EXPERT REVIEWS

Spectrum of mast cell activation disorders

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Mast cell (MC) activation disorders present with multiple symptoms including flushing, pruritus, hypotension, gastrointestinal complaints, irritability, headaches, concentration/memory loss and neuropsychiatric issues. These disorders are classified as: cutaneous and systemic mastocytosis with a c-kit mutation and clonal MC activation disorder, allergies, urticarias and inflammatory disorders and mast cell activation syndrome (MCAS), idiopathic urticaria and angioedema. MCs are activated by IgE, but also by cytokines, environmental, food, infectious, drug and stress triggers, leading to secretion of multiple mediators. The symptom profile and comorbidities associated with these disorders, such as chronic fatigue syndrome and fibromyalgia, are confusing. We propose the use of the term 'spectrum' and highlight the main symptoms, useful diagnostic tests and treatment approaches.

KEYWORDS: antihistamines • brain • c-kit mutation • IgE • inflammation • mast cell • mastocytosis • mediators • tryptase

Mast cells (MCs) are immune cells derived from hematopoietic precursors and mature in tissue microenvironments [1,2].

MCs are considered both as sentinels of the innate immune system (host defense against infectious pathogens, neutralization of toxins) and of the adaptive ones, regulating it either positively or negatively, as they are located in tissues that interface with the external environment [3]. MCs are activated primarily by IgE-dependent mechanism (allergen, anti-IgE) and also by IgE-independent mechanism (IgG, bacterial and viral components, complement fragments [C3a, C5a]) cytokines, chemokines, drugs, physical stimuli and hormones [2,4]. Upon stimulation, MC release preformed mediators, β-hexosaminidase, histamine, TNF and tryptase, through rapid degranulation, as well as newly synthesized cytokines, chemokines (TNF, CCL2, CCL8) and phospholipid products such as prostaglandin D_2 (PGD_2), $PGF_{2\alpha}$ and leukotrienes [5–7] typically 6–24 h after stimulation. These mediators contribute to the late phase reactions and inflammation directly and through the recruitment and activation of other immune cells [7].

Each mediator could lead to specific clinical features. For instance, histamine is associated with headaches, hypotension and pruritus; tryptase with inflammation and fibrinogen lysis; cytokines and chemokines with constitutional symptoms of generalized inflammation, fatigue and PGD_2; PGD_2 with flushing; and leukotrienes with bronchoconstriction and cytokines with inflammation. Many MC mediators may also be released selectively without degranulation, making it difficult to recognize these activated MC with routine histology [8]. We recently showed that activated MC secreted mitochondrial components extracellularly, and these acted as 'autopathogens' triggering autoinflammatory responses [9,10]. The ability to release multiple mediators allows MC to actively interact with other cell types in their surrounding environment, especially T cells [11,12] and participate in the induction and/or propagation of various immune and inflammatory responses. MC is probably the only cell type that stores preformed TNF [13], which is rapidly released and influences T cell recruitment and activation [14]. MC-derived CCL2 and CCL8 enhance recruitment of other immune cells to the site of inflammation [15]. As a result, MCs are not only involved in allergic diseases, asthma and mastocytosis but also in inflammatory conditions such as atopic dermatitis, psoriasis, atherosclerosis and possibly in other conditions, such as chronic fatigue syndrome, fibromyalgia, obesity and autism [2,16–19].

The severity of MC activation symptoms depends on many factors such as the capacity of MC to release mediators, the amount of

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Box 1. Diagnostic criteria for mast cell activation disorders.

Clinical signs and symptoms related to recurrent or chronic systemic MC activation (affection of at least two organ systems)

- Dermatologic: *flushing*, pruritus, urticaria pigmentosa, angioedema, *dermatographism*
- Respiratory: wheezing, sore throat, stridor
- Cardiovascular: chest pain, hypotension, tachycardia
- Gastrointestinal: *abdominal pain*, nausea, vomiting, *diarrhea*, *bloating*, malabsorption, esophagitis
- Naso-ocular: nasal stuffiness, pruritus
- Neurologic: *headache, memory and concentration difficulties/brain fog*, paresthesia, peripheral neuropathy
- Musculoskeletal: bone/muscle pain, degenerative disc disease, osteoporosis/osteopenia
- Systemic: anaphylaxis, fatigue

Documentation of an increase of a validated urinary or serum marker of MC activation

