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Adverse Drug Reactions
Analysis & Consulting

EVALUATING IMMEDIATE TYPE DRUG ALLERGY AND IMMUNOSTIMULATION IN VITRO WITH THE BASOPHIL ACTIVATION TEST (BAT)

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AGENDA

- **Immediate type drug reactions**
  - Symptoms
  - Mechanisms
  - Consequence for the patients
- **Basophil Activation Test (BAT)**
  - Background & utility
  - Examples
- **Conclusions**
  - IgE vs non IgE-mediated activation
  - Hypersensitivity to the drug or to the excipient
  - Cross-reactivity assessment

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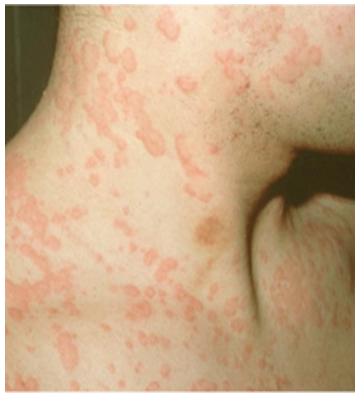
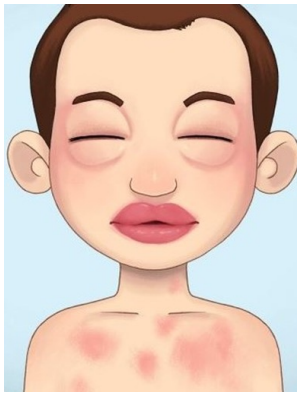
Immediate type drug reactions

Within minutes (< 1hour)



Urticaria

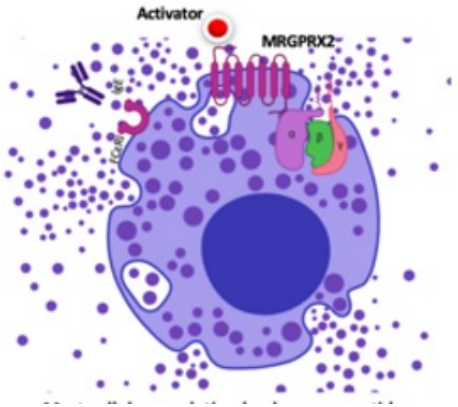


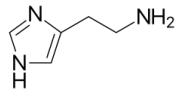
Angioedem

Anaphylaxis

Mechanism

- Degranulation of mast cells
- Release of inflammatory mediators





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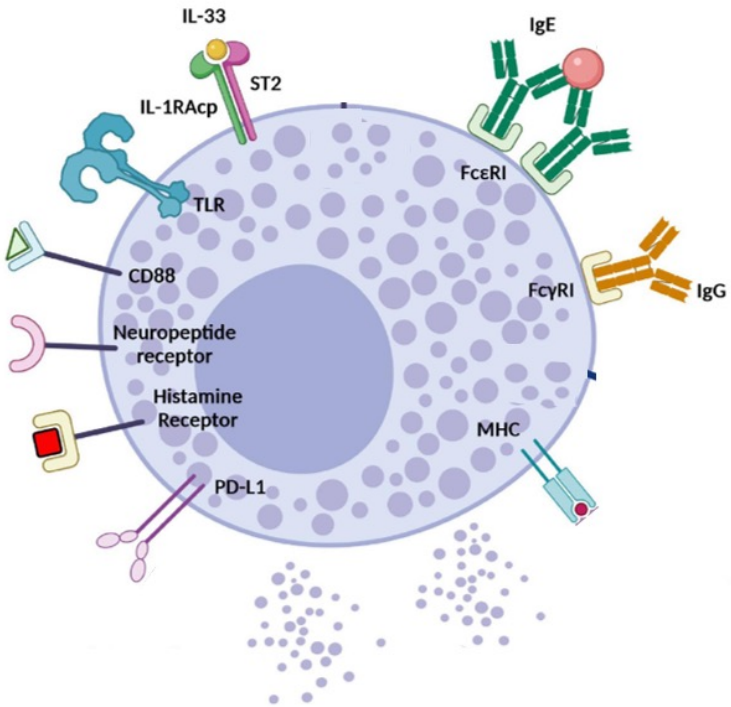
Degranulation of mast cells

