Learning Objectives & Disclaimers

- Learning Objectives
  - Review basic concepts regarding mast cell activation disorders (MCAD, including mast cell activation syndrome (MCAS))
  - Review current evidence of association between MCAD and hypermobile Ehlers Danlos Syndrome (hEDS)
  - Review mechanisms by which MCAD might cause hEDS
  - General approach to diagnosis and treatment of MCAS
- Conflicts of Interest
  - None
- Note that there are not yet any FDA-approved treatments for MCAS, and not even (yet) any "well-designed, non-randomized clinical trials," let alone (yet) any "high-quality randomized controlled clinical trials" (there just hasn't been time yet for such trials!), so by definition, all treatment options discussed in this presentation are ACCME Level Of Evidence (LOE) "C" ("consensus viewpoint or expert opinion").

Outline

- What is mast cell activation disease (MCAD)?
  - What we've long known:
    - Allergic diseases.....
    - Mastocytosis.....
  - What's new:
    - Mast cell activation syndrome (MCAS).....
      - Basic behavior of the disease
      - General approach to diagnosis and treatment
      - Key issues in nursing care for MCAS patients
  - Research issues

General Clinical Theme
Allergy ± Inflammation
MC Neoplasia ± Allergy ± Inflammation
Inflammation ± Allergy ± Aberrant Growth (Dystrophism)

Three ~~Two~~ cases…

"Polycythemia vera"

"Pure red cell aplasia"

and "Burning Mouth Syndrome"?

"Polycythemia vera"

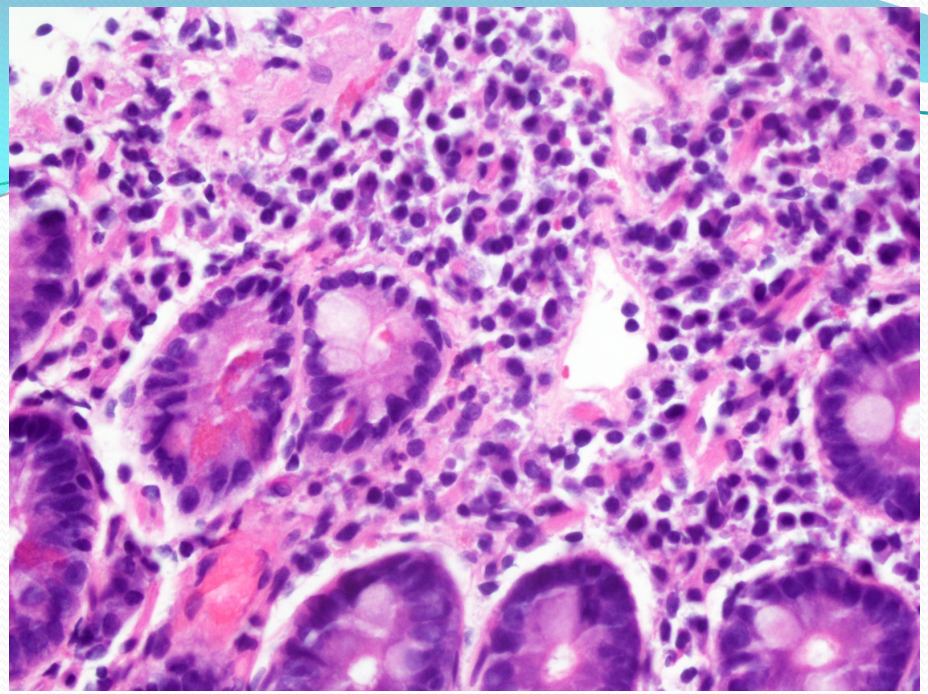
- 1980s: healthy 30ish woman notices migratory rash
- Over time: fatigue, itching, vertigo, falls; evals negative
- 2005: mildly elevated hemoglobin; polycythemia vera (PV) diagnosed (incorrectly), standard therapy begun
- Steadily worsened (migratory GI symptoms, labile BP/pulse, poor healing, episodic shortness of breath, frequent upper respiratory "infections" with no infectant ever found, rashes to all antibiotics), had to close her business
- 2008: self-referred for further eval

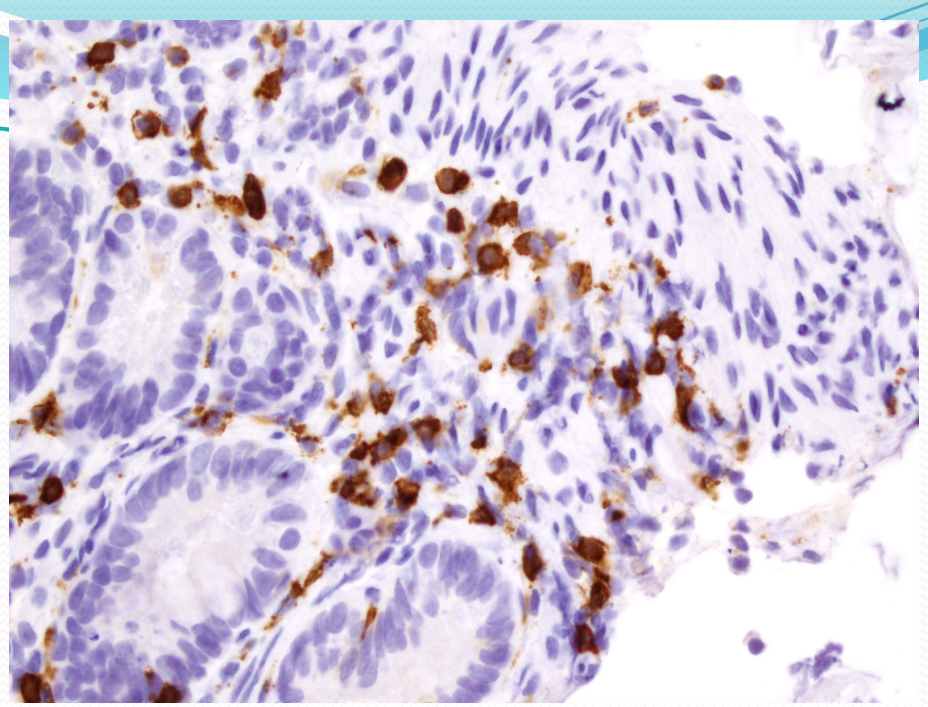
Afrin LB. Polycythemia from Mast Cell Activation Syndrome: Lessons Learned. *Am J Med Sci* 2011;342:44-49.

"Polycythemia vera"

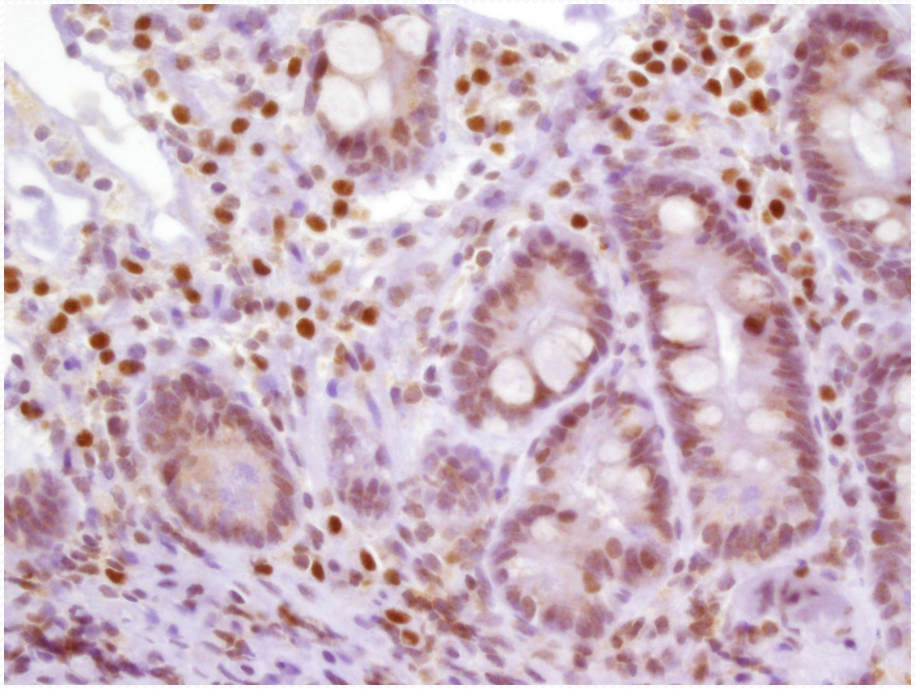
- No sleep apnea, no relevant family/social/medication history
- Exam: looked tired, nothing else
- Doesn't fit PV; what else is driving her issues including ↑hemoglobin?
  - Extensive additional testing proves she doesn't have PV – and fails to find any other known cause of ↑hemoglobin
- Possible fit with mastocytosis; is that what it is?
  - No?: serum tryptase, urine N-methylhistamine normal; marrow and rash biopsies show no mastocytosis
  - Yes?: sl. ↑ urinary prostaglandin D_2
  - EGD/colonoscopy: normal, but biopsies taken anyway…
  - …all "textbook normal" on H&E, but on IHC…

Afrin LB. Polycythemia from Mast Cell Activation Syndrome: Lessons Learned. *Am J Med Sci* 2011;342:44-49.





