (48). Yet, PGE_2 inhibited FcεRI-induced histamine release from human lung mast cells (49). Strangely, PGE_2 stimulated skin mast cell degranulation in older but not in younger mice (50). Stromal-cell-derived factor-1α can selectively produce IL-8 from human mast cells also without degranulation (51). Activation of human cultured mast cells by CD40 ligand was recently shown to lead to release of the chemokines IL-8 and MIP-1 without degranulation (52). Interestingly, nanoreceptor-type protein kinase-deficient mast cells could not generate IL-6, TNF, or MCP-1 during FcεRI aggregation, but IL-13 production was intact, suggesting divergent regulatory pathways (53).

Toll-like receptors (TLRs) are critical in innate and acquired immunity (54–56). Rodent mast cells express TLR4, which binds LPS and induces the release of TNF-α without degranulation, while peptidoglycan induces degranulation and histamine release through TLR2 (57, 58). LPS also induced secretion of IL-5, IL-10, and IL-13 but not GM-CSF, IL-1, or LTC_4 (58, 59). Activation of TLRs appears to be even more complicated, as LPS produced TNF-α, IL-1, IL-6, and IL-13 but not IL-4 or IL-5, while TLR2 activation produced IL-4, IL-6, and

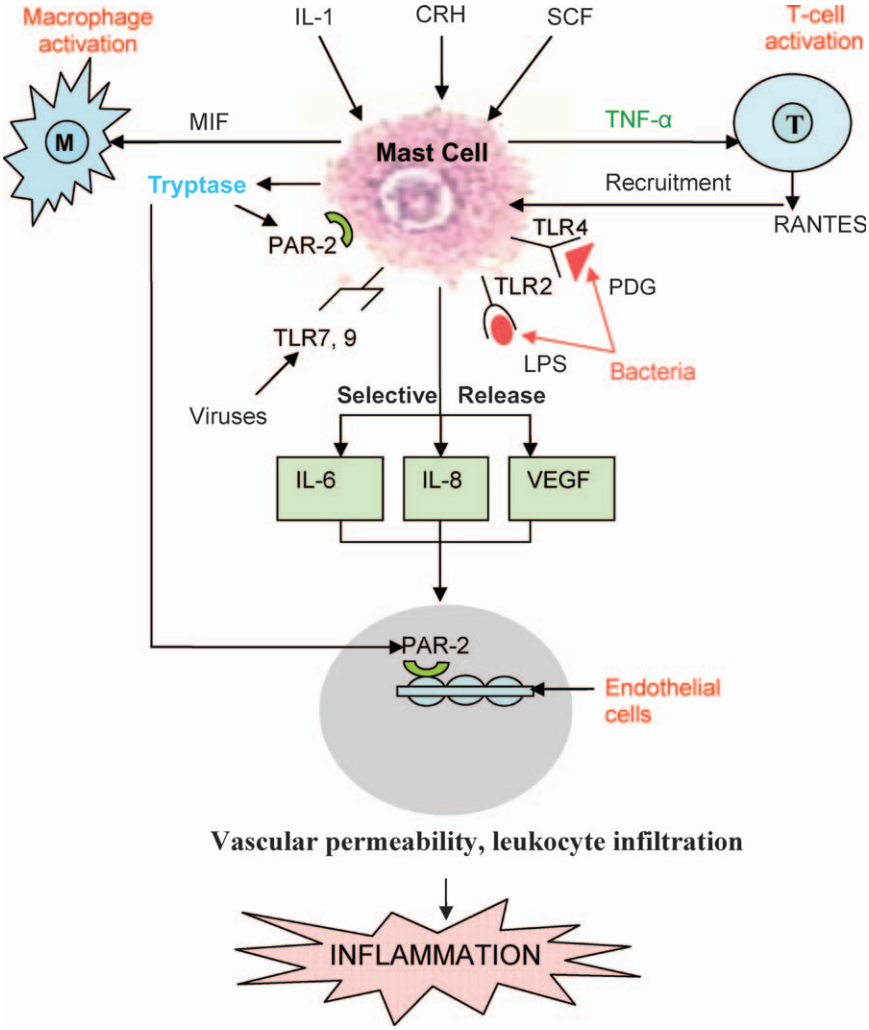

***Fig. 1.* Schematic representation of some of the triggers permitting differential release of mast cell mediators and their contribution to inflammation.** M, macrophages; T, T cells.