- Increased tryptase level (>20 ng/ml) during a symptomatic period in two occasions at least. However, on the occasion of persistent baseline tryptase levels >15 ng/ml, an increase in the tryptase levels above 15 ng/ml even once is also considered a diagnostic criterion. Less specific markers are elevated 24-h urinary histamine metabolites (methylhistamine) or elevated 24-h urinary prostaglandins (PGD_2, 11β-$PGF_{2\alpha}$) (also from basophils)

Response to antimediator therapy (decrease in the frequency or severity or resolution of symptoms)

- H_1 Ra (first and second generation) with or without H_2 Ra, antileukotriene medications (cysteinyl leukotriene receptor blockers or 5-lipoxygenase inhibitor), MC stabilizers (cromolyn sodium)

Words in italics are the most common signs and symptoms.
MC: Mast cell; Ra: Receptor antagonists.
Data taken from [33].

IgE or other triggers and the presence of cytokines and chemokines [20], especially IL-33 [21]. Another important parameter to MC activation is the coexistence of high-risk conditions as this combination may lead to the occurrence of life-threatening episodes. A typical example is the presence of an allergy against hymenoptera venom(s) in patients suffering from mastocytosis [22–25]. The most dramatic clinical reaction mediated by MC that also involves basophils in rodents is anaphylaxis, which is characterized by the sudden onset of skin, cardiovascular, respiratory, gastrointestinal, and sometimes, neuromuscular symptoms that can rapidly lead to death [26]. Blood basophils also participate in allergic and other inflammatory reactions in ways somewhat similar to that of MC [27–29]. Various assays have been proposed to document MC and basophil activation [30,31]. These include identification of CD63 and CD203c [30–32]. However, while basophils are easily accessible for repeated investigations, MCs are only accessible when a tissue biopsy is performed.

MC activation disorders

The term MC activation disorder (MCAD) is used when specific criteria are fulfilled (Box 1) [33]. 'Activation' is usually inferred by increased serum tryptase levels, but this end point could be both inadequate and misleading since MC can release cytokines without any tryptase [8].

Symptoms alone and response or lack thereof to medication are not sufficient for diagnosis as many of the symptoms could derive from other pathological entities, whether they implicate MC or not. For instance, 'flushing' is also associated with menopause, carcinoid syndrome, pheochromocytoma and medullary carcinoma of the thyroid gland, as well as ingestion of niacin; cardiovascular disturbances may be related to myocardial infarction, endocarditis, aortic stenosis, autonomic dysfunction and postural orthostatic tachycardia syndrome; gastrointestinal problems may implicate irritable bowel syndrome, acute inflammatory bowel disease, food intoxication especially from spoiled scombroid fish (e.g., tuna) that generates large amount of histamine and neuroendocrine tumors such as vasoactive intestinal peptide; neurological symptoms may derive from bipolar disease, panic attacks, migraines, epilepsy and CNS tumors; respiratory signs may be part of asthma, 'heartburn' and angioedema (hereditary, acquired); and skin symptoms may be associated with acute toxic dermatosis, psoriasis, high parathyroid hormone, pemphigus vulgaris and acute lupus erythematosus.

In fact, in many cases, two or more of these other disease entities may coexist with MCAD making the diagnosis challenging. Therefore, a detailed medical history including type, duration and triggers of symptoms and careful and focused physical examination, as well as specific laboratory tests are necessary for a proper clinical evaluation. Family history is of great importance because the prevalence of MCAD is higher among relatives of MCAD patients than would be expected by chance [34].

Tryptase is a specific mediator for MC, and it is recommended as the marker of choice even though it is also released in small quantities by basophils [35,36]. Serum tryptase is usually detected with the B12 monoclonal antibody, which is bound with both α- and β-tryptase. The G5mAb is bound with 10-times more affinity to β-tryptase than to α-tryptase, so the α-tryptas is calculated as the difference between total and β-tryptase. The minimal increase in serum tryptase required as indicative of MC activation results from the following equation: 20% of baseline tryptase level + 2 ng/ml + the baseline tryptase level = the value above which the definition of MC activation is met [37], usually, although not necessarily, ≥20 ng/ml.