CD117
40x



CD25
40x

- Dx: “atypical mastocytosis”?
- Low-dose imatinib begun
  - 100 mg/d x 1 week, then
  - 200 mg/d
- The first week: tolerated fine, but no response
- And then, on waking the morning after the fourth dose of 200 mg……

“Polycythemia vera”

- All symptoms acutely gone.
- Improvement sustained >10 years now.
- All labs normalized.
- Resumed exercise and full-time work.

Afrin LB. Polycythemia from Mast Cell Activation Syndrome: Lessons Learned. *Am J Med Sci* 2011;342:44-49.

"Pure Red Cell Aplasia"

- 2004: 50ish woman, worsening fatigue; severe anemia
- Dx: idiopathic pure red cell aplasia (PRCA, confirmed)
- Refractory to all standard treatments for PRCA
- Needing 3 units of blood every 2-3 wks to maintain merely half-normal hemoglobin (Hgb) level
- 2009: 6th opinion: ROS pan-⊕, $uPGD_2$ ↑↑↑, Dx: "MCAS"
- Antihistamines: Good Hgb ↑ in 4 weeks, no transfusions
- Imatinib 200 mg/d added: Hgb normalized in 6 weeks
- "PRCA" relapsed 1 yr later
  - Tried cromolyn (previously precluded by insurer): remission again in 4 wks

Afrin LB. Pure red cell aplasia masquerading as mast cell activation disorder. *Int J Hematol* 2010 Jun; 91:907-908.

"Burning Mouth Syndrome"

- 2004: 50ish woman, new constant "burning" pain throughout GI tract, pain score 10/10 in mouth
- Very extensive evaluations over a year all negative except for finding mild chronic stomach inflammation and, finally, a 100-fold elevated serum chromogranin A (CgA) (not on PPIs)
- Neuroendocrine (NE) malignancy?
  - Miserable from pain, but didn't look like she had cancer of any sort
  - Extensive cancer search negative
  - Top five U.S. NE cancer experts consulted
    - Unanimous opinion: ↑↑CgA must be due to NE cancer, keep looking
- MC disease?
  - Blood/urine markers normal; marrow, oral mucosa biopsies normal

Afrin LB. Burning Mouth Syndrome and Mast Cell Activation Disorder. *Oral Surg Oral Med Oral Path Endodontol* 2011;111:465-472.

"Burning Mouth Syndrome"

- Early '09: Revisited old gastric biopsy with CD117 staining, showing ↑↑MCs (but not in pattern suggestive of mastocytosis)
- Dx: mast cell activation syndrome (MCAS)
- Antihistamines/NSAIDs: Pain ↓ to 1/10 overnight
- MCAS found in every subsequent "idiopathic" BMS patient I've examined
  - Different abnormal MC mediator patterns in blood/urine in different patients
  - ↑MCs in GI tract biopsies when checked
  - All responding to various MC-targeted therapies

Afrin LB. Burning Mouth Syndrome and Mast Cell Activation Disorder. *Oral Surg Oral Med Oral Path Endodontol* 2011;111(4):465-472.

Highly divergent presentations, but…

- …same root disease?
- How can “one disease” (MCAS) do this?
- Could other “weird” presentations be possible?

But hold on a second. Before talking more about this “new” mast cell disease, let’s back up to look at what we’ve long known about diseases of the mast cell…

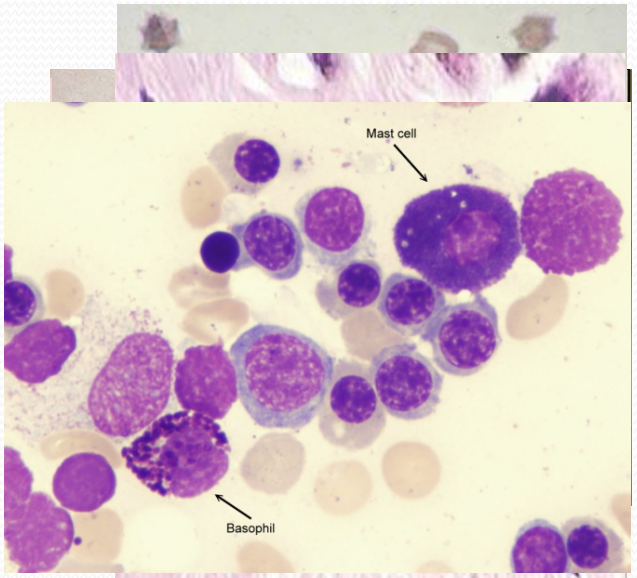
Allergic Diseases

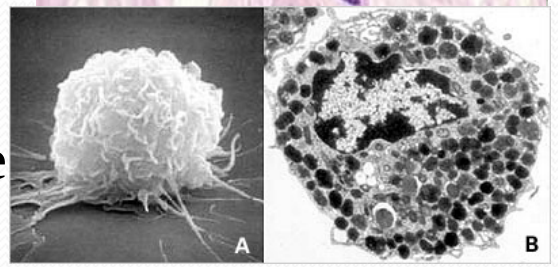
- Allergy, asthma, angioedema, urticaria, anaphylaxis
- 2013: 700 million suffer allergic diseases worldwide
  - 10% of preschoolers worldwide now have food allergies
- Steadily increasing incidence/prevalence across all ages
  - e.g., China (prevalence): 1999: 3.5%; 2009: 7.7%
  - Greatest increases in children < 5 years old
- Allergic diseases are conditioned by a number of genes and influenced by environmental factors
  - Incidence of allergic disease in children if…
    - …neither parent suffers allergic disease: 5-15%
    - …only one parent suffers allergic disease: 20-40%
    - …both parents suffer allergic disease: ≥ 60%
- Relatively little mortality, but significant QoL effects

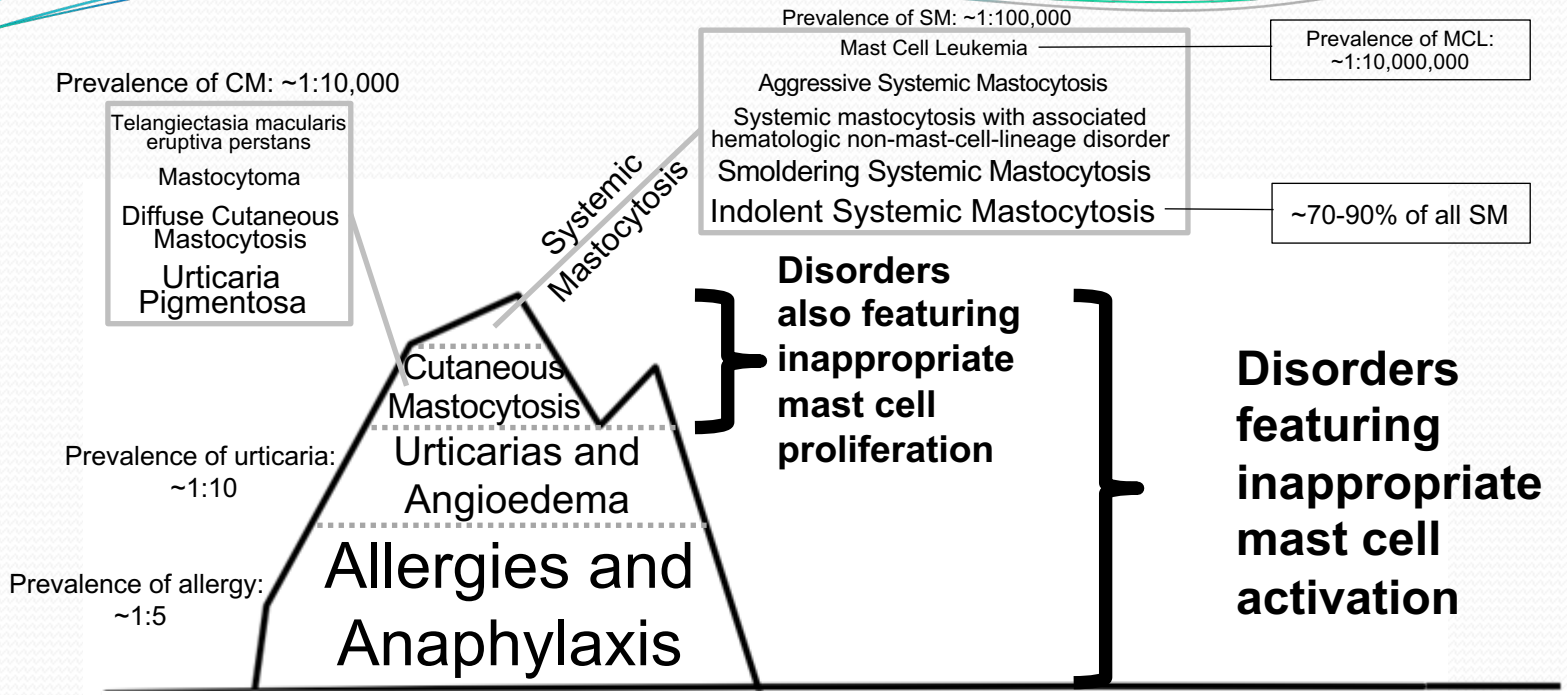
1. Prescott SL et al. A global survey of changing patterns of food allergy burden in children. World Allergy Organiz J 2013;6:21, pp. 1-12.
2. Pawlinska-Chmara R et al., Effect of Socio-Economic Status on Quality of Life in People Affected with Respiratory Allergy, pp. 385-392, in M. Pokorski (ed.), *Neurobiology of Respiration*, Advances in Experimental Medicine and Biology 788, DOI 10.1007/978-94-007-6627-3_52, Springer Science+Business Media Dordrecht 2013.