Antigen non-specific

Anaphylatoxin receptors
(C3a, C5a)
MRGPRX2, TLR,
Opioid receptors

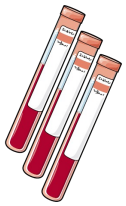


- *First encounter reaction*
- *Independent of the adaptive immune system*
- *No immune memory*
- *Density of receptors*
- *Dose dependence*
- *Polymorphism*

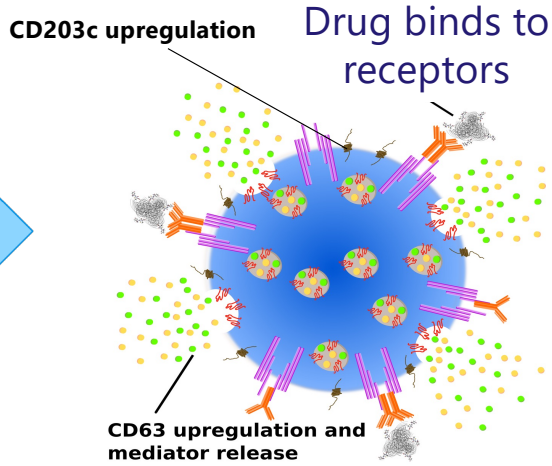
Basophil Activation Test (BAT)

Blood draw

100µL whole blood per test condition



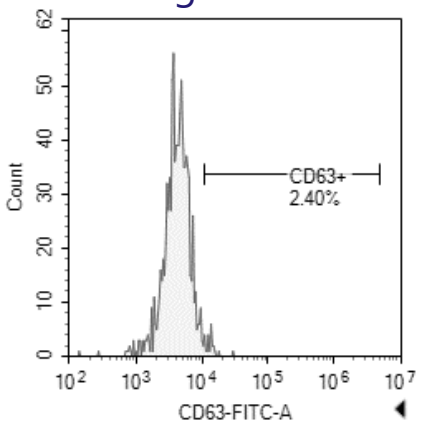
In vitro activation



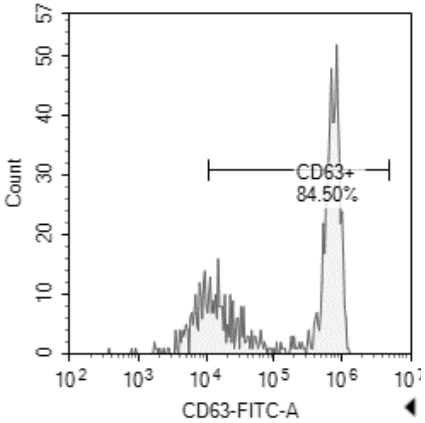
Flow cytometry analysis

Gated on CCR3⁺ single cells:

Neg. control



Pos. control



Stimuli	
Neg.	Culture buffer
Pos. 1	Anti-IgE
Pos. 2	fMLP (N-formyl-met-leu-phe)
Drugs	tested in multiple concentrations

Activation based on

- CD63: degranulation marker
- CD203c: activation marker

Stimulation Index : SI

$$SI = \frac{\%\ \text{of CD63}^+\ \text{events with drug}}{\%\ \text{of CD63}^+\ \text{events in buffer}}$$

SI> 2 in 2 drug concentration. Considered positive

(Similar calculation for CD203c)

Examples on BAT utility with different drugs

1. **Small molecule drugs**
   - Cross-reactivity: chlorhexidine & alexidine.
   - No commercially available specific IgE test for proton-pump inhibitors (PPIs).