However, tryptase measurement could be affected by a number of conditions leading to misleading results: Rheumatoid factor, on rare occasions (<1% of assays), influences the tryptase assay leading to false-positive results. It was shown that the use of heterophile antibody blocking tubes resulted in the normalization of the elevated levels; however, the recent commercial immunoassays may correct these false-positive results; the levels of tryptase must be measured within 0.2–4 h after appearance of symptoms [37]. If a pretherapeutic baseline level

of serum tryptase is available, then a rapid increase is considered as a reliable index; if there is not, then the baseline has to be assessed after the patient's complete recovery or during a symptom-free interval at least 24–48 h after complete resolution of all symptoms [38–40].

Serum histamine is not particularly useful because it is metabolized very quickly and its assay results in false-positive tests. Its serum level may also be increased due to its release from basophils [35,36]; tissue injury, venous stasis, hemolysis or clotting during blood collection and sample preparation or improper centrifugation/storage; a diet rich in histamine [41]. The most informative test is the measurement of the metabolite *N*-methylhistamine (or 1-methyl imidazole acetic acid if available) in 24 h urine kept cold during collection while the patient avoids consumption of foods containing biogenic amines such as bananas and cheese.

Serum PGD_2 may be useful, but cannot be considered as a valid marker because its release can come from cellular elements through processing of peripheral blood samples due to a diet rich in niacin or niacin products used for atherosclerosis [42] and there are no proposed criteria for its minimal physiological increase.

In the absence of any inflammatory disease, serum IL-6 has been shown to correlate with disease severity [43,44].

Classification & diagnostic features of MCADs

MCADs are classified into three general categories (**Box 2**) [33,37].

Box 2. Classification of mast cell activation disorders.

Primary

- Systemic mastocytosis (indolent, aggressive, AHNMD)
- Cutaneous mastocytosis (urticaria pigmentosa, diffuse, telangiectasia macularis eruptiva perstans)
- Mast cell leukemia
- Mast cell sarcoma
- Extracutaneous mastocytoma (benign)
- Monoclonal mast cell activation syndrome

Secondary

- IgE-mediated hypersensitivity reactions (e.g., food, insect anaphylaxis)
- Drug induced (e.g., vancomycin, opioids, taxanes, muscle relaxants, adenosine, nonsteroidal anti-inflammatory)
- Mast cell hyperplasia (related with chronic infections, neoplasia, autoimmune conditions due to a possible excess of stem cell factor)

Idiopathic

- Mast cell activation syndrome or nonclonal mast cell activation disorder
- Idiopathic anaphylaxis

AHNMD: Associated with a hematological nonmast cell lineage disease.
Data taken from [33,37].

Primary

Systemic mastocytosis

Systemic mastocytosis (SM, indolent, aggressive, associated with a hematologic non-MC lineage disease) is defined by one major and one minor or three minor criteria established by the WHO (**Table 1**) [45].

SM is defined as histologically proven MC involvement of at least one extracutaneous tissue, and it is usually diagnosed in the bone marrow (BM). A recent publication of 21 patients evaluated for mastocytosis and 2 patients seen for follow-up of known mastocytosis who underwent bilateral iliac crest aspirations and biopsies were reviewed retrospectively to determine whether mastocytosis could be confirmed in each of a patient's biopsy specimens; 83% of patients had evidence of mastocytosis in each of their two iliac crest biopsies, but in 17% of them, there was evidence in only one of the two [46]. On the other hand, bilateral biopsies are not recommended because BM biopsy is a painful procedure and necessitates physicians' experience, and in some cases (40%), the number of MCs is low, and so, BMMC aggregates are absent; in addition, the low number of MCs at BM smears makes it difficult to identify the abnormal MC. Consequently, in such cases, very sensitive and specific methods must be used in order to identify abnormal MC (multiparameter flow cytometric immunophenotyping, analysis of the KIT mutational status on highly purified BMMC).The BM biopsies, which are performed only if it is very necessary and sensitive methods are available, are characterized by clusters (≥10–15) often being spindle-shaped, identified by immunohistochemistry for tryptase or CD_{117} (c-kit), the surface receptor for stem cell factor (SCF) (**Figure 1**). The markers of choice for screening tissue sections are antibodies against tryptase, CD_{117} and CD_{25}. Specifically, immunohistochemical findings of BM trephine biopsy specimens (**Table 2**) [45] have been proven as the main site for diagnosis. Instead, findings of GI tract mucosa are variable and not reliable [47]. CD_{117} is considered as the marker of choice for the GI tract, especially in the portal triads [47].

