WISC 2012

PG course: Food allergy track

Basophil activation markers

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Basophil activation tests (BAT)

- Measurement of mediator release
  - Histamine
  - Cysteinyl leukotrienes
  - Cytokines
- Flowcytometric measurement of "activation" molecules
  - CD63
  - CD203c
  - CD107a, CD107b (similar kinetics with CD63)*
  - CD13, CD164 (similar kinetics with CD203c)*

* Hennersdorf F,. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. Cell Res 2005; 15:325-35.

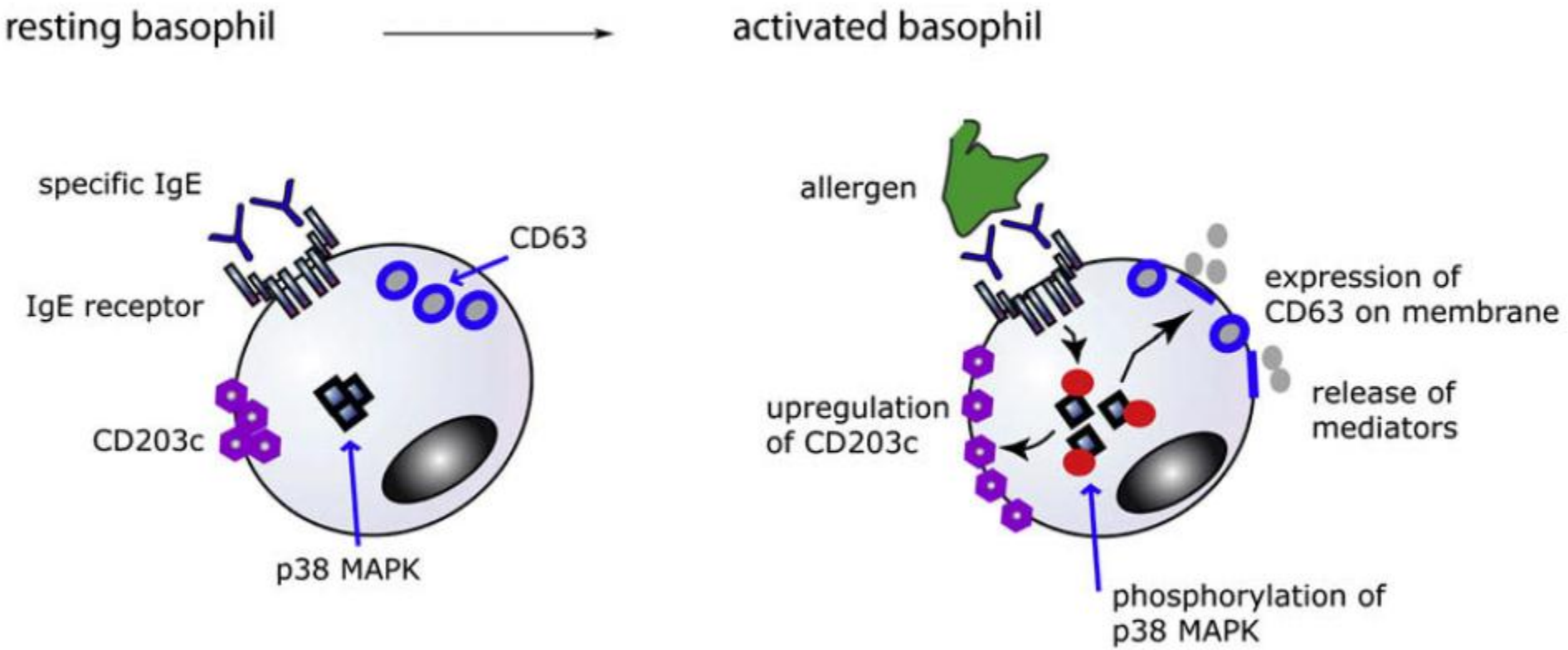
CD63

- 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 "anaphylacic degranulation" of basophils
- Parallel kinetics with histamine release

CD203c

- Ectonucleotide pyrophosphatase/phosphodiesterase (ENPP)-3
- Constituitively 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

