Non-IgE Mediated Mechanisms of Anaphylaxis

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1. *Background*
2. *Biomarkers*
3. *MRGPRX2*: promiscuous GPR
4. *c-KIT*^{GOF} mutations: clonal MCs
5. TPSAB1 CNV (HaT)

Disclosure Slide: Lawrence B. Schwartz, MD, PhD

Employment
- VCU/VCUHS

Research Grants
- NIH
- Deciphera, Blueprint

Consulting
- Blueprint, Astra-Zeneca, GLG, Celldex, Invea, Third Harmonic

Speaker
- Blueprint, Thermo Fisher

Other Financial Interests
- VCU Royalties/Licensing Fees: **ThermoFisher-Phadia (tryptase test);** Millipore, Santa Cruz, BioLegend, Hycult Biotech (mAbs); **Genentech (tryptase inhibitor)**
- Up-To-Date Card (royalties)
- Cecil's Textbook of Medicine Anaphylaxis chapter (royalties)

Mast Cells (MCs) and Anaphylaxis

Paul Ehrlich

Nobel Laureate-Immunology, 1908

Discovered & Named Mast Cells

Cells that ate too much to fatten & nourish surrounding tissue

Charles Richet

Nobel Laureate-Anaphylaxis, 1913

Discovered & Named Anaphylaxis

Sacrifice individuals to preserve humanity by avoiding interspecies cross-contamination

>50 years to realize mast cell activation causes anaphylaxis!

Personal Background

1978: Introduced to rat mast cells (MCs) & the guinea pig ileum bioassay for histamine

1979/1981: β-hexosaminidase, MC degranulation biomarker *in vitro*, rat/human MCs
J Immunol 123:1445-50, 1979; J Immunol 126:1290-1294, 1981

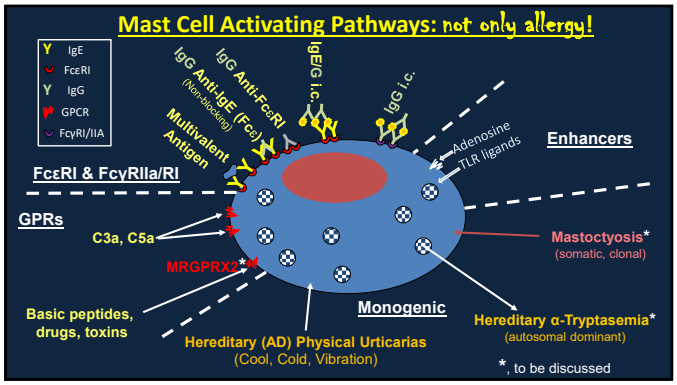
1981: discovery/purification of tryptase from human lung MCs
J Biol Chem 256:11939-43, 1981

1985, 1986, 1987: Anti-tryptase mAbs, selective localization to MCs (~200-fold >basophils)
J Immunol 134:526-31, 1985; Proc Natl Acad Sci 83:4464-68, 1986; J Immunol 138:2184-89, 1987

1987: Serum tryptase (immunoassay) as a biomarker for mastocytosis and anaphylaxis
New Engl J Med 316:1622-126, 1987

1997, 2006: B12 anti-tryptase mAb is a selective allosteric tryptase inhibitor
J Immunol 159: 3540-48, 1997; 176:3165-72, 2006

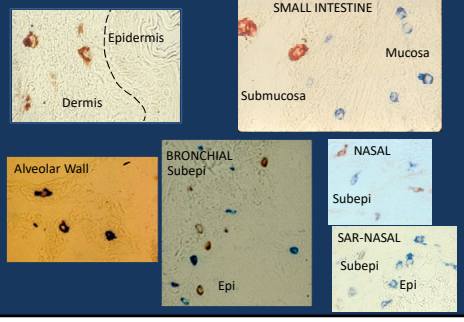
2019: discovery of α/β-tryptase heterotetramers
J Exp Med *216:2348-61, 2019*



Mast Cell Heterogeneity

MC Subtypes	MC_T	MC_{TC}
Proteases	**T**ryptase+/Chymase-	**T**ryptase+/**C**hymase+
Receptors	↑A3aR	C3aR/C5aR/MRGPR-X2
Normal Distribution	Lung parenchyma, intestinal mucosa	Vascular wall, skin, intestinal submucosa
Asthma		↑bronchial SM & mucus glands