Patients who have one of the following characteristics should also be considered candidates for BM biopsy: tryptase >20 ng/ml; tryptase >11.4 ng/ml or greater than the upper limit of normal; in patients with previous hypotensive episodes and/or syncope; urticaria pigmentosa (UP) present in adult patients; unexplained or explained anaphylaxis provoked with hymenoptera sting even when specific IgE testing to hymenoptera is negative [46,48,49], indicating that the most important key factor for the BM biopsy is not the specific IgE in the patients with hymenoptera anaphylaxis, but their level of tryptase (>11.4 ng/ml). Specifically, recent large series of patients with indolent SM (ISM) without skin lesions associated with hymenoptera venom anaphylaxis ($ISMs^-$) have shown coexistence of both negative skin tests and, usually, low total serum IgE levels. These patients may represent a unique clinical subtype of SM who showed clinical, biological and molecular characteristics that differed significantly from classical ISM presenting with skin lesions ($ISMs^+$). Patients with $ISMs^-$ are predominantly male, with hypersensitivity to hymenoptera venom, serum

Table 1. Diagnostic criteria for the establishment of systemic mastocytosis.

Major criterion	Multifocal, dense infiltrates of mast cells (≥ 15 mast cells in aggregates) detected in intramedullary biopsy sections and/or extramedullary organ(s).
Minor criteria	In intramedullary biopsy sections or other extramedullary ones, >25% of the mast cells in the infiltrate are spindle shaped or have atypical morphology, or of all mast cells in bone marrow aspirate smears, >25% are immature or atypical. Activating point mutation of c-KIT at codon 816 (usually KIT D816V) in bone marrow, blood or other extracutaneous organ. Aberrant immunophenotype of mast cells of CD_2 and/or CD_{25} in bone marrow, blood or other extracutaneous organ, in addition to normal mast cell markers. Persistently elevated baseline serum total tryptase (>20 ng/ml). In the occasion that a clonal myeloid disease exists, this criterion is considered invalid.

Data taken from [45].

baseline tryptase levels <20 ng/ml, less MC-related symptoms in between acute episodes and KIT mutation typically restricted to MC. They present with a wide range of heterogeneous MC activation-related symptoms that frequently overlap with those of allergic diseases. Therefore, these cases may be misinterpreted as just presenting an allergic condition with no further investigation being indicated to rule out an associated mastocytosis, which in turn is a well-known risk factor for adverse reactions to venom immunotherapy [50]; presence of unexplained osteoporosis, hepatomegaly, splenomegaly in patients with MCAD (suspicion for an aggressive variant of SM) [46]; abnormalities on peripheral blood count in patients with a MCAD (several hematological non-MC lineage disorders may be related with SM, chronic myelomonocytic leukemia and myelodysplastic syndrome, but also with other myeloproliferative neoplasms and lymphoproliferative diseases) [51].

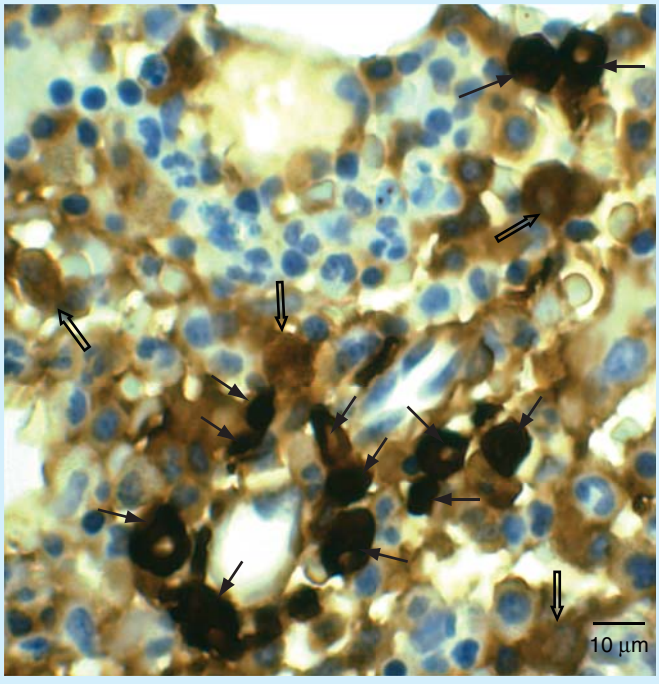

Figure 1. A photomicrograph of bone marrow biopsy from a patient (JMS) with indolent systemic mastocytosis stained for tryptase showing pathological aggregated clusters of mast cell (⟶) for systemic mastocytosis, some of which are at different stages of degranulation (⟹).

