Basophil activation markers

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Basophil activation tests (BAT)

- Measurement of mediator release
  - Histamine
  - Cysteinyi leukotrienes
  - Cytokines

- Flowcytometric measurement of “activation” molecules
  - CD63
  - CD203c
  - CD107a, CD107b (similar kinetics with CD63)*
  - CD13, CD164 (similar kinetics with CD203c)*

CD63

- Lysosomal-associated membrane protein-3 (LAMP-3)
- Located in the intracellular granule membrane of resting basophils and translocated to cellular membrane upon activation
- Related to “anaphylactic degranulation” of basophils
- Parallel kinetics with histamine release
CD203c

- Ectonucleotide pyrophosphatase/phosphodiesterase (ENPP)-3
- Constitutively expressed in resting cells and highly and rapidly upregulated upon activation
- Expressed specifically in basophils and mast cells
Expression/upregulation of basophil activation markers upon crosslinking of membrane-bound IgE

Basophil activation test

CLINICAL APPLICATIONS
Gating Strategy for CD203c detection

- Histogram 1 displays all events and gates all leukocytes.
- Position Region “A” to include all leukocytes while excluding debris.

- Histogram 2 excludes T lymphocytes and focuses on basophils.
- Displays events from region “A” (i.e. Leukocytes).
- Region “B” is adjusted to include lymphocytes and monocytes while excluding CD3\textsubscript{pos} events (i.e. T lymphocytes).
Histograms 3a, 3b and 3c display events from gate “A” and “B” (AB).

Analysis:

- The Y-Median fluorescence intensity (CD203c fluorescence intensity) parameter of non-activated and activated basophils can be determined from Region C.
- Data can be expressed by comparing Y-Median of
  - Activated (allergen) versus Activated (Positive Control) or
  - Ratio « Activated (allergen)/Neg Ctrl » versus Ratio « Activated (Positive Control)/ Neg Ctrl »
Clinical utility of CD203c BAT

- Egg allergy
  - Predicting OFC outcomes
  - Predicting prognosis

- Wheat allergy
  - Epidemic of wheat-dependent exercise-induced anaphylaxis in Japan
Clinical utility of CD203c BAT

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Diagnosis of egg allergy (EA)

OFC-positive v.s. OFC#-negative

Area under the ROC curve

<table>
<thead>
<tr>
<th></th>
<th>EW CAP IgE</th>
<th>EW CD203c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut off</td>
<td>&gt;8.8</td>
<td>&gt;12.75</td>
</tr>
<tr>
<td>Likelihood ratio</td>
<td>2.62</td>
<td>5.63</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>61.6</td>
<td>82.3</td>
</tr>
<tr>
<td>Specificity</td>
<td>76.5</td>
<td>85.3</td>
</tr>
<tr>
<td>PPV</td>
<td>93.8</td>
<td>97.0</td>
</tr>
<tr>
<td>NPV</td>
<td>25.5</td>
<td>45.3</td>
</tr>
</tbody>
</table>
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Egg allergen components

- Gal d 1 : ovomucoid
- Gal d 2 : ovalbumin
- Gal d 3 : ovotransferrin/conalbumin
- Gal d 4 : lysozyme
- Gal d 5 : livertininprotein in egg yolk, chicken serum albumin
Ovomucoid (Gal d 1)

- Heat/digestion-stable protein
- Allergenic in small amount
- Higher ovomucoid IgE indicates intolerance to both raw and heated eggs.
- Absence or lower ovomucoid IgE may predict tolerance development.
The Third domain of ovomucoid (OM-DM3)

N-terminal amino acid of ovomucoid sequence and primary structure (Kato et al., 1978)
Natural history of egg allergy and Basophil activation test at initial challenge test

Entry → 1 year → 2 years

OFC

intolerant → intolerant → intolerant

tolerant

tolerant

tolerant

Initial BAT with CD203c
- egg white
- ovomucoid
- OM-DM3

* Intolerance to egg was confirmed by OFC or apparent egg-induced immediate symptoms within 1 month before entry

N=42
N=21
N=28
Low response to OM-D3 may predict future tolerance

Egg white

Ovomucoid

OM-D3

Kruskal-Wallis test

Dunn's Multiple Comparison Test

P value

Intolerant 2 years 1 year

CD203c high%

Intolerant 2 years 1 year

CD203c high%

Intolerant 2 years 1 year

CD203c high%

Egg white

Ovomucoid

OM-D3

Kruskal-Wallis test

Dunn's Multiple Comparison Test

P value 0.0003

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Egg white

Ovomucoid

OM-D3

Kruskal-Wallis test

Dunn's Multiple Comparison Test

P value < 0.0001

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Egg white

Ovomucoid

OM-D3

Kruskal-Wallis test

Dunn's Multiple Comparison Test

P value < 0.0001

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Oral immunotherapy for severe egg allergy: a randomized controlled trial

Study design

Egg allergy → Rush OIT → Maintenance

Delayed control → Rush OIT → Maintenance

Primary endpoint: DBPCFC

Ito N, Fujisawa T, Shimojo N, Iwata T, manuscript in preparation
Oral immunotherapy for severe egg allergy: a randomized controlled trial

Ito N, Fujisawa T, Shimojo N, Iwata T, manuscript in preparation
BAT with CD203c

Ovomucoid domain 3

Repeated Measures ANOVA
P value
0.0169

Dunnett's Multiple Comparison Test
*

CD203c high%
pre post 2m 12m
0 20 40 60 80 100
rush phase maintenance phase

Ovomucoid (Gal d 1)

Repeated Measures ANOVA
P value
< 0.0001

Dunnett's Multiple Comparison Test
*

CD203c high%
pre post 2m 12m
0 20 40 60 80 100
rush phase maintenance phase
Clinical utility of CD203c BAT

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- Wheat allergy
  - Epidemic of wheat-dependent exercise-induced anaphylaxis in Japan
Epidemic of wheat-dependent exercise-induced anaphylaxis due to hydrolyzed wheat-containing facial soap

Very popular among ladies

Typical ad says;
“This soft and tender foam makes your skin shiny.”
“Don’t give up!!”

Skin/mucosal sensitization

Spaghetti at lunch with friends then Aerobics exercise at gym

Angioedema

Anaphylaxis

Generalized urticaria
Abdominal cramp
Hypotension
Epidemic of wheat-dependent exercise-induced anaphylaxis due to hydrolyzed wheat-containing facial soap


Nealy 1000 cases in 3 years have been reported!!

Spaghetti at lunch with friends then Aerobics exercise at gym

Angioedema

Anaphylaxis

Generalized urticaria Abdominal cramp Hypotension
Application of “raw” material to BAT

HWP-soap

Regular soap
CD203c expression by HWPs
HWP-soap-sensitized patients did not respond to omega-5 gliadin
Two types of wheat-dependent exercise-induced anaphylaxis

Summary

- Basophil activation test (BAT) measuring activation molecules in basophils is useful for diagnosis of food allergy.

- Possible advantages of BAT over serum specific IgE measurement may be:
  - It directly measure basophil activation, a major effector cell in allergy.
  - It reflects not only IgE levels but cellular activation status and other factors in serum including “blocking IgG antibodies”.
  - Very small amount of possible allergen can be applied to the assay, enable to test “rare” allergens.