MC_T and MC_{TC} Mast Cells (Irani et al)



Clinical Criteria for Diagnosing Anaphylaxis

Brighton[1]	NIAID[2]	WAO[3]
• sudden onset AND rapid progression of signs and symptoms AND • involving multiple (≥2) organ systems		
• Dermatologic AND • Cardiovascular OR Respiratory	• Dermatologic OR • Cardiovascular OR • Respiratory OR • Gastrointestinal	• Dermatologic AND • Cardiovascular OR • Respiratory OR • Gastrointestinal
	OR after exposure to allergen	
	• Hypotension alone	• Hypotension alone OR • Respiratory alone

1. https://brightoncollaboration.us/anaphylaxis-case-definition-pictorial-algorithm/ (3/2021)
2. Sampson H et al. JACI 117:391, 2006
3. Cardona et al. WAO J. 10.1016/j.waojou.2020.100472 (10/2020)

But the differential diagnoses of such presentations might include…

Differential Diagnosis of Clinically-diagnosed Anaphylaxis

Vasovagal, Panic attacks, Vocal cord dysfunction, POTS

Flushing disorders (benign, carcinoid syndrome, neuroendocrine tumors)

Angioedema: Plasma prekallikrein activation → bradykinin (HAEI,II;AAE; HAEIII)

Complement activation → C3a, C5a

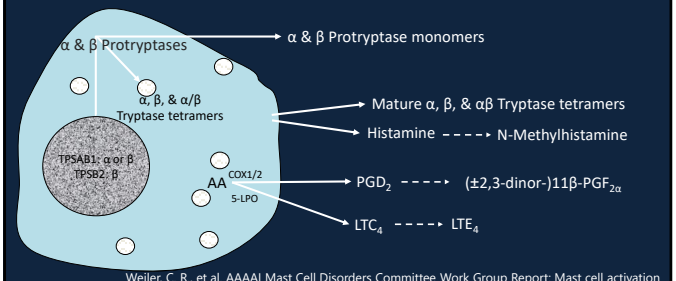
Scombroidosis (ingested histamine)

Pulmonary/Cardiogenic disorders

Other shock syndromes (septic, toxins, …)

Can we be more precise with biomarkers?

Commercially-Available Biomarkers Secreted in Response to Mast Cell Activation



Weiler, C. R., et al. AAAAI Mast Cell Disorders Committee Work Group Report: Mast cell activation syndrome (MCAS) diagnosis and management. J Allergy Clin Immunol 144(4): 883-896, 2019.

Commercial Biomarkers for Mast Cell Activation

PREFORMED		NEWLY-GENERATED	
Histamine →			
Tryptase	(N-methylhistamine)	PGD_2→11β-$PGF_{2\alpha}$	LTC_4→LTE_4

Cell source

Time course for secretion and removal/metabolism

Acute vs Baseline

Migration from tissue into blood

Plasma vs serum vs urine

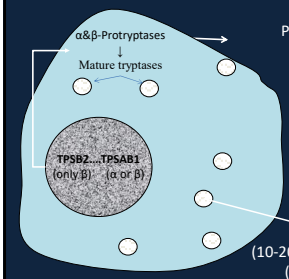
Pharmacologic interference

Sensitivity/Specificity/predictive value

Typical Laboratory Work-up of an ANA Patient at VCU

1. Acute (1 (0.5)-2(3)h after onset) & baseline (before or >24h after MCA) serum tryptase levels
2. 24-hour or spot urinary 11β-$PGF_{2\alpha}$, LTE_4 and N-methylhistamine/creatinine (?acute vs baseline).
3. ± D816V c-Kit: high-sensitivity, quantitative, allele-specific PCR of gDNA from PB.
4. ± TPSAB1 CNV genetic test for hereditary α-Tryptasemia (GeneByGene, $169).
5. ± Work-up for systemic mastocytosis or hereditary α-tryptasemia as clinically appropriate.
6. ± Hymenoptera venom kin testing or IgE panel

α/β-Tryptase Secretion: Unstimulated and Stimulated Mast Cells



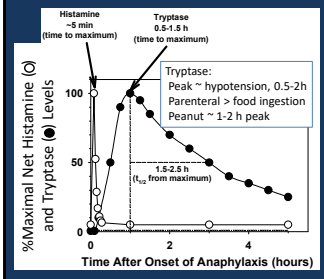
Protrypase(s): ≤11 ng/mL, median ~ 4 ng/mL (genetic)
(baseline serum tryptase (BST))
↑BST: HaT, RF, clonal myelocytic disorders (SM)