The most common mutation found in MCADs is the point mutation found at codon 816 of c-KIT that affects MC in different ways: it promotes the autonomous growth and expansion of the neoplastic MC [52]; it can trigger MC to release IL-6 in high concentrations [53]. The D816V mutation is also expressed in people in whom neither criteria for SM nor for cutaneous mastocytosis (CM) are fulfilled [22,33,49,54–56] and may contribute to the symptoms of MC activation syndrome (MCAS); specifically, in MCAS cases, in which only KIT mutation is presented, they are defined as monoclonal MC activation syndrome (MMAS). There are other rarer, but well characterized, functionally activating mutations of KIT (e.g., D419H, V560G), which do not induce detectable morphological alterations of affected MC. Nevertheless, these mutations have been reported to influence the evolution of the disease and/or the manifestation of certain symptoms [57]. Finally, a novel oncogenic mutation (*FIP1L1–PDGFRA*), which results in a constitutively activated PDGFRA, has been invariably associated with a primary eosinophilic disorder. In an associated study, 10 (56%) of 19 patients with systemic MC disease associated with eosinophilia carried the specific mutation. *FIP1L1–PDGFRA* is a relatively infrequent but treatment-relevant mutation in primary eosinophilia that is indicative of an underlying SM [58].

CM (UP, diffuse, telangiectasia macularis eruptive persistans)

In this subcategory of the primary MC disorders, the only organ that is affected is the skin, typically presenting as UP. CM is rarely associated with systemic disease (even if systemic symptoms appear due to local release of mediators from skin MC), and therefore BM biopsy is not recommended. Noteworthy, CM in most children regresses before their puberty [59]. However, increased serum baseline total tryptase, in association with extensive cutaneous involvement, identifies children at risk for severe MC activation events [57]. On the contrary, most adults with CM have an underlying SM and so they should undergo a BM biopsy regardless of the presence of associated systemic symptoms of mediator release [60]. Finally, CM is never present in MMAS and MCAS.

Table 2. Mast cell activation disorders and related immunohistochemical findings of bone marrow trephine biopsy specimens.

MCADs	Immunohistochemical findings of bone marrow trephine biopsy specimens
SM (mainly indolent/bone marrow mastocytosis, rarer aggressive or leukemic)	Multifocal compact mast cell infiltrates
SM (indolent/bone marrow mastocytosis)	Increase in loosely scattered spindle-shaped mast cells with CD25 expression, KIT D816V mutation and chronically elevated serum tryptase, but without compact tissue infiltrates
MMAS	Increase in loosely scattered spindle-shaped mast cells with KIT D816V mutation, ambiguous presence of CD25 (±) and normal serum tryptase
Secondary (mast cell hyperplasia) and Idiopathic MCAD	Increase in loosely scattered round mast cells without CD25 and KIT D816V

MCAD: Mast cell activation disorder; MMAS: Monoclonal MC activation syndrome; SM: Systemic mastocytosis.
Data taken from [45].

MC activation is profoundly affected by stress. In one case, UP expanded dramatically after emotional stress [61]. Our laboratory showed that corticotropin-releasing hormone stimulates human MC directly [62,63].

Monoclonal MC activation syndrome

MMAS is limited to patients with systemic reactions to hymenoptera stings and increased baseline serum tryptase or in patients with unexplained episodes of anaphylaxis. In these occasions, BM biopsy detects monoclonal MC that carries the mutant KIT D816V also detectable in BM aspirate MC [64] and/or aberrant expression of CD_{25}; however, criteria for diagnosis of SM are not fulfilled as only one or two minor criteria are met [46].

In conclusion, while the symptoms of MC mediators are present in all of the primary MCADs, the features below appear specifically: multifocal MC aggregates and possible UP only in patients with SM; D816V KIT mutation and aberrant CD_{25} expression on BM MC in patients with SM, MMAS; baseline tryptase and baseline 24-h urine methylhistamine or PGD_2/11β-$PGF_{2\alpha}$ in patients with SM and maybe in those with MMAS (and MCAS) and the response to antimediator therapy is different as it is beneficial in patients with SM, MMAS and possibly MCAS, but variable in patients with idiopathic anaphylaxis (IA) [33].