IL-13 but not IL-1 (60). Antigen apparently could have a synergistic action with TLR2 and TLR4 in enhancing cytokine production from rodent mast cells (61).

Fetal rat-skin-derived mast cells express viral TLR3, TLR7, and TLR9, and activation by polyoligodeoxynucleotide and CpG induced release of TNF-α, IL-6, RANTES, and MIP, again without degranulation (62). Human mast cells can produce IL-6 through viral TLR9 activation (63), while they produce interferon (IFN) in response to double-stranded RNA through TLR3 (64). We showed that HMC-1 expresses TLR3, TLR5, TLR7, and TLR9 and that TLR9 expression was increased in response to its activation (65). No specific ultrastructural or biochemical events have so far been defined in TLR-induced release of cytokines, although they may be able to predispose or enhance allergic responses. These results suggest that bacterial or viral infections could lead to aberrant inflammatory responses through mast cell activation without systemic signs of allergy.

Low-intensity stimulation of FcεRI with IgE plus anti-IgE or IgE plus low antigen positively regulates degranulation and cytokine production, whereas Lyn (an sarcoma inducing gene of rous sarcoma virus (Src) family kinase) works as a negative regulator of high-intensity stimulation with IgE plus high antigen (66). However, $Lyn^{-/-}$ mice had increased FcεRI expression, circulating histamine, and eosinophilia (67). Suboptimal antigen challenge of human mast cells led to FcεRI unresponsiveness that correlated to reduced spleen tyrosine kinase (Syk) levels (68). Suboptimal IgE concentrations could induce actin assembly that blocked degranulation (69). However, low antigen sill permitted MCP-1 release (70).

Mizolastine, a histamine-1 receptor antagonist, inhibits LTC_4 synthesis in human mast cells and basophils, but it enhances histamine and IL-4 release only from anti-IgE-stimulated basophils (71). LPS enhances production of IL-9 and IL-13 but not IL-4 from primary murine bone-marrow-derived mast cells activated with ionomycin or IgG–antigen (72). IL-4 enhances

Table 3. **Examples of differential release of mast cell mediators**

Stimuli	Mast cell type used	Mediators released	Mediators not released	Physiological importance	References
Endogenous					
CD4 ligands	hCBMC	IL-8, MCP-1	Histamine	Leukocyte chemotaxis	Fischer *et al.* (52)
CD8 ligands	RPMC	TNF-α, NO	Histamine	T-cell interactions	Lin *et al.* (185)
CRHR-1	hCBMC	VEGF	H, tryptase, IL-8	Inflammation	Cao *et al.* (144)
CRHR-2	hCBMC	IL-6	H, tryptase, IL-8, VEGF	Inflammation	Papadopoulou *et al.* (186)
Endothelin-1-3	RMMC	TNF-α, IL-12↑*	IL-4, IL-10, IL-13↓*	Th1 immunity	Coulombe *et al.* (187)
IL-1	hCBMC	IL-6, IL-8, TNF	H, tryptase	Inflammation	Kandere-Grzybowska *et al.* (45)
IL-1β	RPMC	NO	PAF, H	Inflammation	Hogaboam *et al.* (188)
IL-12	RPMC	IFN-γ	Histamine	Th1 immunity	Gupta *et al.* (189)
LTC_4/LTD_4	IL-4-primed hCBMC	TNF-α, MIP-1α, IL-5	H	Inflammation	Mellor *et al.* (190)
Monomeric IgE	BMMC	IL-6	H, LTC_4	Mast cell survival	Kalesnikoff *et al.* (191)
PGE_2	RPMC	IL-6	H, TNF-α	Inflammation	Leal-Berumen *et al.* (192)
PGE_2	hCBMC	MCP-1	No degranulation	Angiogenesis	Nakayama *et al.* (48)
SCF	BMMC	IL-6	H, LTC_4, TNF-α	Mast cell development	Gagari *et al.* (43)
SDF-α	hCBMC	IL-8	H, GM-CSF, IFN-γ, IL-1β	Endothelial transmigration	Lin *et al.* (51)
Suboptimal FcεRI stimulation	BMMC	MCP-1, H low	IL-10, H	Chemokines >> Cytokines /HA	Gonzalez-Espinosa *et al.* (70)
Thrombin	BMMC	IL-6	5-HT, TNF-α	Coronary inflammation	Gordon *et al.* (193)
Exogenous					
Amitriptyline	RPMC	Serotonin	Histamine	Headaches	Theoharides *et al.* (33)
Cholera toxin	RPMC	IL-6	TNF-α	Inflammation	Leal-Berumen *et al.* (194)
CpG DNA	BMMC	TNF-α, IL-6	H, IL-4, IL-12, GM-CSF, IFN-γ	Host response to bacteria	
Helicobacter pylori VacA toxin	BMMC	IL-6, IL-8, TNF	H	Gastric injury	Supajatura *et al.* (60)
LPS	RPMC	IL-6	H	Bacterial infection	Leal-Berumen *et al.* (41)
PMA	BMMC	VPF/VEGF	5-HT	Angiogenesis	Boesiger *et al.* (10)
Peptidoglycan	hCBMC	GM-CSF, IL-1β, RANTES, LTC_4	β-hexosaminidase, IL-6	Exacerbation of asthma	McCurdy *et al.* (58)