Mastocytosis: A Long History

- 1869: Urticaria pigmentosa (UP) first described
- 1877: First description of the *mastzelle*
- 1887: UP linked with *mastzelles*
- 1933: Suggestion of linkage with internal dz
- 1939: MC heparin identified
- 1949: Definitive linkage with systemic dz
- 1953: MC histamine identified
- 1984: First conception "MCAS" might exist
- 1987: MC tryptase identified
- 1988: Travis classification
- 1995: KIT activating mutation D816V identified
- 1998: Unique flow cytometric signature found
  - CD117 + (CD25 and/or CD2)
- 2001: WHO classification and imatinib
- MC <u>neoplasia</u> is morbid only in rare, aggressive forms; MC <u>activation</u> is what causes symptoms





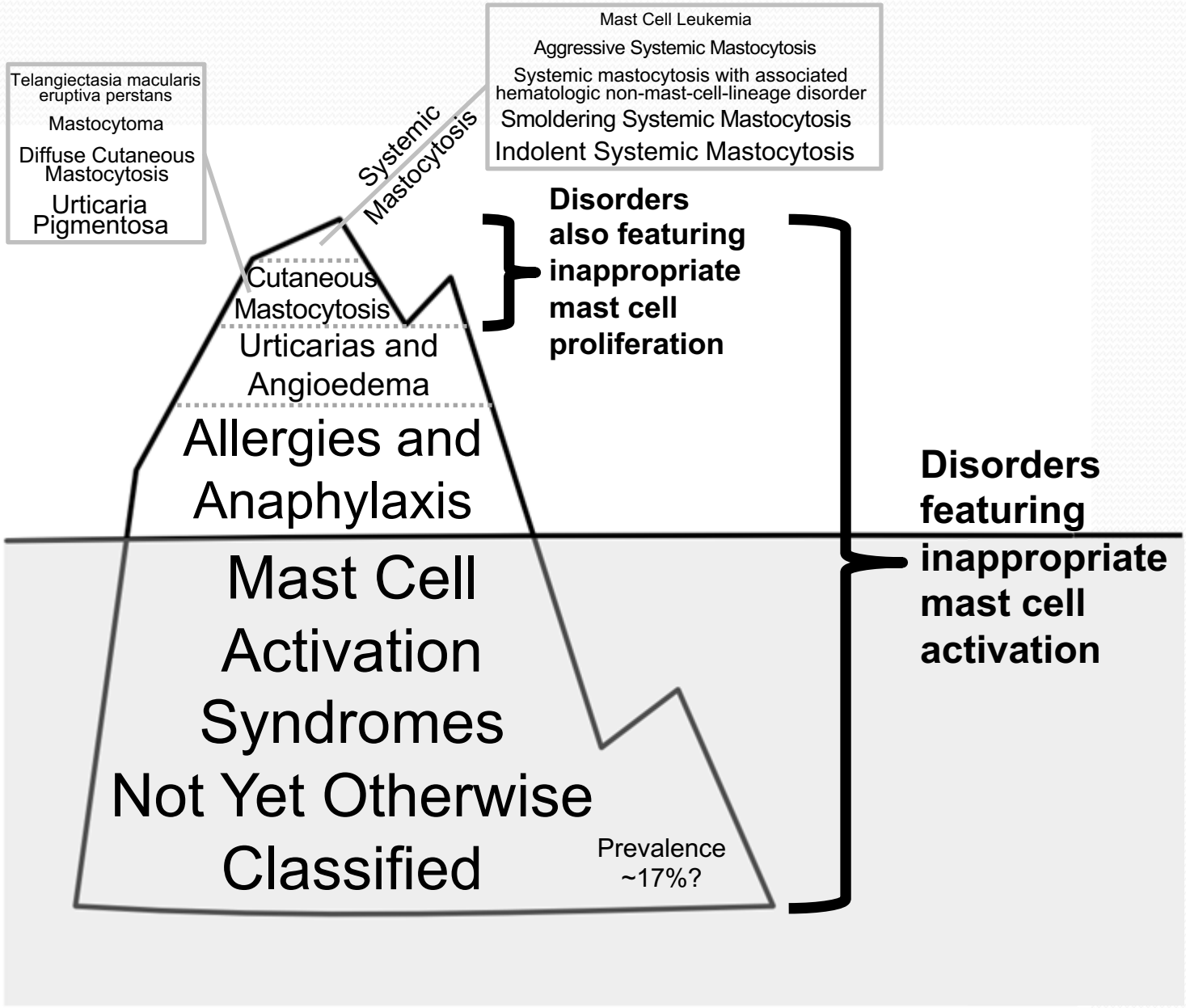


The Spectrum of Mast Cell Disease We've Long Known

MCAD: A Brief History

- 1984-91: 1st published hypotheses that MCAS ought to exist
- 2007: 1st case reports of MCAS
  - Some with KIT-D816V, some without
- 2007: 1st study showing other KIT mutations in most MCAS (Bonn)
- 2008-: Non-KIT mast cell regulatory gene mutations found in SM
- 2010: 2nd study showing KIT mutations in most MCAS (Bonn)
  - KIT-D816V rare
  - Few mutations in controls
- 2010: Proposal of "MCAD" (Harvard, Vienna, NIH)
  - Includes 1st proposal for MCAS diagnostic criteria
- 2011: Alternative proposal for MCAS diagnostic criteria (Bonn, MUSC)
  - Revised 2016, 2017
- 2012: Revised (Vienna et al.) proposal for MCAS diagnostic criteria
  - Still problematic
  - "Updated" again in 2019 – no different than 2012
- 2016: Revised WHO diagnostic criteria for SM
  - Mastocytosis now separate from the myeloproliferative neoplasms (MPNs)
  - "Smoldering SM" added; "SM-AHNMD" shortened to "SM-AHN"
  - No statement regarding MCAS

MCAD: Emerging Understanding




Mast Cell Activation Disease
Mast Cell Leukemia
Aggressive Systemic Mastocytosis
SM-AHNMD
Indolent Systemic Mastocytosis
Cutaneous Mastocytosis
Mast Cell Activation Syndrome
0.0000001%?
Increasing prevalence
17%?

Normal mast cell biology

- Hematopoietic origin, brief circulation
  - Normally 0.05% of marrow nucleated cells
  - Typically < 2% even in systemic mastocytosis
  - Unique flow cytometric signature (incl. CD117+, CD25/2-)
- Maturation completed in all vascularized tissues
  - Especially abundant beneath environmentally exposed mucosal/epithelial surfaces and adjacent to blood and lymphatic vessels and nerves, permitting sentinel function
- Relatively immobile once localized in peripheral tissue
- Lifespan typically several months to a few years

Kalesnikoff J, Galli SJ. New developments in mast cell biology. *Nature Immunology* 2008;9:1215-23.
Ribatti D. The development of human mast cells: an historical reappraisal. *Exp Cell Res* 2016;342(2):210-5.

Normal mast cell biology

- Functions (when appropriately stimulated):
  - Synthesize active substances
    - Some stored in granules of highly heterogeneous content
  - Release various mediators upon various triggerings
  - Phagocytose particulate material including bacteria, erythrocytes, schistosomes, metals, etc.
- KIT stem cell factor receptor and tyrosine kinase (on 4q11-12) is expressed at high levels on the mastocyte surface
  - Critical for many mast cell functions including survival, differentiation, chemotaxis, and activation

Akin C, Metcalfe DD. The biology of Kit in disease and the application of pharmacogenetics. *J Allergy Clin Immunol* 2004; 114:13–19.
Gordon JR *et al.* Mast cells as a source of multifunctional cytokines. *Immunol Today* 1990;11:458.
Bradding P *et al.* Heterogeneity of human mast cells based on cytokine content. *J Immunol* 1995; 155:297.