2. **Biologics (Antibody)**
   - Dupilumab
   - Cross reactivity with excipients

3. **Paclitaxel**
   - Importance of the formulation
   - Liposomes vs albumin-based nanoparticles

4. **mRNA vaccines**
   - Assessment of activation mechanism

5. **Antigen non-specific stimulation**
   - Influence of high local dose

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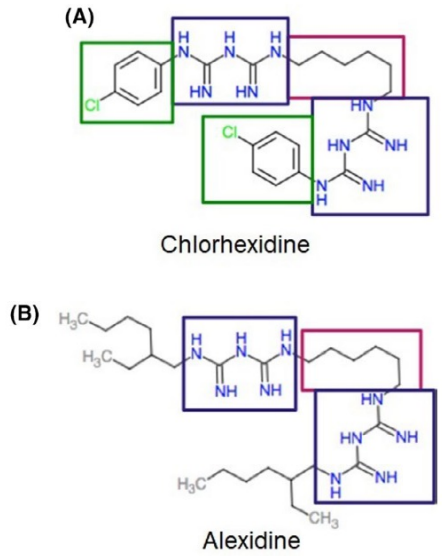
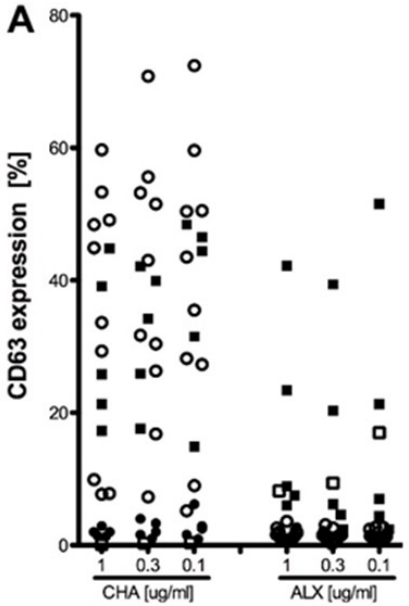
Cross-reactivity: chlorhexidine & alexidine

Chemical similar structures



Cross-reactivity evaluation



IL-3 primed basophiles

CHA = chlorhexidine acetate;
ALX = alexidine;

- IgE response to chlorhexidine can be polyclonal.
- Cross-reactivity with alexidine is possible.
- Safety of alexidine in non-cross-reactive cases

Mueller-Wirth *et al.* (2020). *Allergy* **75(12)**

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Proton-pump inhibitors (PPI)

Stimulant	Conc. µg/ml	with IL-3 CD63	with IL-3 SI	no IL-3 CD63	no IL-3 SI	no IL-3 CD203c	no IL-3 SI
Esomeprazol	50	8.4 %	3.4	3.1 %	1.5	14.8 %	7.4
	10	6.2 %	2.5	2.7 %	1.3	10.1 %	5.1
	1	2.8 %	1.1	1.8 %	0.9	2.6 %	1.3
Lansoprazol	50	8.4 %	3.4	3.0 %	1.4	10.2 %	5.1
	10	4.6 %	1.9	1.5 %	0.7	2.2 %	1.1
	1	3.3 %	1.3	0.9 %	0.4	1.8 %	0.9
Omeprazol	50	26.8 %	10.9	21.9 %	10.4	33.6 %	16.8
	10	9.9 %	4.0	3.1 %	1.5	12.4 %	6.2
	1	4.8 %	2.0	2.1 %	1.0	2.6 %	1.3
Pantoprazol	50	14.5 %	5.9	5.5 %	2.6	16.5 %	8.3
	10	11.2 %	4.6	3.7 %	1.8	12.3 %	6.2
	1	5.5 %	2.2	3.8 %	1.8	8.1 %	4.1
Controls							
Buffer		2.4 %		2.1 %		2.0 %	
Anti-IgE		87.2 %		80.4 %		49.1 %	
fMLP		67.4 %		65.4 %		43.4 %	

Patient's symptom: anaphylaxis

Skin test:	
Pantoprazole	++
Omeprazole	+
Esomeprazole	+
Lansoprazole	-

$$\textbf{SI} = \frac{\%\ \text{of CD63}^+\ \text{events with drug}}{\%\ \text{of CD63}^+\ \text{events in buffer}}$$

(Similar calculation for CD203c)

- ➔ No commercially available assay for specific IgE
- ➔ Skin test feasible only for i.v. formulation
- ➔ High degree of cross-reactivity inside the PPI class