BMMC, bone marrow-derived mast cells (rodent); CRH, corticotropin releasing hormone; GM-CSF, granulocyte-macrophage-colony stimulating factor; H, histamine; 5-HT; 5-hydroxytryptamine; LPS, lipopolysaccharid; LTD_4, leukotriene D_4; RPMC, rat peritoneal mast cells; PMA, phorbol myristate acetate; SCF, stem cell factor; SDF, stromal cell-derived factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VPF, vascular permeability factor.

whereas INF-γ inhibits the FcεRI-mediated production of MIP-1α, IL-8, and GM-CSF from human mast cells (73). B-cell lymphoma 10 (Bcl10) and mucosal-associated lymphoid tissue-1 (MALT-1) are identified as a key regulators of mast cell signaling. Mice deficient for either protein show severely impaired IgE-dependent late-phase anaphylactic reactions. Mast cells from these animals neither activate NF-κB nor produce TNF-α or IL-6 upon FcεRI signaling, while degranulation and LT secretion are normal. Thus, Bcl10 and MALT-1 are essential positive mediators of FcεRI-dependent mast cell activation that selectively uncouple NF-κB-induced proinflammatory cytokine production from degranulation and LT synthesis (74).

Mechanisms involved

FcεRI-induced mast cell degranulation requires calcium-independent granule translocation to the surface but calcium-dependent exocytosis (75). This process involved SNAP-23 phosphorylation (76). Mast cells can undergo ultrastructural alterations of their electron-dense granular core indicative of secretion but without degranulation, a process that has been termed 'activation' (77–79), 'intragranular activation' (80), or 'piecemeal' degranulation (81) (*Fig.* 2). Piecemeal degranulation was recently shown to involve vesicular transport of secretory granules contents (82). However, these ultrastructural observations have not been linked to release of a specific mediator. We had shown that differential release of serotonin involved its being sequestered from secretory granules inside vesicles containing high-affinity serotonin-binding proteins from which it was then released (83). More recently, we showed that IL-6 release in response to IL-1 involves 40–80 nm vesicles unrelated to the secretory granules (800–1000 nm), and IL-4 release from these vesicles did not require extracellular calcium ions (45). Selective mediator segregation in and release