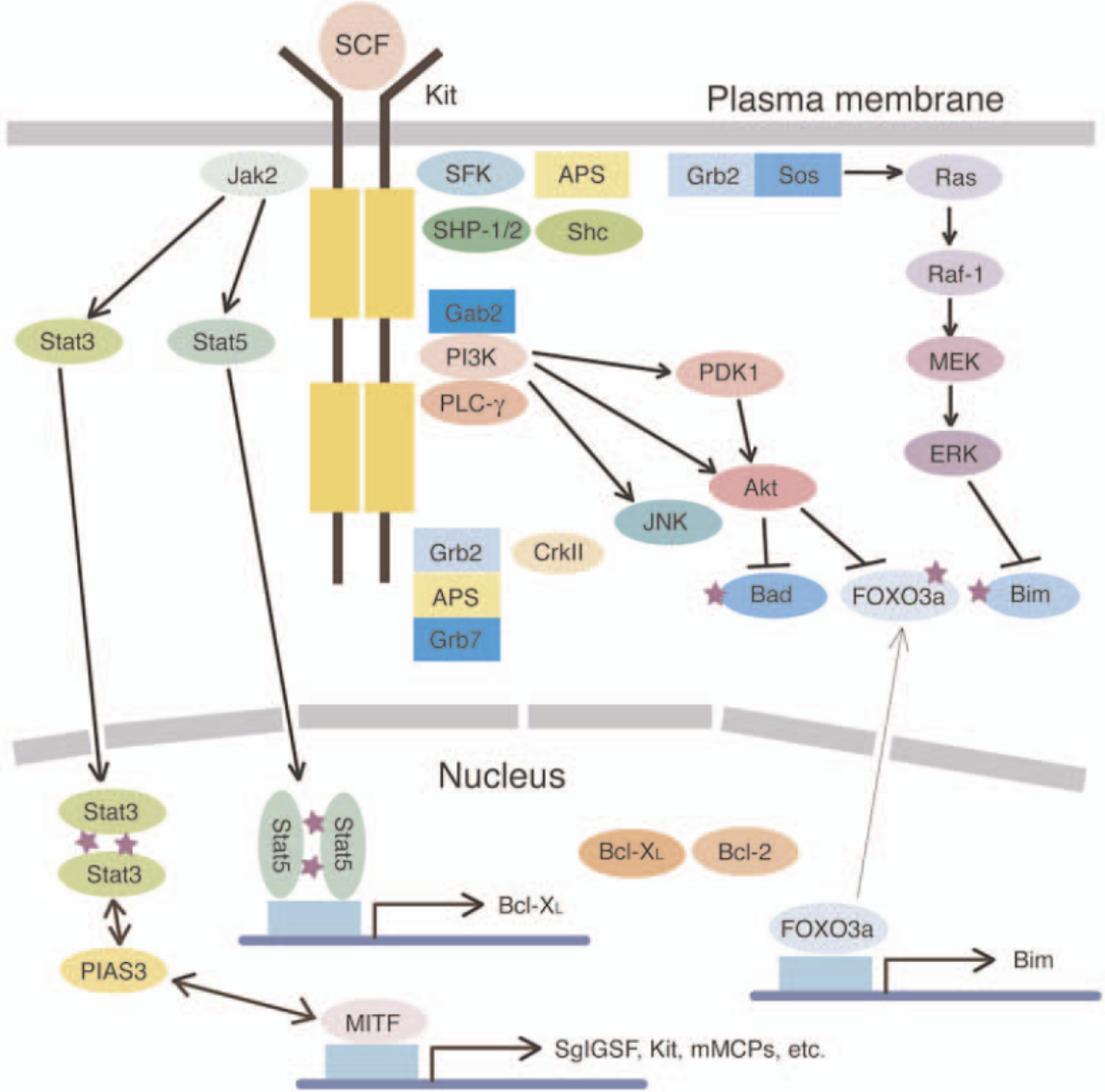


Fig. 2. Kit signaling pathways in mast cells. This figure summarizes signaling proteins activated by Kit. Filled boxes of Kit indicate split kinase domains. Abbreviations: APS, adaptor containing PH and SH2 domains; Grb, growth factor receptor-bound protein; JAK, Janus kinase; JNK, c-Jun NH2-terminal kinase; MEK, mitogen-activated protein kinase kinase; ERK, extracellular regulated protein kinase; MITF, microphthalmia transcription factor; PI3K, phosphatidylinositol 3-kinase; PLCγ, phospholipase Cγ; SCF, stem cell factor; SFK, Src family kinases; Shc, SH2-containing transforming protein C1; SHP, SH2 domain-containing phosphatase; Stat, signal transducers and activators of transcription.

Okayama Y, Kawakami T. Development, migration, and survival of mast cells. *Immunologic Research* 2006;34(2):97-115.

Theoharides TC, *et al. New Engl J Med* 2015;373:163-72, with permission.

Normal mast cell biology

- Capable of synthesizing and releasing many mediators
  - Many expressible at very high levels
  - Some stored in fully active form in electron-dense secretory granules, tightly packaged with serglycin proteoglycans
  - A small sample:

- Pro-inflammatory cytokines
  - IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-16, IL-18, IL-21, IL-23, IL-25, IFN-γ, TNF-α
- Chemokines
  - MCP-1, IL-8, RANTES, eotaxin, leukotrienes B_4, C_4, D_4, E_4 (SRS-A), CCL2, CCL3, CCL4, CCL5, CCL11, CCL19, CCL20, CCL21, CXCL8, CXCL10, XCL-1
- Proteases
  - Tryptase, chymase, ACE, carboxypeptidase, cathepsin G, cysteinyl cathepsins, metalloproteinases
- Growth factors
  - IL-3, GM-CSF, bFGF, VEGF, TGF-β, PDGF, EGF, NGF, SCF, angiopoietin
- Vascular permeability, vasodilatation
  - Histamine, 5-hydroxytryptamine, tryptase, NO, VLA4
- Platelet aggregation and thrombosis:
  - PAF, thromboxane
- Heparin proteoglycan
- Chondroitin sulfate proteoglycan
- Superoxide dismutase
- Acid hydrolases
  - Glucuronidase, galactosidase, hexosaminidase, peroxidase
- Arylsulphatase A
- Prostaglandin D_2, thromboxane
- Serotonin
- Antimicrobial agents
  - IFN-α, IFN-β, IFN-γ, cathelicidin, LL-37
- CRH
- TSLP
- Want more? See http://www.cells-talk.com/index.php/page/copelibrary?key=mast%20cells

Theoharides TC *et al.* Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunological Reviews* 2007;217(1):65-78.

MCAS: Emerging Understanding

- Increasing estimates of prevalence
  - 1-17% of the general first-world population?
- Increasing evidence of critical mast cell involvement in…
  - Irritable bowel syndrome (11%) Est. Global Prev.
  - Chronic fatigue syndrome (3%)
  - Fibromyalgia (1-10%)
  - Diabetes mellitus (2-20%)
  - Asthma (4-20%) Est. Global Prev.
  - Obesity (37%)
  - Depression (5%)
  - Atherosclerosis (?)
  - Etc. etc. etc. etc.

What Portions of These Populations Bear Clonal Mast Cell Disease?

Theoh
Afrin L
Haenis
Pejler
Theoh
Theoharides TC, *et al. Trends Pharmacol Sci* 2011;32(9):534-542.

Frieling T, *et al., Z Gastroenterol* 2011;49:191-194.

MCAS: Emerging Understanding

- And might there be critical mast cell involvement in hypermobile EDS, too?

MCAS: Emerging Biology

- May be clonal in most cases…
  - More than 50 mutations (mostly heterozygous, but still functionally dominant) found scattered across all domains of KIT
  - Most patients have multiple KIT (and other) mutations
  - No commercial assays yet for most of these mutations

Molderings GJ *et al.* Multiple novel alterations in Kit tyrosine kinase in patients with gastrointestinally pronounced systemic mast cell activation disorder. *Scand J Gastroenterol* 2007; 42(9):1045-1053.
Molderings GJ *et al.* Comparative analysis of mutation of tyrosine kinase Kit in mast cells from patients with systemic mast cell activation syndrome and healthy subjects. *Immunogenetics* 2010;62:721-727.

MCAS: Emerging Biology

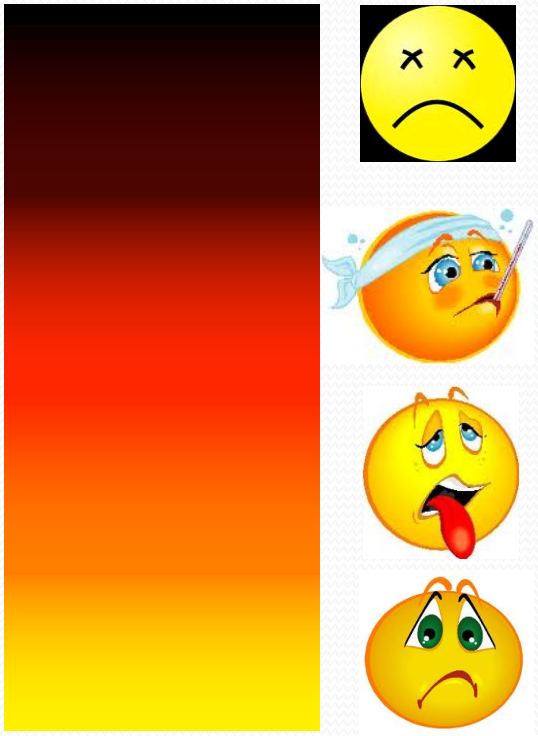
- KIT mutations found thus far in MCAS:
  - Ligand-binding domain: W8R, C12S, del(nt a153), E53K, insertion 71 ?seq(400bp), E73R, T74R, exon 3 & 5 del and ins/del, ins nt 248a, ins Q252, K259E, H265Q, E270K, L276S
  - Dimerization domain: E338K, Q346L, M351E, F355L, E359V, exon 7 ins/del, del (aa 378-390)
  - Proteolytic cleavage site: L416Q, D419H, ins(nt 1282g), exon 8 del
  - Membrane-spanning region: del 510-513, exon 10 ins, M541L
  - Juxtamembrane (autoinhibitory) region: F584C
  - Kinase insert sequence K1: S709A, del(S715), A736V, D751Y
  - Kinase domain K2: F782S, N787D, H790R, D816V (rare!), S821F, A829T, A837V, L862V
  - C-terminus: complex insertions

Molderings GJ *et al.* Multiple novel alterations in Kit tyrosine kinase in patients with gastrointestinally pronounced systemic mast cell activation disorder. *Scand J Gastroenterol* 2007; 42(9):1045-1053.
Molderings GJ *et al.* Comparative analysis of mutation of tyrosine kinase Kit in mast cells from patients with systemic mast cell activation syndrome and healthy subjects. *Immunogenetics* 2010;62:721-727.