BAT with Biologic antibody
dupilumab and excipient (polysorbate 80)

		with IL-3		no IL-3		no IL-3	
Stimulant	**Conc. µg/ml**	**CD63**	**SI**	**CD63**	**SI**	**CD203c**	**SI**
Dupilumab (Dupixent)	1000	3.1 %	1.5	9.5 %	5.0	21.3 %	11.2
	500	5.5 %	2.6	9.0 %	4.7	21.7 %	11.4
	100	0.9 %	0.4	2.3 %	1.2	7.5 %	3.9
Polysorbat 80	50	16.8 %	8.1	13.3 %	7.0	18.4 %	9.7
	10	13.7 %	6.6	5.8 %	3.1	12.6 %	6.6
	1	0.6 %	0.3	14.1 %	7.4	32.3 %	17.0
Controls							
Buffer		2.1 %		1.9 %		1.9 %	
anti-IgE		76.2 %		75.8 %		59.7 %	
fMLP		81.1 %		78.0 %		54.4 %	

$$\textbf{SI} = \frac{\%\ \text{of CD63}^{+}\ \text{events with drug}}{\%\ \text{of CD63}^{+}\ \text{events in buffer}}$$

(Similar calculation for CD203c)

- Clear sensitisation detectable to polysorbate 80.
- Sensitisation to dupilumab (dupixent which contains polysorbate 80) is actually due to the excipient of the formulation.

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Paclitaxel: importance of the formulation

- Paclitaxel in two different formulations → different basophil reactivity.

Albumin nanoparticle bound

Cremophor EL solvent (polyethoxylated castor oil)

		with IL-3		no IL-3		no IL-3	
Stimulant	**Conc. µg/ml**	**CD63**	**SI**	**CD63**	**SI**	**CD203c**	**SI**
Abraxane (Paclitaxel)	1	2.9 %	1.4	0.6 %	0.3	1.0 %	0.4
	.1	2.2 %	1.1	2.2 %	0.9	1.8 %	0.7
	.01	2.0 %	1.0	2.1 %	0.9	1.5 %	0.6
Taxol (Paclitaxel)	1	20.2 %	10.0	13.3 %	5.7	25.1 %	9.3
	.1	30.0 %	14.8	19.9 %	8.5	49.6 %	18.4
	.01	25.8 %	12.8	15.2 %	6.5	40.3 %	14.9
Controls							
Buffer		2.0 %		2.4 %		2.7 %	
anti-IgE		84.8 %		75.0 %		89.0 %	
fMLP		32.2 %		22.8 %		31.9 %	

- BAT detects reactivity differences in drug formulation changes.

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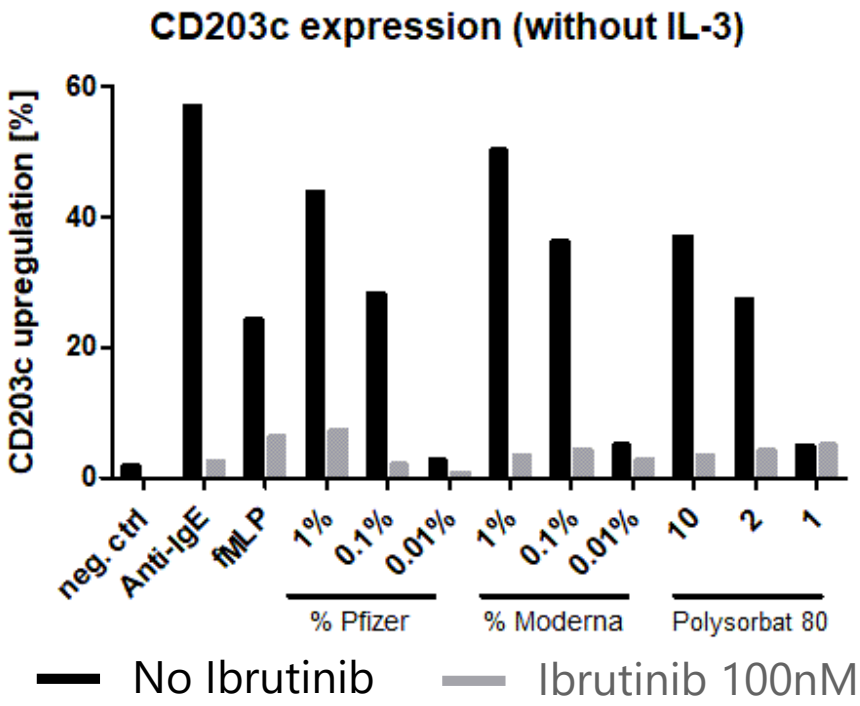
mRNA vaccine to SARS-CoV2
Assessment of activation mechanism

- Both COVID-19 mRNA vaccines activated basophils in sensitized patients.
- Polysorbate 80 has cross-reactivity in (~15%) of the vaccine-sensitised individuals.