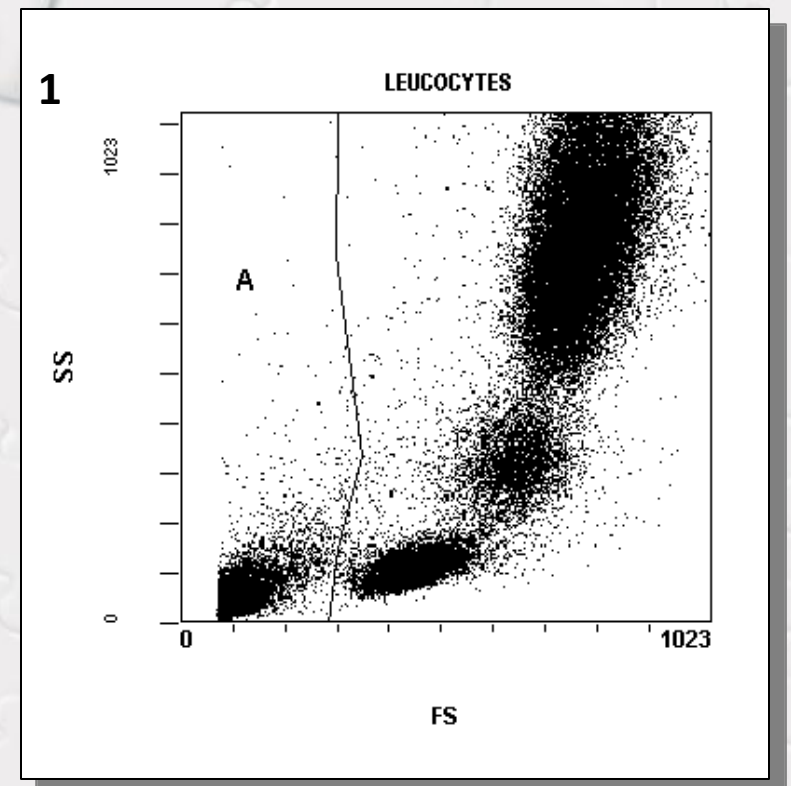


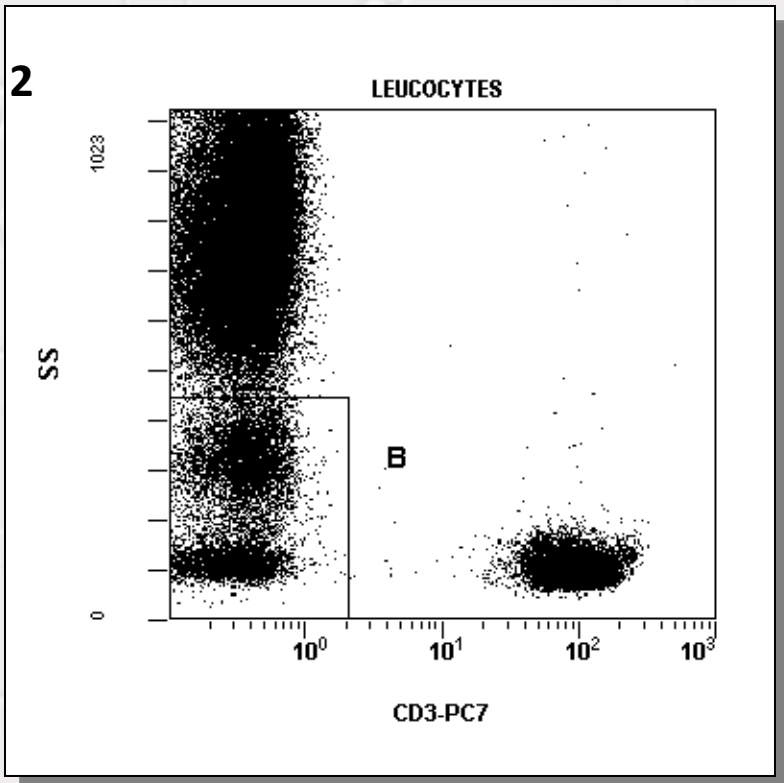
Adapted from Hausmann OV, et al. The basophil activation test in immediate-type drug allergy. Immunol Allergy Clin North Am 2009; 29:555-66.

Basophil activation test

CLINICAL APPLICATIONS

Gating Strategy for CD203c detection



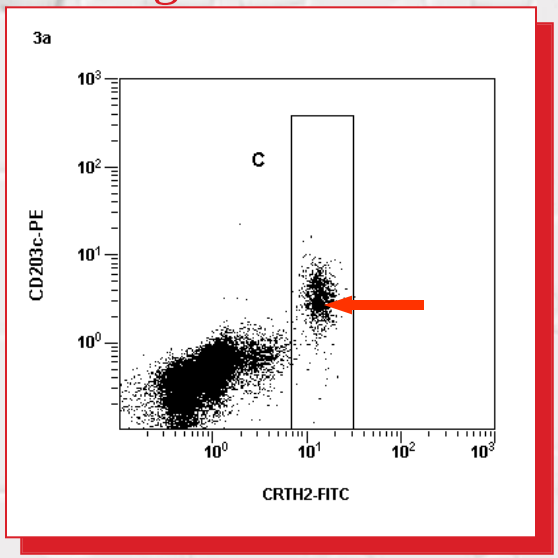


- Histogram 1 displays all events and is gates all leukocytes.
- Position Region "A" to include all leukocytes while excluding debris.