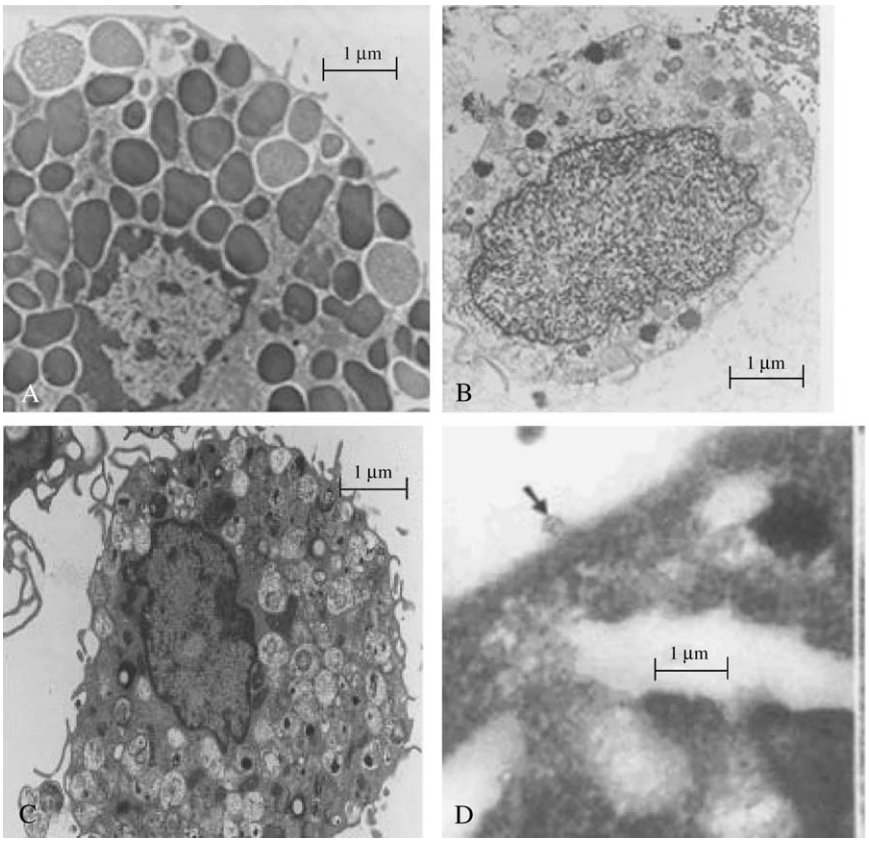

Fig. 2. Mast cells in inflammatory diseases. (A) Degranulated human LAD2 cell in response to 0.01 mM MBP. (B) Activated bladder mast cell from a patient with the inflammatory disease interstitial cystitis. (C) An activated brain mast cell from monkey EAE, with prominent intragranular changes. (D) Section of one hCBMC after cryoimmunoelectron microscopy showing a vesicle (arrow) releasing a group of gold-labeled antibody-recognized selectively release IL-6 molecules in response to IL-1 (100 nM).

from specific vesicles could be accomplished through corresponding 'non-functional' receptors expressed on such vesicles, as shown for eosinophils (84).

The downstream pathway steps involved in FcεRI-induced or peptide-induced degranulation are well known and appear to be distinct from those necessary in differential secretion (*Table* 4). For instance, FcεRI aggregation requires extracellular calcium ions as well as PI3K, extracellular-signal-regulated kinase, c-Jun N-terminal kinase (JNK), NF-κB, and protein kinase C (PKC) activation, although PKC isozyme-ε was recently shown to be redundant (85, 86). FcεRI aggregation leads to production of phosphatidyl inositol-3,4,5-triphosphate through the action of PI3K, which is inhibited by phosphatase and the molecule called 'tensin homologue deleted on chromosome ten' (PTEN); PTEN knockdown induced constitutive cytokine production without degranulation along with phosphorylation of AKT, p38 mitogen-activated protein kinase (MAPK) and JNK (87). Secretion in response to NGF appears to be regulated by tyrosine kinase (TK), PI3K, and PKC, but not MAPK, while secretion by compound 48/80 requires PLC, TK, MAPK, and PKC (88). In contrast, IL-1 stimulation of mast cells was extracellular calcium independent and involved p38 MAPK, NF-κB, and PKCθ isozyme activation (89). CRH activation of selective mast cell VEGF release was also extracellular calcium independent but involved only protein kinase A (PKA) and p38 MAPK activation (46). Adapter complexes appear to segregate FcεRI-dependent activation of mast cells; for instance, the Bcl10–MALT-1 complex permits IL-6 and TNF-α release without degranulation (90).