Secondary

The disease presentation varies from sporadic to chronic, and the observed symptoms can be temporary or frequent. In these patients, no mutation of KIT D816V is found, and flow cytometry shows that MCs are CD_{25} negative [37].

- IgE-mediated hypersensitivity reactions (e.g., food, insect) due to aggregation of high-affinity IgE receptors (FcεRI) by allergen-bound IgE.
- Drug induced (e.g., adenosine, muscle relaxants, nonsteroidal anti-inflammatory, opioids, taxanes, vancomycin).

Such drugs can cause MC activation symptoms either directly or indirectly through G-activated proteins [65].

- MC hyperplasia (related with chronic infections, neoplasia, autoimmune conditions due to a possible excess of SCF).

This subvariant includes different disease states that can cause MC activation through different mechanisms. Psoriasis and inflammatory bowel disease are characterized by aberrant IFN-γ, which triggers MC degranulation, through upregulation of high-affinity IgG receptors [66]. MC in patients with rheumatoid arthritis is activated through C3a and C5a by their direct binding to their respective receptors on the MC surface [66]. Patients with infectious diseases involve MC activation through the direct activation of toll-like receptors that recognize molecular patterns common to microbial or viral pathogens. These reactive states are rare causes of MCADs [37].

Idiopathic

The disorders in this subcategory have no identifiable causes, or at least their causes have not been identified yet. In these cases, the criteria for MCADs are fulfilled, but there is no detectable clonal MC, no underlying reactive disease and no allergen-specific IgE. Environmental or endogenous stimuli, intrinsic MC defects or both may be possibly considered as causes of the idiopathic MCADs.

MCAS or nonclonal MCAD

In nonclonal MCAD, MC activation may be caused by various external stimuli, lower threshold that MC possess for their activation, or even a normal release of MC mediators, but with an abnormal response of the surrounding tissues (e.g., deficiency of catabolic enzymes such as diamine oxidase and histamine *N*-methyltransferase).

This condition can be best described by aberrant MC activation [67]. Diseases (carcinoid syndrome, pheochromocytoma, gastrinoma) that could explain symptoms have to be ruled out through specific diagnostic tests. Then, MC clonality is examined in order to classify the disease as one of the primary MCADs. If clonality is absent, then MC mediators (serum tryptase, *N*-methylhistamine, PGD_2) are measured in symptomatic patients. If these are not elevated, then further clinical evaluation is necessary [60]. MCAS may be an underlying cause

of various other frequent clinical presentations like in subsets of patients with chronic fatigue syndrome, fibromyalgia [68,69] and irritable bowel syndrome [47,70].

Idiopathic anaphylaxis

These patients are characterized by: life-threatening anaphylactic reactions; increased baseline levels of tryptase between the episodes; absence of MC clonality and acute onset of the disease. Anaphylaxis may be different in patients with IA and in patients with indolent SM because urticaria is absent during anaphylactic episodes and serum tryptase levels are significantly elevated, while in patients with IA, urticaria is present in higher frequency and serum IgE is significantly elevated [54].

Treatment of MCADs

Unfortunately, no curative treatment exists for MCADs at the present time. Recognition and avoidance of triggers and good control of symptoms for increased quality of life are the first aim of treatment. Prevention of MC proliferation and release of MC mediators is the ultimate goal.

The first step in these patients' management is the avoidance of causative agents of MC activation, if they can be identified. Patients should be advised to keep track of provoking factors. Alcohol, preservatives, spices, heat, cold, drugs (antibiotics, nonsteroidal anti-inflammatory drugs [NSAIDs], narcotics, neuromuscular blocking agents – ketamine, propofol and tramadol are better tolerated), radiocontrast media, certain general anesthetics, hymenoptera stings, infections, physical stimuli (pressure, friction), sex hormones and stressful conditions are examples of such triggers. Some of these triggers may be related more with one of the MCADs such as alcohol and heat with MCAS [55]. In some occasions, these triggers may be the only manifestation of a MCAD as in hymenoptera stings, MMAS and MCAS [71].

Patients are advised to carry diphenhydramine (Benadryl, preferably liquid gels) and self-administered intramuscular epinephrine.

