ACAAI 2021
Owning Anaphylaxis:
Non-IgE Mechanisms of Anaphylaxis-like Reactions

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1. c-Kit GOF mutations: clonal MCs
2. MRGPRX2: promiscuous GPR
3. α/β-tryptase heterotetramers: a new tryptase., hiding in plain site

Disclosure Slide: Lawrence B. Schwartz, MD, PhD

Employment
• VCU/VCUHS

Research Grants
• NIH
• Novartis, GSK, Merck, Dyax-Shire-Takeda, CSL Behring, Deciphera, Blueprint

Consulting
• Genentech, Deciphera, Dyax-Shire-Takeda, CSL Behring, Deciphera, Blueprint, Allakos, Astra-Zeneca, GLG, Celldex

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)
• NIH Study Section (honoraria)
Human Mast Cells

Paul Ehrlich
Nobel Laureate-Immunology, 1908
Discovered
Mast Cells

Charles Richet
Nobel Laureate-Anaphylaxis, 1913
Discovered
Systemic Anaphylaxis

>50 years to realize mast cell activation causes systemic anaphylaxis!

Mast Cell Activation Disorders

1. Clinical presentation of episodic anaphylaxis
2. Elevated acute MC biomarker(s): tryptase; histamine, PGD₂, LTC₄ metabolites
3. Response to anti-MC mediator/activation therapies
   ? Inherited/Acquired genetic trait

**PRIMARY** (Intrinsic to MCs)
Heritable - genetic trait
Clonal - gene mutation
Idiopathic - ?somatic/inherited trait?

**OVERLAP**
↑MC Responsiveness
to Agonists
↑Tissue response to MC mediators

**SECONDARY** (Extrinsic to MCs)
Allergen:IgE:FceRI
Antigen:IgG:FcyRIIa
GPR MC Activators
Autoimmune

Triggers of Mast Cell Activating Pathways

FcRs

- IgE
- FcεRI
- IgG
- GPCR
- FcγRI/IIA

Non-FcRs

- IgE/Gi.c.
- Y
- FcεRI
- Y
- IgG
- GPCR
- FcγRI/IIA

IgG Anti-FcεRI (Non-blocking)

IgG Anti-FcγR IIa

IgG Anti-FcγR Ic

Multivalent Antigen

TPSAB1 CNV

PLCG2 GOF

ADGRE2 GOF

EMR2, CD312

Cryopyrin GOF

PLAID

Vibration (dominant)

Caps (cold; Autoinflam, dominant)

HaT (somatic, clonal)

α-tryptasemia (dominant, αCNV)

Hereditary

Demonstration of aberrant clonal MC population in a subset of patients with "idiopathic" anaphylaxis

~no allergen trigger identified


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mastocytosis</th>
<th>Clonal MC:</th>
<th>Idiopathic</th>
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<tbody>
<tr>
<td></td>
<td>SA: cutaneous</td>
<td>SA, s</td>
<td>SA, s UP or MC</td>
</tr>
<tr>
<td>n=</td>
<td>12</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>UP or BM MC aggregates</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Kitmut, CD25+, or morphology</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tryptase &gt;20</td>
<td>+</td>
<td>2/5</td>
<td>?</td>
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</table>
A distinct biomolecular profile identifies monoclonal mast cell disorders in patients with idiopathic anaphylaxis

Clonal MCs ($KIT^{GOF}$) predispose an individual to severe anaphylaxis triggered by an insect sting

Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels

Clonal mast cell disorders in patients with severe Hymenoptera venom allergy and normal serum tryptase levels
**Case 1**

45 y/o M: episodic watery diarrhea & hives → syncope. PMH: systemic 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:**

i) clonal MCs ~ somatic Kit<sup>GOF</sup>,
ii) systemic anaphylaxis
(spontaneous/insect sting allergy)
~40-50% prevalence.

**Diagnosis of Systemic Mastocytosis**

(1:10,000 prevalence)

<table>
<thead>
<tr>
<th>Major Criterion</th>
<th>MC Aggregates (BM bx, &gt;15 MC/hpf)</th>
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<tbody>
<tr>
<td>Minor Criteria</td>
<td>Abnormal MC morphology;</td>
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<tr>
<td></td>
<td>Activating c-KIT mutation*;</td>
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<tr>
<td></td>
<td>CD25+ MC;</td>
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<tr>
<td></td>
<td>Baseline serum tryptase &gt;20 ng/ml</td>
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Diagnosis: 1 major + 1 minor OR ≥3 minor


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**Drug agonists of MRGPRX2**

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<tr>
<th>Drug</th>
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<tr>
<td>Vancomycin</td>
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<tr>
<td>Non-depolarizing neuromuscular blockers</td>
</tr>
<tr>
<td>atracurium &amp; mivacurium, cisatracurium, &amp; rocuronium</td>
</tr>
<tr>
<td>Narcotics: morphine, codeine...*</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
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<tr>
<td>Antidepressants</td>
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<tr>
<td>Icatibant</td>
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MRGPRX2 vs IgE-Mediated Reaction

1. Can occur with first exposure
2. Higher concentration than allergen:IgE
3. ~peak level ... lowering rate of infusion may prevent future reactions
4. Positively charged amphiphiles, less stereospecificity

* fentanyl OK
Endogenous & Exogenous Peptide Agonists of MRGPRX2

### Endogenous Peptides/Proteins

<table>
<thead>
<tr>
<th>Proteins</th>
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<tbody>
<tr>
<td>EPO/eMBP</td>
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<table>
<thead>
<tr>
<th>Neuropeptides</th>
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<tbody>
<tr>
<td>SP, VIP, PACAP, neurotensin</td>
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<table>
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<tr>
<th>Defense Peptides</th>
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<tr>
<td>β-defensins-2 &amp; -3, LL-37</td>
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### Exogenous Peptides/Proteins

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

### MRGPRX2 Consideration

1. Anxiety-associated MC activation?
2. MC ↔ Eos crosstalk?
3. Infection/toxin-associated MC activation?

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

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

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

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**Hereditary α-Tryptasemia** (aut. dom., 5-6% European ancestry)

Flushing/Pruritus/Vibratory Urticaria
Dysautonomia: IBS-C/D, POTS
MSK: EDSIII→arthritis
Retained primary dentition
Anaphylaxis: ↑severity

HaT in 10% severe insect sting systemic anaphylaxis
HaT in 12% systemic mastocytosis (↑SA from 40-50% in SM to 90% in SM+HaT)
HaT in 17% Idiopathic systemic anaphylaxis

How might ↑α-tryptase expression account for any of these signs or symptoms?

How does α-tryptase overexpression relate to clinical features in HaT?

Heterotetramers of tryptase increase PAR-2–dependent vascular endothelial monolayer permeability in vitro, but homotetrameric tryptases do not.

Concluding Comments

1. Clonal D816V c-Kit MCs, MRGPRX2, and an $\frac{\alpha/\beta}{\beta}$ tryptase ratio have each been associated with non-IgE-mediated anaphylaxis.

2. Monogenic disorder 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 anaphylaxis that are now considered idiopathic.

3. Further evidence to support the non-IgE-mediated pathways discussed today being involved in anaphylaxis will require studies of the effectiveness of targeted therapies, which might include D816V c-Kit inhibitors now approved for advanced systemic mastocytosis, or of MRGPRX2 or tryptase inhibitors that are in development.