Gene array analysis of human mast cells activated by IgE showed overexpression of numerous, mostly inflammation-related genes (91). In contrast, among the genes that were upregulated more than 1.5-fold after CRH stimulation (6 h) were those related to vesicular trafficking and release (*Table* 5) (unpublished data).

Multiple sclerosis

One disease where mast cells have been implicated without degranulation is multiple sclerosis (MS), a demyelinating condition (92, 93) involving brain infiltration by lymphocytes (94, 95). The role of $CD4^+$ T cells is well documented in MS, but this $CD4^+$ Th1 model has recently been questioned (96). Increasing evidence implicates Th2 processes typically associated with allergic reactions (97–99). For instance, myelin basic protein (MBP) induced homogeneic mast cell activation and brain demyelination (100). Virally induced encephalomyelitis could not develop in W/W^v mast-cell-deficient mice (101, 102), and experimental allergic encephalomyelitis (EAE) was

***Table* 4. Signaling steps involved in mast cell degranulation and differential secretion**

	Triggers				
Steps involved	FcεRI	NGF	48/80	CRH	IL-1
Ca^{2+}*	+	+	−	−	−
PI3K	+	+	−	?	?
PKA	−	−	−	+	−
TK	+	+	+	−	−
p38/MARK	−/+	−	−	+	+
ERK1/2	+	?	?	−	−
JNK1/2	+	?	?	−	−
NF-κB	+	+	+	−	+
PKC isoforms	+ (α, β, δ, ε, θ, μ)	+	−	?	+ (θ)

ERK, extracellular signal-regulated protein kinase; PKA, protein kinase A.
*Extracellular.

attenuated and delayed in these mice (103). Subsequent studies suggested that the inability of mast-cell-deficient mice to fully develop EAE may also depend on reduced T-cell activation (104, 105). Ig-free light chains can sensitize mast cell release of cytokines that induce T-cell-mediated immune reactions critical in MS (20). Mast cell contact with activated T cells leads to secretion of matrix metalloproteinase-9 and IL-6 from human mast cells (106). Moreover, mast cells can promote IgE-dependent and T-cell-independent proliferation and activation through TNF-α release (107). We recently showed that mast cell contact with activated T cells stimulates the latter to produce 30-fold more IL-2, which is further increased when mast cells are activated by MBP (108).

Mast cells could, therefore, participate in the pathogenesis of MS in different ways (*Table* 6). They could be activated by bacterial or viral antigens and release cytokines/chemokines, selectively inducing T-cell/macrophage recruitment and activation. Myelin damage could then release fragments and other

Table* 5. Secretion-related genes upregulated during CRH activation of HMC-1 cells

Code	Gene name	Fold increase
SPIR-1	Spir-1 protein	23.60
CDC42BPB	CDC42-binding protein kinase β (DMPK-like)	12.65
PCANAP7	Synaptotagmin VII	7.20
MTMR7	Myotubularin-related protein 7	5.15
SYNGR2	Synaptogyrin 2	4.70
STX18	Syntaxin 18	2.34
SYP	Synaptophysin	2.30
PCLO	Piccolo	1.93
RAB3A	RAB3A, RAS oncogene family member	1.80
STX1B2	Syntaxin 1B2	1.76
STXBP6	Syntaxin-binding protein 6 (amisyn)	1.70

*Our unpublished results. DMPK, dystrophy protein kinase.

***Table* 6. Possible mast cell actions in MS**

- Adhesion molecule induction
- BBB disruption
- Demyelination
- Fibrosis and plaque formation
- Macrophage recruitment
- PAR-related inflammation
- T-cell activation
- T-cell infiltration

neuropeptides that could stimulate degranulation and release of the protease tryptase, which could in turn enhance demyelination and inflammation through stimulation of protease activated receptor-2 (PAR-2) (*Fig.* 1).