Heparin: Mature Tryptase ↑

Anaphylaxis
ECNM: AST> 2 + 1.2*BST
NIH: AST> 1.685*BST
(pro + mature = total tryptase)
(AST, acute serum tryptase)

Mature Tryptase
(10-20% MC protein, <1% Bas protein)
(≥95% intracellular tryptase)

Net Tryptase (& Histamine) Levels in Serum (Plasma) During Insect Sting-Induced Anaphylaxis



Schwartz et al. J Clin Invest 83:1551, 1989

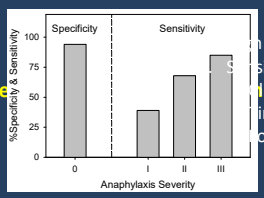
Clinically significant rise in tryptase:
Acute level > 2 + 1.2*Baseline level
>1.685*Baseliine level
(regardless whether acute level ≤ or > 11)

BST: before onset or >24 h after all signs & symptoms of anaphylaxis have resolved

AST: collect 0.5-3 h after onset, ~1-2 h optimal

*Valent et al. Int Arch Allergy Immunol **157**:215-25, 2012;*
De Schryver et al. J Allergy Clin Imm 137:1138-42, 2016 (peds ED);
Baretto RL et al. Allergy 72:2031-4, 2017 (periop) ;
Valent et al. J Allergy Clin Immunol Pract 7:1125-33, 2019

So how we



n specificity (>90%)
sitivity varies
ical severity ~↓BP
iming of collection
ood vs sting/drug

Overall Non-Mastocytosis PV
53% NPV
98% PPV

*Valent et al. Int Arch Allergy Immunol **157**:215-25, 2012: European Competency Network on Mastocytosis (ECNM)*

Emergency Department ANA in children

De Schryver et al. J Allergy Clin Imm 137:1138-42, 2016
(81% food (TN>PN>milk)>?>venom>drug>other)

≥2 organ systems (CV, Respiratory, GI, skin/mucosa)
&/or
↓BP with likely allergen exposure

Compared [sAT>1.2xsBT +2] vs [sAT>11.4]

Sensitivity:
Algorithm > 11.4 cut-off
Severe (86%) > Mild-Moderate ANA.

Perioperative SA

Baretto RL et al. Allergy 72:2031-4, 2017

Sensitivity	78%
Specificity	91%
PPV	98%
NPV	44%

Acute and Baseline Tryptase Algorithms

1. Variability of BST levels over time can be problematic in HαT and SM for tryptase MCA thresholds, more so for 2+1.2*BST than 1.685*BST. Uncertain whether this issue is due to between assay variability or to inherent variability of BST levels or to both.
2. If possible, BST serum should be collected within a few days before or 24-48 h after all signs and symptoms of anaphylaxis have cleared, and the BST and AST assays performed at the same time.
3. Whichever algorithm is used, clinical judgement is critical.

The Tryptase Algorithm for Systemic Anaphylaxis

acute > [2 + (1.2 x baseline)]

1. High specificity; a negative result does not r/o local MCA
2. Sensitivity ~ clinical severity (↓BP) & collection timing
   Acute samples obtained 30 min - 4 hour after clinical onset, 1-2 hours best; sensitivity diminishes over time
   Food allergens raise serum tryptase levels less and peak a little later than other triggers, like insect stings
3. sAT collection tips:
   Prescription or future order
   Order BMP; then call lab to add tryptase
   Retrieve plasma/serum drawn in ED & stored in pathology

MRGPR-X2 provides an IgE:FcεRI-independent pathway for MCA/ANA

Drug agonists of MRGPRX2

Drug
Vancomycin
Narotics: morphine, codeine…*
Non-depolarizing neuromuscular blockers atracurium & mivacurium, cisatracurium, & rocuronium
Fluoroquinolones
Antidepressants
Icatibant
*fentanyl OK

MRGPRX2 vs IgE-Mediated Reaction

1. Can occur with first exposure, no worse with later exposures
2. Higher concentration than allergen→IgE
3. Intensity ~ peak level … lowering rate of infusion may prevent future reactions
4. Positively charged:lipophilic agents, low stereospecificity

Endogenous & Exogenous Agonists of MRGPRX2

Endogenous Peptides/Proteins
Proteins EPO/eMBP
Neuropeptides SP, VIP, PACAP, neurotensin
Defense Peptides β-defensins-2 & -3, LL-37

Exogenous Peptides/Proteins
Wasp mastoparan, Gila monster lizard helodermin, Snake sarafotoxins, Tick defensin peptides
QSMs of g^+ bacteria
Viral/Parasitic: ?