Antihistamines & MC membrane 'stabilizers'

Standard treatment in any patient with MCAD includes H_1, H_2 receptor antagonists (Ra). Second-generation H_1 Ra (loratadine, cetirizine, fexofenadine) are generally preferred because of less sedation and are the drugs of the choice used once or twice daily [72]. However, for neuropsychiatric symptoms, or before or during invasive procedures (radiology studies), the first-generation H_1 Ra hydroxyzine may be used. Ketotifen, an H_1 Ra and weak ocular MC inhibitor [73], is useful. A number of patients with MCADs present with eosinophilic esophagitis in which case, the H_1 Ra, antiplatelet-activating factor and antieosinophilic drug rupatadine may be useful [74].

Disodium cromoglycate (cromolyn sodium), a 'MC stabilizer', controls mostly GI symptoms, such as abdominal bloating and diarrhea, although it may be beneficial for other MC activation manifestations like dermal and neuropsychiatric ones. Recent data have cast some doubt on the ability of cromolyn to stabilize MC [75], and its beneficial effect on pruritus might be mediated by inhibition of C-fiber sensory nerves rather than MC [76]. Cromolyn is administered orally and is divided into doses with weekly titration [77]. H_2 Ra (ranitidine, famotidine) is used either additionally to enhance the results of H_1 Ra or supportively to block gastric hypersecretion, and they are administered once or twice daily [77].

Leukotriene blockers

Increased secretion of leukotriene C_4 has been reported in mastocytosis [78]. The cysteinyl leukotriene receptor H_1 Ra montelukast may be used in patients with refractory symptoms, especially those with pulmonary symptoms (asthma, wheezing).

Acetylsalicylic acid & other NSAIDs

Some patients with likely diagnosis of MCAS have overproduction of prostaglandins instead of histamine [78]. These patients were refractory to antihistamines, but respond in addition to or treatment with acetylsalicylic acid. The effects of this therapy may be ambiguous. Patients with refractory flushing and elevated urinary 11β-$PGF_{2\alpha}$ may benefit, but up to 650 mg twice daily may be required for clinical improvement [77,79]. Others, however, may experience hypersensitivity reactions [78,80]. Drug challenge and possible desensitization should therefore be considered if necessary. Treatment with H_2 Ra should be recommended if symptoms with gastric reflux persist. Patients who do not tolerate acetylsalicylic ac may be able to use other NSAIDs at similar doses.

Omalizumab

Omalizumab consists of an anti-IgE-humanized murine monoclonal antibody that has been already used with success in disorders in which MCs are pathogenetically involved (allergic asthma, drug allergies, idiopathic angioedema, chronic urticaria). Therefore, in the occasions of MCAD, where symptoms are frequently resistant to treatment, therapy with this antibody seems worth considering.

Omalizumab reversibly binds to the Fc portion of the free serum IgE molecules, forming noncomplement-fixing complexes. Therefore, free serum IgE is depleted and so is its binding to the high-affinity FcεRI expression on MCs and basophils. As a consequence, FcεRI expression is downregulated by both basophils (faster) and (dermal) MCs (slower), and the possibility of reactivity of these cells is also reduced. The fact that the symptoms in MCAD patients' are slowly improved indicates that omalizumab in these patients acts predominantly at MCs. In the occasion that the reduced binding of IgE to MCs may not only inhibit the MCs for a subsequent antigen challenge, but may also by itself inhibit MCs to trigger other immune effector cells [81], then this is an additional way that omalizumab, through the free IgE decrease, may antagonize the trigger effects *in vivo*. Finally, it is likely that the regulatory influences of omalizumab on basophils or other immunoregulatory cells are not non-IgE

mediated, given that omalizumab therapy is beneficial in MCAD patients' in whom IgE levels were not pathologically increased [82]. However, omalizumab treatment targets components of the immune system and therefore may potentially increase the risk of infections or neoplasms.

Taken together, omalizumab treatment can successfully alleviate high intensity symptoms of MCAD, but that is not a curative therapy. Since treatment with omalizumab has an acceptable risk–benefit profile, it should be considered in cases of MCAD at an early stage as an experimental therapeutic option in resistant to evidence-based therapy.

Corticosteroids

Corticosteroids are of great importance in patients with refractory signs and symptoms especially in the event of anaphylaxis. Resuscitation from an acute episode of anaphylaxis is achieved with the use of epinephrine and the control of symptoms with H_1, H_2 Rα, along with corticosteroids β-agonists (when bronchospasm presents) [77]. However, there are reported instances of sensitivity to steroids and they should be avoided to the extent possible.