Affected brains in MS areas fill with fibrotic tissue forming the MS plaque (109) that also contains activated mast cells (110–112). Gene array analysis of MS plaques showed overexpression of genes for FcεRI, the histamine-1 receptor, and tryptase, all of which are associated with mast cells (113–115). Mast cell tryptase is increased in the cerebrospinal fluid (CSF) of patients with MS (116), can activate peripheral mononuclear cells to secrete TNF and IL-6 (117), and stimulate PARs that can lead to microvascular leakage and widespread inflammation (118–121).

BBB breakdown (122) precedes any pathological or clinical signs of MS (112, 123, 124), as shown by trans-BBB leakage of albumin (125) and magnetic resonance imaging-gadolinium studies (124). Mast cells have been proposed as the 'immune gate to the brain' (126). They line the BBB (77) and are activated in EAE, but they show ultrastructural signs of activation without typical degranulation (127) (*Fig.* 2). Mast cells migrate from the meninges (128) and from blood to brain (129). Mast-cell-derived products can enter neurons, a process termed transgranulation, indicating a novel form of brain–immune system communication (130). Brain mast cells could be activated by non-allergic stimulation, such as myelin basic protein (MBP) (100), as well as by acute stress (131). Restraint stress activated brain mast cells and led to CSF elevation of rat mast cell protease I (132), effects abolished by polyclonal antiserum to CRH (132) and the CRH receptor-1 (CRHR1) antagonist antalarmin (132, 133). Acute stress also increased BBB permeability through the action of CRH (134) on brain mast cells (134, 135). Acute restraint stress also shortened the time required for the development of EAE in mice and increased BBB permeability (136). $CRH^{-/-}$ mice with EAE were shown recently to have decreased clinical disability and decreased brain infiltration by immune cells (137). Restraint stress was also reported to increase mortality rates and lead to higher central nervous system viral load during Theiler's virus infection (138). Stressed mice had increased inflammatory lesions in spinal cord

and developed autoimmune antibodies to MBP (139). Acute restraint stress (140) as well as CRH (141) and its structurally related peptide, urocortin (142), can activate mast cells and induce mast-cell-dependent vascular permeability in rodent as well as in human skin (143), another neuroectodermal tissue. We recently showed that human mast cells express CRHR, activation of which leads to selective release of VEGF (144). The frequency, chronicity, severity, and timing of stressors appear to be important (145).

The effect of stress and CRH on mast cell activation and BBB permeability may help explain clinical findings in patients with MS. The symptoms of MS and the appearance of new lesions have been repeatedly shown to be precipitated by psychological stress (109, 146–151). In one study in Denmark (151), parents who had unexpectedly lost a young child had a significantly increased risk of MS compared with other bereaved parents. Meta-analysis of 14 prospective studies showed a significantly increased risk of MS exacerbations after stressful events (152). The role of 'stress-response systems for the pathogenesis and progression of MS' was reviewed recently, and it was proposed that MS is associated with glucocorticoid-insensitive immune cells (153). Such a finding has never been documented. An experimental study argued that stress does not affect MS because the function of peripheral blood leukocytes in patients with MS is apparently unaffected by stress (154). However, this reasoning is faulty, as stress may predominantly affect mast cells and T cells but not peripheral leukocytes. In fact, restraint stress induced mast-cell-dependent increase in mouse serum IL-6 levels (155), while examination stress dramatically increased serum TNF-α levels in medical student volunteers (156). These results suggest that mast-cell-derived cytokines in response to stress, or other triggers, may be involved in MS exacerbations.