MRGPRX2 Considerations

1. MC↔Eos crosstalk?
2. Anxiety-associated MC activation?
3. Infection/toxin-associated MC activation?
4. Synergy with IgE:FcεRI pathway?

Possible Cases of MRGPRX2-mediated Anaphylaxis

30 y/o admitted for elective cholecystectomy.
Fentanyl/propofol induction, sevoflurane; NMBD, vancomycin (1 g/5-10 min)→ tachycardia, hypotension, responded to fluids + epinephrine – acute tryptase 25 ng/mL.
~Vancomycin-triggered anaphylaxis
Schwartz et al, unpublished case

52 y/o renal tx on propranolol admitted for cystoscopy.
Fentanyl/propofol induction, sevoflurane; atracurium 25 mg iv→bradycardia, hypotension, arrest/coded/died – acute serum tryptase 102 ng/mL(no BST).
~Possible atracurium-triggered anaphylaxis
Schumacher. A&A Practice 12:145-6, 2019

Other disorders for which non-IgE-dependent MRGPR-X2-dependent ANA should be considered and studied:

1. SIDS: Platt et al. J Allergy Clin Immunol 94:250-56, 1994
2. Apnea in infant with DCM: Arnout & Schwartz. J Allergy Clin Immunol 100:850-51, 1997
3. Anticipated, traumatic deaths in adults: ~1:20 with markedly elevate tryptase in postmortem plasma, personal observations
4. Adrenergic urticaria: Shelley Lancet 2:1031-3, 1985; stress-induced asthma …

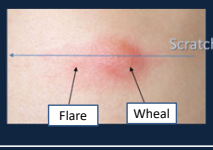
Excessive secretion of endogenous neuropeptides might cause MCA, locally in skin of CU patients or airways of asthmatics, or systemically with severe stress.

Acquired or inherited mutations associated with non-IgE (and IgE) dependent ANA?

Adult Indolent Systemic Mastocytosis: Presenting Scenarios
(a/w c-*KIT*^{GOF} somatic mutations, e.g., D816V-KIT)

30 y/o F with pruritic rash

Urticaria pigmentosa with a positive Darier's sign



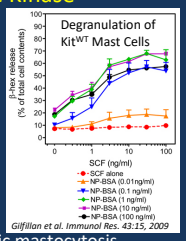
Prevalence: ~1:100,000 → ↑Baseline Serum Tryptase → ~1:10,000

PB D816V c-*KIT*+

Implications of Constitutively Activated D816V Kit Tyrosine Kinase

Functionally:
1. ↑MPP to MCs
2. ↑MC survival
3. ↑MC accumulation
4. ↑MC activation

(no ↑proliferation)



Gilfillan et al. Immunol Res. 43:15, 2009

Practically:
1. Minor criterion for diagnosis of systemic mastocytosis.
2. Indicates MC clonality.
3. **↑Risk for anaphylaxis** (severe spontaneous & insect sting triggered)

Case 1

45 y/o M: spontaneous episodes of anaphylaxis. PMH: severe anaphylaxis to wasp sting. FH: negative → suspicion of SM

Serum baseline tryptase (sBT) =60 ng/mL (<12)
acute =95 ng/mL (>2+1.2*60=74)
D816V c-*KIT*+ (PB allele-specific PCR)

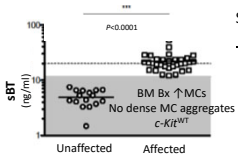
Systemic mastocytosis:
Severe anaphylaxis
(spontaneous/insect sting allergy)
~40-50% prevalence.

