Le basophile n’est pas un mastocyte circulant: ce que MRGPRX2 nous apprend

(Basophils are not circulating mast cells: lessons from MRGPRX2)
Content

What do we know?
Distinct MC (B) activation pathways – outcomes
IDHR ~ off-target occupation MRGPRX2 on MC

Basophils in IDHR (BAT and HistaFlow: principles)

Do basophils express MRGPRX2?

Can BAT be applied to diagnose IDHR from MRGPRX2 occupancy?

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Conclusions
Distinct activation pathways - outcomes

Antigen specific
Compound exocytosis
“Delayed” – sustained (5-30-60 min)
Local – regional – systemic (granule trafficking)
Inflammatory mediators

Not antigen specific
“kiss and run”
Rapid – transient (<5-30 min)
Local
<<< inflammatory mediators

Espinosa E et al, Curr Opin Imm 2018
### IDHR ~ off-target MRGPRX2 occupancy

<table>
<thead>
<tr>
<th></th>
<th>CIPRO</th>
<th>MOXI</th>
<th>ICATIBANT</th>
<th>ROCU</th>
<th>ATRA</th>
<th>OPIATE</th>
<th>CETROR</th>
<th>ELIX</th>
<th>VANCO</th>
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<td>Nat Chem Biol 2017</td>
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<td>J Clin Invest 2016</td>
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</table>

- Mrgprb2 WT/null mutant mice
- LAD2 (intermediately differentiated hMC^1^)
- MRGPRX2-HEK cell line (human embryonic kidney)
- Human MC cultures (CD34)

**Ca++ influx! ~ degranulation/mediator release/ ....**
**In vitro** human mast cell culture (UA)

- CD34+ progenitor cells
- 50 mL peripheral blood

**Weeks 1, 2, 3**

- **Week 1**
  - Day 0: IL-3, SCF, LDL
- **Week 2**
  - Day 3: IL-3, SCF
  - Day 7, 10: IL-3, SCF
- **Week 3**
  - Day 14: SCF

**Mature mast cells**

**Flow cytometric analysis:**
- Phenotypic
- Functional
Immunophenotyping: cell membrane
Immunophenotyping: granule content

A

Histamine

CD117-
DAO+
CD117+
DAO+

DAO

10^0

10^1

CD117

10^0

10^1

B

Tryptase

CD117-
Tryptase+
CD117+
Tryptase+

Tryptase

10^0

10^1

CD117

10^0

10^1

C

Chymase

CD117-
Chymase+
CD117+
Chymase+

Chymase

10^0

10^1

CD117

10^0

10^1

DAO = diamine oxidase
MRGPRX2 expression

FMO = fluorescence minus one
Clone K125H4 (Biolegend San Diego, USA)
  Gupta et al Infect Imm 2017
  Manorak et al Respir Res 2018
Clone 477533 (R&D)
  Fujisama et al JACI 2014
MRGPRX2-dependent activation
### IDHR ~ off-target MRGPRX2 occupancy

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Can BAT be applied to diagnose IDHR from MRGPRX2 occupancy?

Conclusions
Flow Cytometric Allergy Diagnosis: Basophil Activation Techniques

Chris H. Bridts, Vito Sabato, Christel Mertens, Margo M. Hagendorens, Luc S. De Clerck, and Didier G. Ebo
staining with fluorochrome conjugated DAO