MCAS: Do the Biology Math

- MCs produce and release scores of mediators
- 1 mutation ⇒ aberrant release of N mediators
- Multiple KIT mutations in most MCAS patients?
- Multiple genes mutated in most MCAS patients?
- Each mediator has its own unique array of direct and indirect, local and remote effects

Potential for Multisystem Polymorbidity and Clinical Heterogeneity

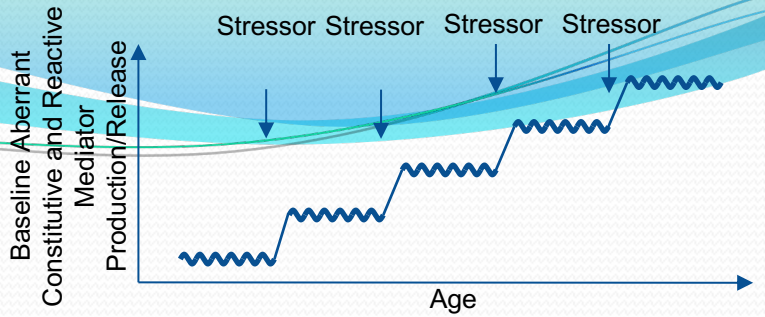


MCAS: Presentation

- MCAS is a chronic multisystem polymorbidity of general themes of:
  - Inflammation – the universal constant in MCAS
  - ± allergic-type phenomena
  - ± aberrancies in growth/development (i.e., dystrophisms), far more commonly benign than malignant in potentially any tissue
    - can be difficult to recognize given typically slow pace of development and, often, lack of clinical significance

Afrin LB, Butterfield JH, Raithel M, Molderings GJ. *Ann Med* 2016;48(3):190-201.

MCAS: Presentation

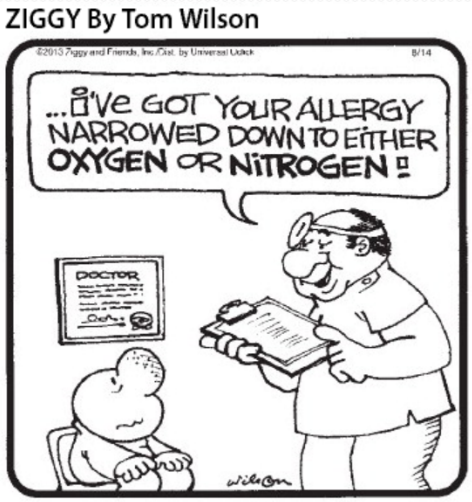


- Typical presentation
  - Age of onset: typically < 20 but unrecognized for decades
    - Escalations of baseline MC misbehavior may shortly follow (by a few days to a few months) major physical or psychological/emotional stressors
  - Usually **MULTISYSTEM**; can affect every system
  - Symptoms often (but not always) "inflammatory"
  - Perplexingly inconstant course:
    - Abnormalities often externally inapparent ("she looks fine!")
    - Chronic or waxing/waning or episodic ("flares", "spells")
    - Different symptoms at different times
    - Often no apparent triggers
  - Mediators:
    - Tryptase (total & mature) usually normal (reflects MC load >> activation)
    - Heparin, CGA, PGD_2 and histamine (& metabolites), LTE4 often elevated
  - Many MDs, many dx's (often non-specific, idiopathic, "somatic")
  - Patients commonly cease reporting symptoms – ROS important!

1. Afrin LB, Butterfield JH, Raithel M, Molderings GJ. *Ann Med* 2016;48(3):190-201.
2. Zenker N, Afrin LB. *Blood* 2015;126:5174.
3. Schwartz LB. *J Immunol* 2003;170(11):5667-73 and *Immunol Allergy Clin N Am* 2006;26:451-63.
4. Hamilton MJ et al. *J Allergy Clin Immunol* 2011;128;147-52.
4. Vysniauskaite M et al. *PLoS One* 2015 Apr 24;10(4):e0124912.
5. Ferrer M et al. *Clin Exp Allergy* 2010 Dec;40(12):1760-6.
6. Sala-Cunill A et al. *Int Arch Allergy Immunol* 2013;160(2):192-9.

MCAS: Presentation

- Constitutional
  - Fever, chills, fatigue, sweats, weight ↑ or ↓ or ↑↓, pruritus
  - Odd and prolific sensitivities (drugs, foods, environs)
- Eyes
  - Irritation, episodic inability to focus vision, blepharospasm
- Ears
  - Irritation, hearing deficit and/or tinnitus
- Nose
  - Irritation, sores, epistaxis, coryza
- Oral/esophageal
  - Irritation, sores, dysphagia, globus



Afrin LB, Butterfield JH, Martin R, Molderings GJ. *Ann Med* 2016;48(3):190-201.

MCAS: Presentation

- Nodes
  - Borderline pathologic, waxing/waning, migratory adenopathy
  - Left upper quadrant (splenic?) discomfort common
  - Path: usually reactive lymphocytosis, occ. sinus histiocytosis
- Pulmonary
  - Waxing/waning migratory edema/inflammation (e.g., cough)
  - Dyspnea (normal PFTs; "I just can't catch a deep breath")
- Cardiovascular
  - Unprovoked presyncope/syncope, labile BP/pulse, palpitations
  - Chest pain: coronaries usually clean, but occ. aggressive CAD
  - Arterial, venous malformations; episodic migratory edema
  - Takotsubo (acute balloon CHF), Kounis (allergic angina) synd.

Afrin LB, Butterfield JH, Martin R, Molderings GJ. *Ann Med* 2016;48(3):190-201.

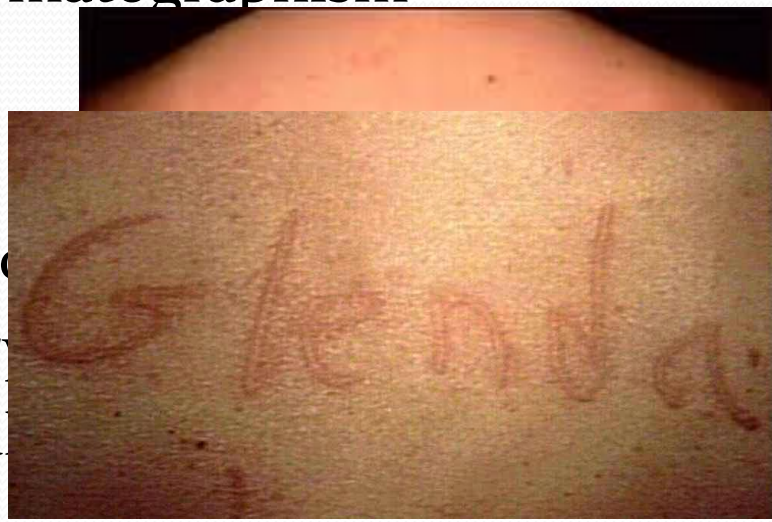
MCAS: Presentation

- GI
  - Inflammation (any/all luminal segments, solid organs)
  - Refractory GERD, IBS, mild ↑LFTs common
  - Diarrhea ⇔ constipation
  - Queasiness, nausea, vomiting (sometimes "cyclical")
  - Malabsorption common (gen., or selected micronutrients)
  - Hepatic involvement common, usually inflamm./fibrosis
- GU
  - Inflammation (any/all luminal segments, solid organs)
    - e.g., "interstitial cystitis"
  - ↓ libido, infertility

Afrin LB, Butterfield JH, Martin R, Molderings GJ. *Ann Med* 2016;48(3):190-201.

MCAS: Presentation

- Musculoskeletal and Joints
  - Myositis, osteopenia and/or osteosclerosis
  - Diffusely migratory soft tissue pain; "fibromyalgia," "CRPS"
    - NSAIDs/narcotics often unhelpful (may trigger flares!)
- Skin/Integument
  - Lesions (many types), rashes (many types, often migratory), pruritus, flushing, angioedema, dermatographism
  - Hair/nail/dental dystrophy
- CNS
  - Headache, vertigo, syncope, tic/treme
  - Migratory paresthesias, insomnia ver
  - Wide range of psychiatric disorders a



Afrin LB, Butterfield JH, Martin R, Molderings GJ. *Ann Med* 2016;48(3):190-201.