Ibrutinib significantly reduces the basophil reactivity

→ *suggests the involvement of the IgE-specific pathway*.

Stehlin *et al.* (2022). *Front Allergy* **3**

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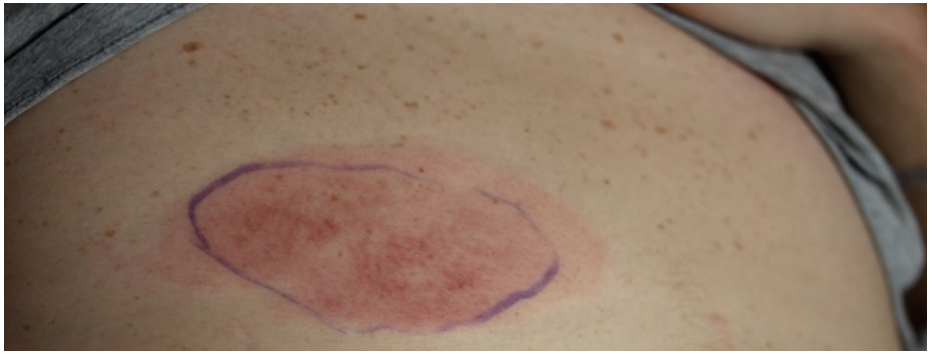
BAT in unexposed donors (n=5)
All reacted to drug X in different formulations

Overall BAT Innocuity SI (n=5)			SI values, positive if >2
Drug / conc.	with IL-3 **CD63** **SI**	without IL-3 **CD63** **SI**	without IL-3 **CD203c** **SI**
anti-IgE	32.8	21.5	23.6
fMLP	18.0	17.9	17.6
Drug X, form. A, 5000 ug/ml	11.5	12.1	9.8
Drug X, form. A, 1000 ug/ml	12.1	13.2	9.6
Drug X, form. A, 200 ug/ml	9.0	10.6	6.4
Drug X, form. A, 40 ug/ml	3.1	4.3	2.8
Drug X, form. B, 5000 ug/ml	14.3	16.1	15.9
Drug X, form. B, 1000 ug/ml	17.7	15.8	16.0
Drug X, form. B, 200 ug/ml	14.9	12.3	12.0
Drug X, form. B, 40 ug/ml	9.8	9.2	6.4
PS-80 100	1.9	0.9	1.8
PS-80 20	1.1	0.8	1.2
PS-80 5	0.8	1.2	1.1
PS-80 1	0.6	0.8	1.2
Formulation A, no drug 5000	1.6	0.9	2.4
Formulation A, no drug 1000	0.7	0.6	1.2
Formulation A, no drug 200	0.7	0.8	0.5
Formulation A, no drug 40	0.7	1.7	0.3



- Strong local reaction after s.c. infusion
- Modified antibody
- Reactivity in unexposed donor (no sensitisation possible)
- s.c. injection in high concentration
- Reactivity to the drug itself and not to the excipient

Conclusion

1. Immediate type reactions:
   - Antigen specific: **IgE-mediated** → BAT positivity in sensitized patients
   - Non-specific stimulation: **non IgE-mediated** → BAT positivity in drug-unexposed individuals
2. Activation mechanism can be evaluated by blocking the different pathways (e.g. Ibrutinib).
3. Evaluation of cross-reactivity (e.g. PPI).
4. Formulation / excipients maybe responsible for reactivity.
5. Alternative for serum sample: **indirect BAT with donor basophiles**.
6. Dosing considerations based on assessments.
7. Basophil reactivity and mediator degranulation:
   - pre-clinical drug development
   - allergy diagnosis

Thank you!

Delighted to discuss further:

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