before activation after
### TABLE IV. BAT in immediate NMBAs hypersensitivity

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Reference test</th>
<th>Activation marker</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>N</th>
<th>Reference</th>
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<tr>
<td>Various NMBAs</td>
<td>H</td>
<td>CD63</td>
<td>64</td>
<td>81</td>
<td>26</td>
<td>92</td>
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<tr>
<td></td>
<td></td>
<td>CD45</td>
<td>43</td>
<td>96</td>
<td></td>
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<tr>
<td>Various NMBAs</td>
<td>H ± ST</td>
<td>CD63</td>
<td>54</td>
<td>100</td>
<td>56</td>
<td>93</td>
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<tr>
<td>Various NMBAs</td>
<td>H</td>
<td>CD63</td>
<td>79</td>
<td>100</td>
<td>31</td>
<td>94</td>
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<tr>
<td></td>
<td></td>
<td>CD203c</td>
<td>36</td>
<td>100</td>
<td></td>
<td></td>
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<tr>
<td>Various NMBAs</td>
<td>H ± ST</td>
<td>CD63</td>
<td>36-86*</td>
<td>93</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Rocuronium</td>
<td>H ± ST</td>
<td>CD63</td>
<td>92†</td>
<td>100</td>
<td>22</td>
<td>96</td>
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<tr>
<td>Various NMBAs</td>
<td>H ± ST ± IgE</td>
<td>CD63</td>
<td>60</td>
<td>100</td>
<td>49</td>
<td>97</td>
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<td>Rocuronium</td>
<td>H + 2 tests‡</td>
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<td>80</td>
<td>96</td>
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<td>Various NMBAs</td>
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<td>68</td>
<td>100</td>
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<td>98</td>
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<tr>
<td>Atracurium</td>
<td>H ± ST</td>
<td>CD63</td>
<td>71‡</td>
<td>100</td>
<td>75</td>
<td>99</td>
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*History: ST, skin test.

*Increasing sensitivity when only the reactions that occurred during the 3 y were taken into account.

†Taking into account the nonresponders sensitivity is 76%.

‡For validation purposes, diagnosis of rocuronium allergy was considered definite when at least 2 out of 3 test results (skin test, BAT, sIgE) were positive, because rocuronium challenges at full dose are not possible.

§Taking into account the nonresponders sensitivity is 63%.
<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Reference test</th>
<th>Activation marker</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Number of patients and controls</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactam</td>
<td>H</td>
<td>CD63</td>
<td>50</td>
<td>93</td>
<td>88</td>
<td>41</td>
</tr>
<tr>
<td>β-Lactam</td>
<td>H + DPT</td>
<td>CD63</td>
<td>39</td>
<td>93</td>
<td>53</td>
<td>51</td>
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<tr>
<td>β-Lactam</td>
<td>H ± ST ± IgE ± DPT</td>
<td>CD63</td>
<td>49</td>
<td>91</td>
<td>110</td>
<td>52</td>
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<tr>
<td>Amoxicillin</td>
<td>H ± ST</td>
<td>CD203c</td>
<td>52</td>
<td>100</td>
<td>41</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD63</td>
<td>22</td>
<td>79</td>
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<tr>
<td>β-Lactam</td>
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<td>CD63</td>
<td>50</td>
<td>89-97</td>
<td>262</td>
<td>54</td>
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<tr>
<td>β-Lactam</td>
<td>H ± ST ± IgE</td>
<td>CD63-CCR3</td>
<td>55</td>
<td>100</td>
<td>39</td>
<td>55</td>
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<td>CD63-IgE</td>
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<tr>
<td>Amoxicillin</td>
<td>H</td>
<td>CD63</td>
<td>29</td>
<td>NA</td>
<td>14 patients, no controls</td>
<td>56</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>H ± ST ± DPT</td>
<td>CD63</td>
<td>50</td>
<td>NA</td>
<td>61 patients, number of controls</td>
<td>57</td>
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<tr>
<td>Amoxicillin</td>
<td>H ± ST</td>
<td>CD63</td>
<td>50</td>
<td>NA</td>
<td>30 patients</td>
<td>58</td>
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<tr>
<td>Amoxicillin</td>
<td>H ± ST ± DPT</td>
<td>CD63</td>
<td>47</td>
<td>93</td>
<td>57</td>
<td>44</td>
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<tr>
<td>Clavulanic acid</td>
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<td>62</td>
<td>89</td>
<td>58</td>
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<tr>
<td>Cefazolin</td>
<td>H + ST</td>
<td>CD63</td>
<td>33</td>
<td>94</td>
<td>16 patients, 17 controls</td>
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<td></td>
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<td>CD203c</td>
<td>67</td>
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*H.* History; *NA,* not available; *ST,* skin test.
### IgE and BAT in Immediate Drug Hypersensitivity