MCAS: Presentation

- Heme
  - Counts often normal, or…
    - ↑ or ↓ H/H (subtle ↑ RDW, MCV, and/or MCH common)
    - ↑ or ↓ WBC (subtle/intermit. ↑ monos, eos, &/or basos common)
    - ↑ or ↓ plts
    - ↑ or ↓ clotting
  - Marrow
    - Usually normal (histology, IHC, cytogenetics, flow, PCR)
    - Most common abnormality: mild dysplasia (“unclass. MDS/MPN”)
- Immunity
  - Hypersensitivities, ↑ risk for malig., autoimm., infection
  - Poor healing

Afrin LB, Butterfield JH, Martin R, Molderings GJ. *Ann Med* 2016;48(3):190-201.

MCAS: Presentation

- Endocrinologic/metabolic
  - Delayed puberty/menarche, dysmenorrhea
  - Osteopenia/osteoporosis, osteosclerosis
  - Hypo/hyperthyroidism, hyperferritinemia (inflammatory)
  - ↑ or ↓ electrolytes, ↑ lipids (often hypertriglyceridemia)
- Growth/Development
  - Poor healing
  - Cysts, fibrosis, endometriosis, vascular anomalies, cancer
  - Connective tissue weakness (e.g., hypermobile Ehlers Danlos Syndrome)?
  - Autism spectrum disorders?

Afrin LB, Butterfield JH, Raithel M, Molderings GJ. *Ann Med* 2016;48(3):190-201.

MCAS and EDS: A Few Notes

- In some portion of the hypermobile EDS (hEDS) population, is that disease rooted in certain patterns of chronic aberrant mast cell mediator expression causing aberrant assembly of normal connective tissue proteins into abnormal connective tissue (similar to how MCAS seems likely to be driving the many other growth/development abnormalities seen in MCAS patients such as cysts, fibrosis, vascular anomalies such as hemorrhoids and aneurysms, neurodevelopmental anomalies such as autism, malignancies, etc.)?

Seneviratne SL, Maitland A, Afrin L. *Am J Med Genet C Semin Med Genet* 2017 Mar;175(1):226-236.

MCAS and EDS: A Few Notes

≠

- A few small studies to date have <u>suggested</u> an <u>association</u> between MCAS and hEDS. For example:
  - Shneerson JM (1977). Atopy in connective tissue disorders. *Clinical Allergy* 7:203.
  - Luzgina NG, Potapova OV, Shkurupiy VA (2011). Structural and functional pecularities of mast cells in undifferentiated connective tissue dysplasia. *Bull Exp Biol Med* 150:616-8.
  - Louisias M, Silverman S, Maitland AL (2013). Prevalence of allergic disorders/mast cell activation syndrome in patients with Ehlers-Danlos syndrome. *Annals of Allergy, Asthma & Immunol*, A12. Baltimore, MD, USA: American College of Allergy, Asthma & Immunology.
  - Cheung I, Vadas P (2015). A new disease cluster: Mast cell activation syndrome, postural orthostatic tachycardia syndrome, and Ehlers–Danlos syndrome. *J Allergy Clinical Immunol* 135(2):AB65.
  - Lyons JJ, Yu X, Hughes JD,…Milner JD (2016). Elevated basal serum tryptase identifies a multisystem disorder associated with increased TPSAB1 copy number. *Nat Genetics* 48:1564-9.
  - Lee D, Mueller (2017). Mast Cell Activation Features in Ehlers-Danlos/Joint Hypermobility Patients: A Retrospective Analysis in Light of an Emerging Disease Cluster. 2017 ACR/ARHP Annual Meeting, abstract 2115.
  - Afrin LB, Self S, Menk J, Lazarchick J. *Am J Med Sci* 2017;353(3):207-215 (online suppl. Table 1).
- Increasing clinical observation, too, suggests an <u>association</u> between MCAS and hEDS.
- BUT…
- **Association ≠ Causation!!!!!!!!!!**
- Much more research is required to PROVE (or disprove) that certain variant(s) of MCAS cause hEDS.

Seneviratne SL, Maitland A, Afrin L. *Am J Med Genet C Semin Med Genet* 2017 Mar;175(1):226-236.

Which MC Mediators Might Be Principal Drivers of hEDS?

- Integrins? Cell adhesion molecules (CAMs)? Elastins? Elastases?
- Drivers/inhibitors of integrin/adhesion/elastin/elastase production?
  - Alpha-1-antitrypsin ("anti-elastase")?
  - SERPINB1 ("elastase inhibitor")?
  - Obviously, many others are possible, too.
- Note there is no requirement that any of the connective tissue proteins themselves be mutated to cause weakened connective tissue permitting hypermobility. It may be sufficient simply for there to be overproduction of an elasticity-promoting protein or underproduction of an elasticity-inhibiting protein to result in hypermobility, i.e., the central issue in hEDS could simple be an aberrant stoichiometric balance among the many proteins needed to ultimately fabricate normal connective tissue.
- There also is no requirement that the mast cells themselves inappropriately express the key aberrantly expressed connective tissue protein (or its promoter or inhibitor). It may be sufficient simply for dysfunctional mast cells to over- or under-express a key promoter or inhibitor of the key proximate protein.

Ibelgaufts H. COPE: Cytokine Online Pathfinder Encyclopedia. http://www.cells-talk.com/index.php/page/copelibrary?key=mast%20cells

If the mast-cell-based somatic mutations of MCAD/MCAS are central to hEDS, what are the chances whole genome/exome sequencing will find them?

- Minimal.
  - Most current WGS/WES technologies/approaches will find and call mutations present in ~20% or more of the sequenced cell population.
  - Mast cells comprise ~0.02% of peripheral blood leukocytes.
    - This is why mast cells are never reported in a leukocyte differential except in the very rare case of mast cell leukemia.

How will we find mast-cell-based somatic mutations central to hEDS?

- Sequence the mast cells – just the mast cells.
  - Extract the mast cells from the sample (blood is probably the easiest/safest tissue to access which will provide the greatest number of mast cells), either by…
    - Immunomagnetic beads (cheap, laborious)
      or
    - CD117-based flow cytometric sorting (expensive machine, quick)
  - Sequence (Sanger, NGS, etc.) the extracted mast cells.
    - Dysfunctional mast cells (all progeny of an identically mutated stem cell or multipotent progenitor cell, of course) certainly don't comprise all the mast cells in an MCAS patient, but prior research has shown repeatedly this sequencing approach reliably detects a menagerie of (mostly somatic) mutations in the mast cells in MCAS patients.

1. Molderings et al., Scand J Gastroenterol 2007. 2. Molderings et al., Immunogenetics 2010. 3. Altmüller et al., Immunogenetics 2017.

How will we find mast-cell-based somatic mutations central to hEDS?

- Barrier: No clinical sequencing lab anywhere on the planet – yet – which offers mast-cell-focused sequencing.
  - Many labs will do sequencing focused on tumor cells or other cells, but none yet are willing to focus on mast cells.
- Therefore, mast-cell-focused sequencing presently can be done only in research labs.
  - There are hEDS research projects under way performing WGS, but none performing mast-cell-focused sequencing.

How will we find mast-cell-based somatic mutations central to hEDS?

- If not already found by research labs, the mast cell mutational profiles common in hEDS likely will quickly become apparent once mast-cell-focused sequencing becomes clinically available.
  - Physicians attending to MCAD/MCAS patients likely will routinely order clinically available mast-cell-focused sequencing, primarily to learn of correlations between mutational profiles and effective therapies (to short-circuit the present "trial and error" approach to finding those therapies effective in the individual patient), but correlations between mutational profiles and clinical symptoms/phenotypes will "fall out" from such data, too.
  - Identification of hEDS driver mutations should permit identification/design of both mechanisms underlying symptoms and rational therapies.

MCAS: Diagnosis

- Best diagnostic aids:
  - Most physicians' best friend: a <u>complete</u> history and exam
  - Faith in Occam's Razor: which scenario is more likely?

    - Multiple diagnoses/problems all independent of each other

    vs.

    - One diagnosis that's biologically capable of causing most or all of the findings (i.e., the simplest solution, even if it's not the most immediately obvious solution)

MCAS Diagnostic Work-Up: 2021

Establish Suspicion:

Signs of mastocytosis (e.g., urticaria pigmentosa, unprovoked flushing or anaphylaxis, wasting, end-organ dysfunction, etc.)?

Symptoms of MC activation (Table 1)? MC mediator release syndrome per validated questionnaire (Figure 4)?

More symptoms/findings than can be explained by definitively established diagnoses? Odd/strange symptoms/findings?

Poor response to treatment of definitively established diagnoses?