Venom immunotherapy

Treatment with lifelong venom immunotherapy is considered necessary by most experts in patients with MMAS after hymenoptera stings, especially if they test positive for venom-specific IgE [83].

Specific agents

Patients with advanced SM without the D816v c-kit mutation may be treated with a tyrosine kinase inhibitor (TKI), Imatinib (Glivec). The importance of this drug is great, given that it acts on a specific target – only cancer cells are killed through its action. Other TKI drugs are dasatinib and midostaurin. On the one hand, dasatinib (Sprycel) inhibits D816V c-kit *in vitro* but has not been proved successful in clinical trials. Midostaurin can inhibit mutated c-kit MC, with early results being promising although not curative, but it can also inhibit non-mutated c-kit MC. Patients who develop complications associated with advanced forms of SM (such as osteoporosis, bone lesions, hepatomegaly, splenomegaly, cytopenias) may also use TKIs. Nevertheless, it is of great importance to highlight that these therapies can have major toxicity and can change the natural course of the disease. This is the reason why these therapies are usually not indicated.

Natural flavonoids

The natural flavonoids quercetin and luteolin have potent anti-inflammatory and MC inhibitory actions [12,84]. Luteolin inhibits oxidative stress [84], inflammation [84], MC degranulation [85], MC cytokine release [86], microglial activation and proliferation [87,88] and autoimmune T cell activation [12,89]. Luteolin is neuroprotective [90] and mimics brain-derived neurotrophic factor [91] that may be useful in those patients with peripheral neuropathy or neuropsychiatric symptoms. A luteolin-containing formulation was recently shown to have significant benefit in ASD children [92,93].

Luteolin and quercetin are generally safe [94] and can even protect against chemically induced liver toxicity, a common consequence of many drugs [95].

Expert commentary

There has been considerable progress in defining c-kit mutations and developing drugs that block tyrosine kinases that are involved in MC proliferation. However, our understanding of non-IgE-mediated MC activation remains poor, and there are still no effective clinically available MC inhibitors. One of the possible reasons for this problem has been the over-reliance on the use of rodent MC, the results from which increasingly have been shown not to reflect the human conditions, as well as the recognition that MC from different tissues behave differently. Moreover, MC lines are derived from patients with MC leukemia that do not behave like normal MC, while BM- or umbilical cord-derived human MC an immature, require 12–15 weeks of growth before use and need SCF for culture, which is increasingly difficult to obtain.

Five-year view

Future research efforts should focus on: establishing normal MC cultures that mimic different tissue MCs; investigating the ability of MC to respond to different triggers with selective release of mediators; identifying the molecular event involved in MC activation and inflammatory mediator synthesis and release; studying the role of growth factors other than SCF such as NGF and PDGF and the role of mammalian target of rapamycin (mTOR); identifying innate triggers such as adrenomedullin corticotropin-releasing hormone, parathyroid hormone; defining synergistic actions of triggers such as SP an IL-33; and selecting/synthesizing luteolin analogs with better inhibitory/absorption profile.

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Key issues

The term MCAD is used when specific criteria are fulfilled. A careful medical history (including the familial history), physical examination and specific laboratory tests are necessary for proper clinical evaluation.

- Tryptase is a rather specific mediator for MCs, and it is recommended as the biomarker of choice even though IL-6 may better reflect disease activity in the absence of inflammatory diseases. Urine (24 h) histamine (serum, urine) metabolites and PGD_2 are helpful.
- MC activation disorders are classified into three categories: primary, secondary and idiopathic.
- The establishment of SM requires one major and one minor or three minor criteria.
- Screening tissue sections for MCs should use antibodies against tryptase, CD_{117} or CD_{25}. Degranulated MC can be assessed with tryptase staining. In certain cases, bilateral BM biopsies might provide higher yield [96].
- Secondary MCADs are triggered by allergic or nonimmune stimuli, but do not involve MC clonality.
- Idiopathic MCADs fulfill the criteria of MCADs, but there is no detectable clonal MC, no underlying reactive disease and no allergen-specific IgE.
- There is no curative treatment for MCADs at the present time. Prevention of the MC triggers and reduction of the effects of MC mediators are main goals. Good control of symptoms with combination of different agents for improved quality of life is the first aim.

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