**Table 3** BAT in immediate quinolone hypersensitivity

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Ref. test</th>
<th>Activation marker</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Number of patients and controls</th>
<th>Ref.</th>
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<tr>
<td>Various quinolones</td>
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<td>CD63</td>
<td>0</td>
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<tr>
<td>Various quinolones</td>
<td>H + ST + DPT</td>
<td>CD63</td>
<td>0</td>
<td>100</td>
<td>18</td>
<td>[38]</td>
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<tr>
<td>Various quinolones</td>
<td>H</td>
<td>CD203c</td>
<td>100</td>
<td>100</td>
<td>5</td>
<td>[41]</td>
</tr>
<tr>
<td>Various quinolones</td>
<td>H + DPT</td>
<td>CD63</td>
<td>71</td>
<td>–</td>
<td>73</td>
<td>[40]</td>
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<tr>
<td>Various quinolones</td>
<td>H + DPT</td>
<td>CD203c</td>
<td>NA</td>
<td>100</td>
<td>34</td>
<td>[42]</td>
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<td>Moxifloxacin</td>
<td>H</td>
<td>CD63</td>
<td>9.1</td>
<td>77.8</td>
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<td>CD203c</td>
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<td>88.9</td>
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<td></td>
<td>CD203c</td>
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<td>0</td>
<td>94.4</td>
<td>6</td>
<td></td>
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<tr>
<td>Moxifloxacin</td>
<td>H</td>
<td>CD63</td>
<td>13.3</td>
<td>100</td>
<td>24</td>
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<td>CD203c</td>
<td>46.7</td>
<td>100</td>
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</table>

_BAT_ basophil activation test, _DPT_ drug provocation test, _H_ history, _NA_ not available, _Ref._ reference, _ST_ skin test, _Unpub_ unpublished data
Moxi IDHR ~ MC (not basophils)
“Steady state basophils”
Moxifloxacin hypersensitivity: Uselessness of skin testing
Astrid P. Uyttebroek, MD, Vito Sabato, MD, Chris H. Bridts, MT, Luc S. De Clerck, MD, PhD, and Didier G. Ebo, MD, PhD

Clinical Implications

- Skin prick and intradermal testing with moxifloxacin are unreliable methods to establish a diagnosis of moxifloxacin hypersensitivity. Higher dilution skin tests remain negative in the majority of patients with prior reactions, whereas higher concentrations are not discriminative between patients and exposed control individuals.
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Roles of Mas-related G protein–coupled receptor X2 on mast cell–mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases

Hariharan Subramanian, PhD,* Kshitij Gupta, PhD, and Hydar Ali, PhD

Philadelphia, Pa

formerly known as MrgX2). Unique features of MRGPRX2 that distinguish it from other GPCRs include their presence both on the plasma membrane and intracellular sites and their selective expression in MCs. In this article we review the possible roles of
MRGPRX2 was exclusively active in MCs ex vivo and exhibited the highest fold change among candidates. It mediates MC degranulation by nonimmunologic secretagogues\textsuperscript{28} and binds cathelicidin.\textsuperscript{29} Thus, MRGPRX2 may play important roles in skin MC activation.
Anti-MRGPRX2: K125H4 Mouse IgG2κ (BioLegend)
(similar results with 577533 (Novus Biologicals: data not shown)
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Is the expression of MRGPRX2 functional?

*Does occupancy activate basophils?*
Content

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Conclusions
Les MC(TC) et basophiles se ressemblent mais ....
Thanks

Labo Immunologie - Allergologie - Reumatologie

Prof. Dr. V. Sabato
Prof. Dr. M. Hagendorens
Dr. M. Faber
Prof. Dr. L. De Clerck
Dr. A. Uyttebroeck
Dr. J. Leysen
Dr. A. Van Gasse
Jessy Elst (MSc)

Chris Bridts (MLT)
Christel Mertens (MLT)
Lisa Capoor (FB)
Kathleen Van Benegem