Inhibition

There is still no curative therapy for MS (157). Mast cells were considered a therapeutic target for MS (158). Unfortunately, very few clinically available drugs can inhibit mast cell secretion. Disodium cromoglycate (cromolyn) was originally shown to inhibit rodent mast cells (159, 160), but it has proven ineffective in human mast cells (161). Chondroitin sulfate, the major constituent of mast cell granules (161), and the flavonoid quercetin (162, 163) are potent mast cell inhibitors. Moreover, quercetin is the only compound shown to inhibit differential release of IL-6 in response to IL-1 by reducing intracellular calcium ions and PKCθ phosphorylation (89). Flavonoids may also inhibit mast cell degranulation through inhibition of the intracellular activation of Syk (164). Flavonoids may also be acting as a 78-kDa protein (165) shown to mediate the inhibitory effect of cromolyn on rat mast cells (160) and found to be homologous to moesin (166). The inhibitory receptor IRp60 (CD300a) was shown to be expressed in human cord blood mast cells, and its neutralization in mice led to increased mediators (167). Human mast cells also express the myeloid cell inhibitory receptor CD200, engagement of which inhibited FcεRI-induced phosphorylation activation (168). Two peptides derived from complement component C3a, $C3a^{+}$, and C3a9 were shown to inhibit FcεRI-induced degranulation and TNF-α release (169). Degranulation in response to FcεRI aggregation or compound 48/80 was

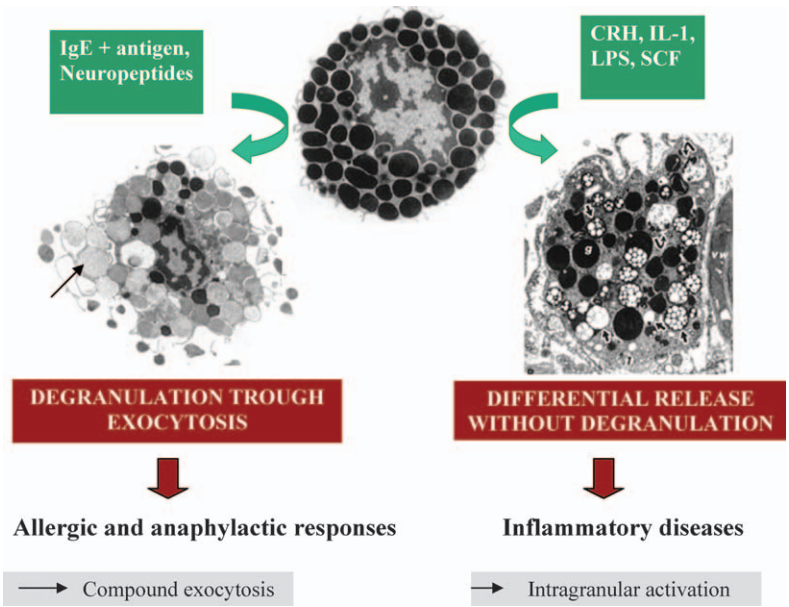

Fig. 3. Summary scheme showing morphological appearances at the ultrastructural level of rat peritoneal mast cells, along with potential triggers and proposed pathophysiologic functions. Intact (center), exocytotic degranulation (left), and intragranular changes (right).

severely impaired in IL-2-inducible T-cell kinase-1 mice (170). Nitric oxide also blocked FcεRI-induced IL-4 and IL-6 production through TNF-α inhibition of Jun (171).

It is of interest that flavonoids known to inhibit mast cell secretion have also been shown to inhibit macrophage myelin phagocytosis (172) and EAE (173, 174). The flavone luteolin was also a strong inhibitor of human autoimmune T cells (175), and we showed that luteolin can inhibit mast cell activation and mast cell-dependent T-cell activation (108).

Conclusion

Mast cells are unique immune cells that can be activated by numerous triggers, including CRH (176). The versatile roles of mast cells, especially in the brain, must utilize the mast cell's ability to secrete specific mediators selectively without degranulation. Differential or selective release of mast cell mediators without degranulation has been stressed repeatedly as critical in the pathogenesis of inflammatory diseases (15, 177), atopic dermatitis and psoriasis (178), chronic fatigue syndrome (179), cancer (26), coronary artery disease (177), fibromyalgia (180), inflammatory arthritis (181), interstitial cystitis (182, 183), migraines (184), and MS (15, 131, 158) (*Fig.* 3). Understanding the mechanism of differential release could permit us to selectively inhibit mast cell involvement in pathological inflammatory conditions, while permitting their function in innate or acquired immunity.

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