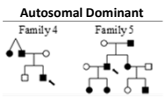
Diagnosis of Systemic Mastocytosis (1:10,000 prevalence)
Major Criterion: MC Aggregates (BM bx, >15 MC/hpf)
Minor Criteria: (1)Abnormal MC morphology; (2) *KIT*^{GOF} mutation; (3) CD25^{+} MC; (4) Baseline serum tryptase >20 ng/ml
Diagnosis: 1 major + 1 minor OR ≥3 minor

Valent et al. Leukemia Res 25:603-25, 2001; Schuch & Brockow Immunol Allergy Clin North Am **37**:153-64, 2017

Jon Lyons, MD
NIAID, NIH

Discovery of Inherited Disorder a/w ↑Serum Baseline Tryptase Levels (sBT)~tryptasemia





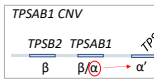
Symptoms: ~1/2 w/o, 1/3 mild, 1/5 moderate/severe
~5% prevalence with European ancestry
Flushing/Pruritus/(**Vibratory**) Urticaria
IBS-C/D
Retained primary dentition
Anaphylaxis: ↑severity

HαT⁺ ~ 10% severe insect sting-triggered ANA
~ 12% systemic mastocytosis
(↑ANA from 40% in SM to 90% in SM+HaT)
~ 17% Idiopathic ANA

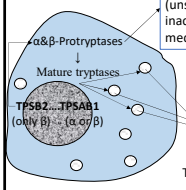
Lyons et al. JACI 133:1471-4, 2014; Nat Genetics 48:1564-9, 2016; Immunol Allergy Clin North Am 38:483-95, 2018

HαT

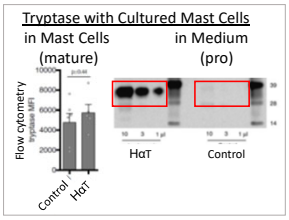
(autosomal dominant, 5-6% European ancestry; rare in Asian or African ancestries)



Baseline ~ PROTRYPTASES
(unstimulated MC,
inactive monomers,
median ~4 ng/mL)



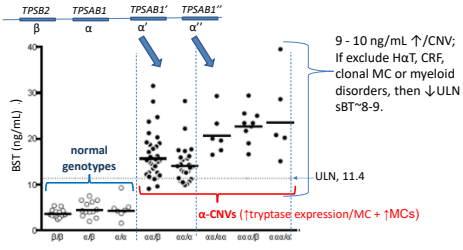
MATURE TRYPTASES
(MC activation-degranulation)



Total Tryptase = mature + pro forms of α & β tryptases, Thermo Fisher Assay

Lyons et al: JACI 133:1471-4, 2014; Nat Genetics 48:1564-9, 2016

↑*TPSAB1*-α CNV→↑Baseline Serum Tryptase (BST)→Hereditary α-Tryptasemia (HαT)



TPSAB1 Tryptase Genotype
~80% with 1 extra copy
~15% with 2 extra copies

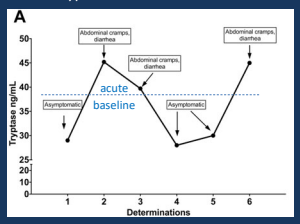
Lyons JJ, et al. Nat Genetics 48:1564-9, 2016.

BST, serum baseline tryptase
ULN, upper limit of normal

Idiopathic Anaphylaxis

Adult F: recurrent episodes diarrhea & abdominal cramps. Similar symptoms in 3|6 sibs. GI studies & bx wnl.

Acute tryptase levels 40-45 > baseline levels 25-30, c/w mast cell activation; neg MC clonality.



40 > 2 + 1.2x30 = 38 ~ clinically-significant MC activation

↑sBT and autosomal dominant inheritance
~Hereditary α-Tryptasemia
(*TPSAB1* α-tryptase quintuplication)

Sabato et al. JACI 134:1448, 2014; JCI 38:457, 2018

Non-IgE-dependent Anaphylaxis

1. Clonal MCs *KIT*^{GOF} (ligand independent), MRGPRX2 receptor (ligand dependent), and HaT (↑α/β-tryptase) have each been associated with severe non-IgE-mediated anaphylaxis, and also may synergize with the IgE pathway.
2. Rare monogenic disorders making mast cells susceptible to activation by physical or other stimuli may help reveal somatic GOF mutations or familial hypomorphous mutations of these genes that could contribute to cases of spontaneous anaphylaxis that are now considered idiopathic.
3. Further evidence to support the non-IgE-mediated pathways discussed today as being involved in anaphylaxis could include examining the effectiveness of targeted therapies against D816V KIT, MRGPRX2, or tryptase.