↓

Initial Testing:

Biopsy of lesions of suspected cutaneous mastocytosis

Serum tryptase persistently > 20 ng/ml:

- bilateral marrow aspiration/biopsy including MC-specific immunohistochemical staining (e.g., CD117, tryptase, toluidine blue, Giemsa, Alcian blue), multicolor flow cytometry for co-expression of CD117/CD25, CD117/CD2, and molecular testing for KIT mutations as available (PCR for KIT^{D816V} at a minimum)
- biopsy of other extracutaneous tissues (e.g., GI tract) as appropriate, for MC-specific testing as above

Complete blood count (CBC) with manual differential
Common serum chemistries
Quant. Ig profile if frequent infections and/or delayed healing
PT/PTT if easy bruising or bleeding or thromboembolic events

↓

Additional MC Mediator Testing:

Serum chromogranin A (avoid PPIs for 5+ days before testing)
Chilled plasma for PGD_2 (and/or 11-β-$PGF_{2\alpha}$) (avoid NSAIDs for 5+ days before testing)
Chilled plasma histamine
Chilled plasma heparin (if not on exogenous heparin products)
Chilled random and 24-hour urine collections for PGD_2 (and/or 11-β-$PGF_{2\alpha}$) and *N*-methylhistamine
Chilled urine for leukotrienes B4, C4, D4, and E4 (if necessary)

Afrin LB, Molderings GJ. A concise, practical guide to diagnostic assessment for mast cell activation disease. *World J Hematol* 2014 Mar;3(1):1-17.

MCAS: Prognosis

- No epidemiologic studies of prognosis yet
- Present gestalt impression:
  - After the first three years, survival curves parallel the general population (similar to indolent systemic mastocytosis (ISM))
- So, like allergic diseases and ISM, reduced survival is a relatively small problem in MCAS, and instead most suffer reduced quality of life (anywhere from mild to severe, variable over time) until the disease is accurately diagnosed and effectively controlled
- Many therapies (targeting many receptors and pathways) found helpful in various MCAD/MCAS patients
  - Most cytotoxic chemotherapy quite unlikely to help MCAS
- Most MCAS pts eventually identify a significantly helpful regimen…
  - …and given they'll likely live a normal lifespan, the improved quality of life they can achieve – once correctly diagnosed – is important!

Roberts LJ, Anthony LB, Oates JA. "Disorders of Vasodilator Hormones: Carcinoid Syndrome and Mastocytosis" in Wilson JD, Foster DW, Kronenberg HM, *et al., eds.*, *Williams Textbook of Endocrinology, 9th ed.*, 1998, W. B. Saunders Company, Philadelphia, pp. 1718-1732.
Lim K-H, *et al.* Systemic mastocytosis in 342 consecutive adults: survival studies and prognostic factors. *Blood* 2009;113:5727-5736.

MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - Identify and avoid triggers
  - Inhibit mediator production
  - Inhibit mediator release
  - Block actions of released mediators
  - Cytotoxic and cellular therapy only for aggressive SM, MCL
  - Secondary issues and comorbidities

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence “C” as detailed in the Disclaimer slide near the beginning of this presentation.

MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - **Identify and avoid triggers, both antigenic (environmental, dietary, medication, etc.) and physical**
    - Or try desensitization therapy if feasible
    - Be aware: medication excipients often are prominent triggers
      - Trying alternative (commercial or compounded) formulations often necessary
    - Low-histamine diets, diamine oxidase supplementation
  - Inhibit mediator production
  - Inhibit mediator release
  - Block actions of released mediators
  - Cytotoxic and cellular therapy only for aggressive SM, MCL
  - Secondary issues and comorbidities

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - Identify and avoid triggers
  - **Inhibition of mediator production**
    - Steroids (long-term issues), NSAIDs (possibly more likely to be helpful in patients with elevated prostaglandin moieties)
      - Caution re: NSAIDs: some patients react to them; avoid them, or start "low and slow," if concerning history present
    - Vitamin C
    - Possibly also hydroxyurea (or even IMiDs?), TKIs
  - Inhibition of mediator release (stabilization)
  - Blockade of released mediators
  - Rarely (if ever): Cytotoxic therapy
  - Hypothetical: Cellular therapy
  - Secondary issues and comorbidities

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - Identify and avoid triggers
  - Inhibition of mediator production
  - **Inhibition of mediator release (stabilization)**
    - Cromolyn (oral and/or inhaled – non-absorbed)
      - Can trigger flares 1st few days; tachyphylaxis can abrogate efficacy
    - Pentosan (especially for interstitial cystitis)
    - Tyrosine kinase inhibitors
    - Interferon (& pegylated form?)
    - Omalizumab (anti-IgE)
    - Azathioprine, other immunosupp.
    - JAK1 and mTOR inhibitors?
    - Benzodiazepines and imidazopyridines; cannabinoids; low-dose naltrexone
      - e.g., lorazepam, clonazepam, flunitrazepam, zolpidem; cannabidiol
  - Blockade of released mediators
  - Rarely (if ever): Cytotoxic therapy
  - Hypothetical: Cellular therapy
  - Secondary issues and comorbidities

Imatinib (FDA approved for CML, mastocytosis)
Dasatinib (FDA approved for CML)
Nilotinib (FDA approved for CML)
Sunitinib (FDA approved for renal cell Ca & GIST)
Midostaurin (FDA approved in AML, ASM/MCL)?
Masitinib, avapritinib (investigational)?

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

Molderings GJ et al. Pharmacological treatment options for mast cell activation disease. *Naunyn Schmiedebergs Arch Pharmacol* 2016 Jul;389(7):671-94.

MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - Identify and avoid triggers
  - Inhibition of mediator production
  - Inhibition of mediator release (stabilization)
  - **Blockade of released mediators**
    - Antihistamines (even cont. IV diphenhydramine in severely afflicted pts)
      - Often impressive benefits even absent rhinosinusitis and dyspepsia
      - Can also stabilize mast cells via their autoexcitatory H_1/H_2 receptors
    - Leukotriene antagonists
    - Calcium/vit. D, bisphosphonates, denosumab for osteoporosis/osteopenia
    - TNF antagonists (etanercept, adalimumab, infliximab)?
    - IL-1 antagonists (e.g., anakinra), IL-1β antagonists (e.g., canakinumab)?
    - In development: inhibitors of tryptase, chymase, H_3 receptors, etc. etc.
  - Rarely (if ever): Cytotoxic therapy
  - Hypothetical: Cellular therapy
  - Secondary issues and comorbidities

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - Identify and avoid triggers
  - Inhibition of mediator production
  - Inhibition of mediator release (stabilization)
  - Blockade of released mediators
  - **Rarely (if ever): Cytotoxic therapy**
    - Hydroxyurea, alkylators, taxanes, etc.
    - Fludarabine, cladribine, cytarabine, etc.
    - Alemtuzumab, daclizumab
  - Hypothetical: Cellular therapy
  - Secondary issues and comorbidities

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MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - Identify and avoid triggers
  - Inhibition of mediator production
  - Inhibition of mediator release (stabilization)
  - Blockade of released mediators
  - Rarely (if ever): Cytotoxic therapy
  - **Hypothetical: Cellular therapy**
    - Allogeneic stem cell transplantation
      - Likely to be extremely challenging
  - Secondary issues and comorbidities

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MCAS: Treatment

- 2021: Largely as for indolent mastocytosis
  - Inhibition of mediator production
  - Inhibition of mediator release (stabilization)
  - Blockade of released mediators
  - Rarely (if ever): Cytotoxic therapy
  - Hypothetical: Cellular therapy
  - **Treatment of secondary issues and comorbidities**
    - Frequent mistake by patients and providers: *Assuming* a symptom (new or old, chronic or acute) is directly due to MCAS
      - MCAS does not render one immune to developing other disease
      - Regardless of the likelihood that a symptom may *ultimately* stem from MCAS, rule out other reasonable diagnostic considerations before assuming MCAS is the (direct) cause!
    - Illnesses secondary to mast cell disease require full treatment until the mast cell disease is controlled, and even then…
    - "…the horse is sometimes already out of the barn": malignancy and autoimmunity rarely, if ever, spontaneously remit simply with control of the underlying mast cell disease

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

MCAS: Treatment

- Note there are "complementary" treatments, too, in these various therapeutic categories. For example:
  - Inhibition of mediator production and/or release
    - Vitamin C (note kidney stone patients may want to avoid this)
    - Vitamin D
    - Alpha lipoic acid
    - N-acetylcysteine
    - Palmitoylethanolamide (PEA)
    - Cannabidiol (CBD)

1. Hagel AF et al. *Naunyn Schmiedebergs Arch Pharmacol* 2013 Sep;386(9):789-93.
2. Molderings GJ et al. *Naunyn Schmiedebergs Arch Pharmacol* 2016 Jul;389(7):671-94.

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

MCAS: Treatment

- Many "natural herbs and supplements," too, have anti-inflammatory activity and have potential to help control MCAS via COX-1/-2, MAPK, NFkB, and other pathways, e.g.:
  - Flavonoids (e.g., quercetin, luteolin, ruten)
  - Stilbenoids (e.g., resvera-trol)
  - Alkaloids (e.g., berberine)
  - Lion's mane
  - Elderberry
  - Omega-3 essential fatty acids
  - White willow bark
  - Turmeric/curcumin
  - Green tea
  - Pycnogenol
  - Boswellia
  - Cat's claw
  - Capsaicin
  - Ginseng

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

1. Maroon JC et al. *Surg Neurol Int* 2010;1:80.
2. Attiq A et al. *Front Pharmacol* 2018 Sep 7; 9:976. doi: 10.3389/fphar.2018.00976.

Again, all treatment options for MCAS discussed in this presentation are ACCME Level Of Evidence "C" as detailed in the Disclaimer slide near the beginning of this presentation.

MCAS: Treatment

- Non-pharmacologic therapies occasionally can be helpful, too
  - For example, certain behavioral re-training programs
  - Typically require longer periods (3-6 months) to see improvement than required by most pharmacological interventions
  - Mechanisms unclear, but seem likely related to the known close interactions – even physical abutment! – of neurons and mast cells throughout the body, with constant mediator "cross-talk" between such dyads

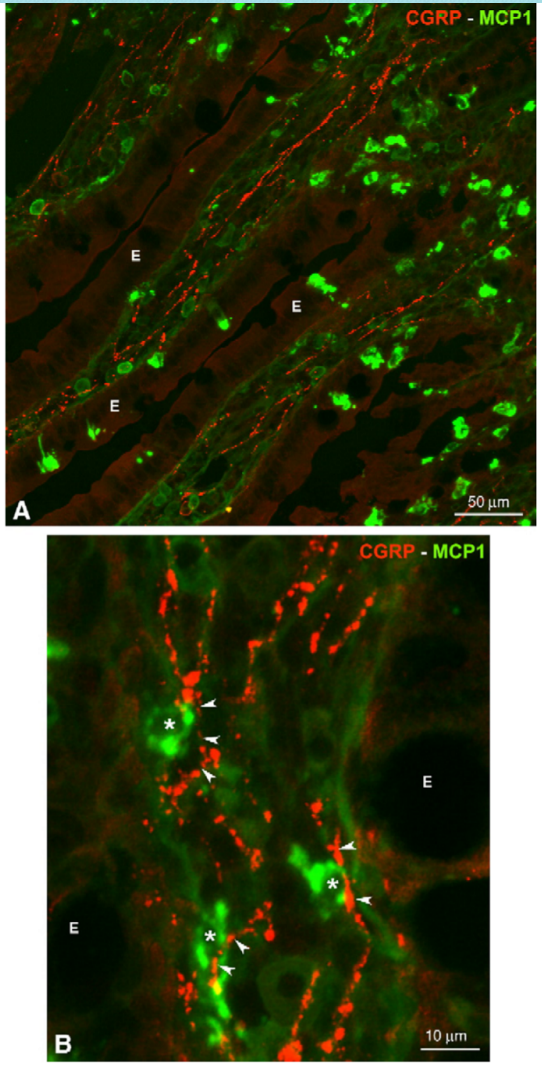


Fig. 1. Low (A) and high (B) magnification of a cryosection double stained for CGRP (red) and mouse mast cell protease 1 (green) of the mucosal layer in the small intestine of a *Schistosoma mansoni*-infected adult mouse, 8 weeks postinfection. Note the close apposition between the afferent CGRP-ir nerve fibres (arrows) and the mucosal mast cells (asterisks). Both nerve sprouting and strong mastocytosis are typical features during the acute and chronic infection stage in this inflammatory model. E: epithelium.

Shown with permission from ref. 6 below.

1. Blennerhassett MG et al. *Cell Tissue Res* 1991 Jul;265(1):121-8.
2. Theoharides TC et al. *Trends Pharmacol Sci* 2004 Nov;25(11):563-8.
3. Theoharides TC. *Life Sci* 1990;46(9):607-17.
4. Barbara G et al. *Gastroenterology* 2007 Jan;132(1):26-37.
5. Newson B et al. *Neuroscience* 1983 Oct;10(2):565-7,569-70.
6. Van Nassauw L et al. *Autonom Neurosci* 2007 Apr 30;133(1):91-103.

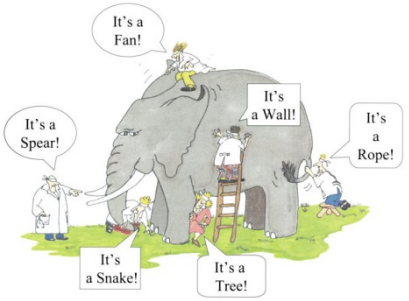
MCAD: Other Research Ideas

- Characterization of Mast Cell Regulatory Gene Mutations in MCAS
- MCAD in Refractory GERD
- MCAD in Asthma
- MCAD in Obesity
- MCAD in Fibromyalgia
- MCAD in Chronic Fatigue Syndrome
- MCAD in Irritable Bowel Syndrome
- MCAD in Hypermobile Ehlers-Danlos Syndrome
- MCAD in Postural Orthostatic Tachycardia Syndrome
- MCAD in Atherosclerotic Vascular Disease
- MCAD in Multiple Chemical Sensitivity (MCS)/Toxicant-Induced Loss of Tolerance (TILT)
- MCAD in Gulf War Illness
- MCAD as a Significant Modifier in Sickle Cell Disease
- Etc. etc. etc. etc.

MCAD/hEDS: What's next?

- **RESEARCH**
  - Clarify the association between hEDS and MCAD/MCAS
    - Larger studies (preferably multi-center rather than single-site, to reduce bias) probing what proportions of the general population and various disease-bearing populations bear hEDS and/or MCAD/MCAS
  - Investigate the possibility of causation
    - Appropriate sequencing/"omic" studies to identify which misexpressions of which mast cell mediators might cause hEDS
  - Improve treatment
    - Identify and test therapies targeted at inhibiting inappropriately expressed mast cell mediators driving hEDS
    - Might it even be possible to correct the phenotype?

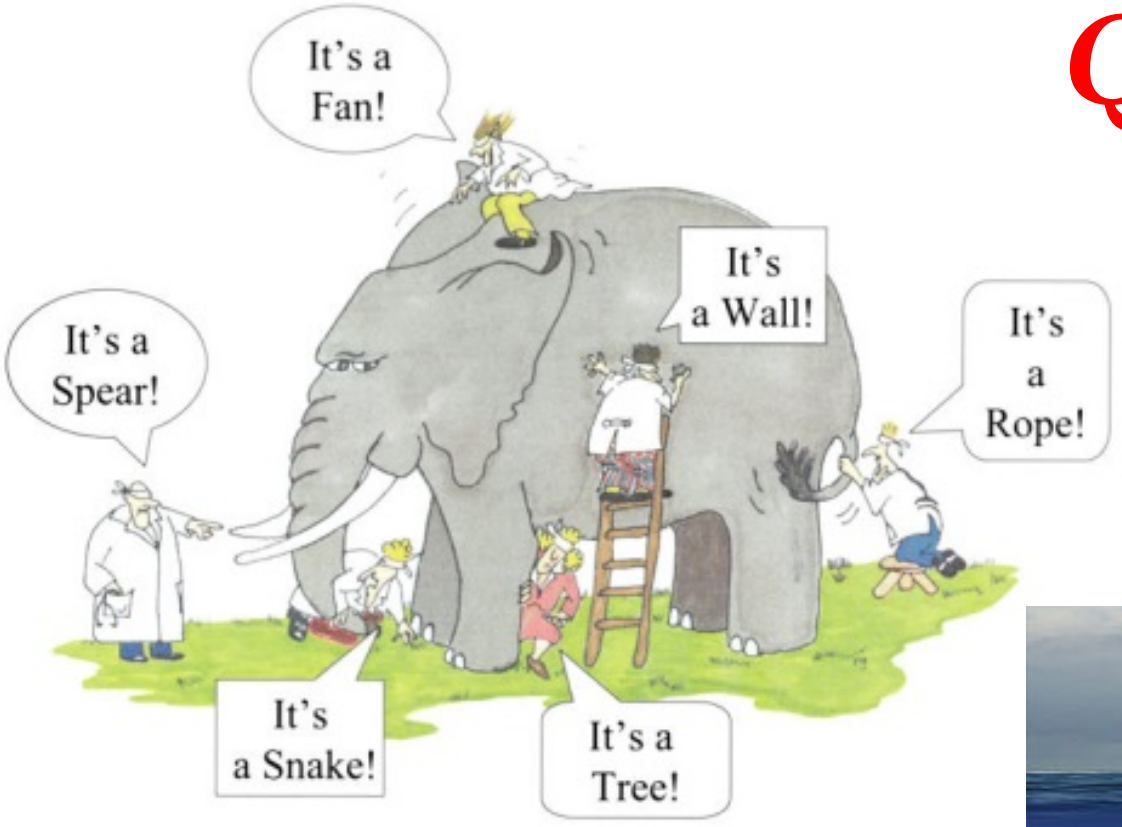
Summary



MCAD Diagnostic Class	General Prevalence	Phenotype	Tryptase usually…
Allergic Diseases	Prevalent	Allergy ± Inflammation	Normal
Mastocytosis	Rare	MC Neoplasia ± Inflamm./Allergy	Elevated
MCAS	Prevalent	Inflamm. ± Allergy ± Dystrophism	Normal

- Tryptase dominantly reflects total body MC load, not activation state
- MCAD symptoms usually from MC activation, not MC load
- Most MCAD patients…
  - …have normal survival, making disease control even more important (QoL!)
  - …can eventually find significantly helpful therapy once diagnosed
- Challenges:
  - Heterogeneity of MCAS (mutational origin?)
  - Many helpful therapies already found, but few biomarkers yet identified which reliably predict helpful therapy; persistence at trial & error needed
  - Education of patients, providers, payers, regulators, grantors, pharma, etc. etc. etc.

Questions?



Questions later?
drafrin@armonkmed.com