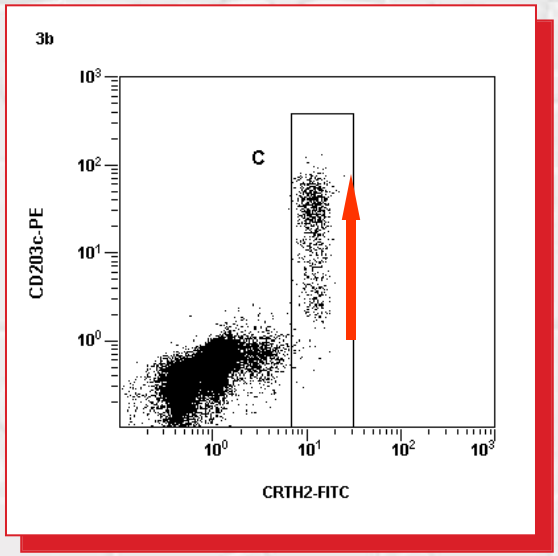
- Histogram 2 excludes T lymphocytes and focuses on basophils.
- Displays events from region "A" (i.e. Leukocytes).
- Region "B" is adjusted to include lymphocytes and monocytes while excluding $CD3^{pos}$ events (i.e. T lymphocytes).

Analysis Strategy for CD203c

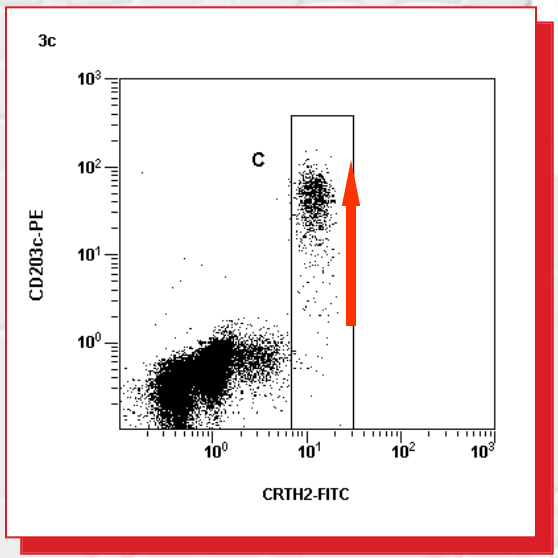
Negative Control



Positive Control



Allergen



- 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 (Postive 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

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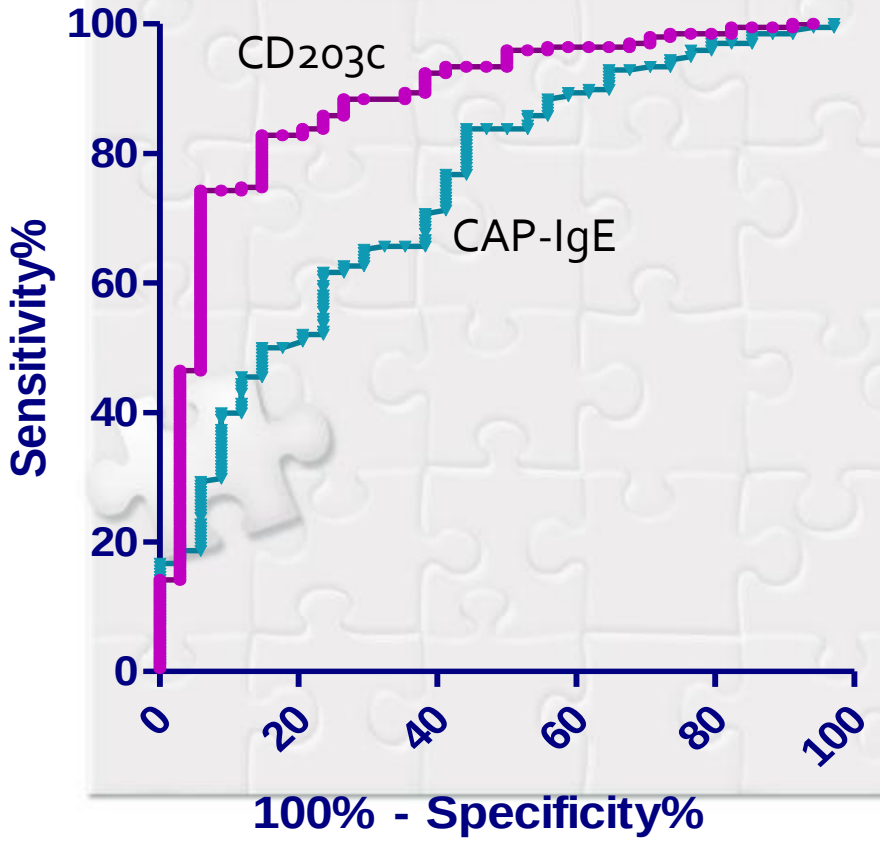
Diagnosis of egg allergy (EA)

OFC with heated egg → failed → EA
OFC with heated egg → passed → OFC with unheated egg → Failed → EA
OFC with unheated egg → passed → Tolerant

OFC-positive v.s. OFC#-negative



	EW CAP IgE	EW CD203c
Area under the ROC curve	0.746	0.886
Cut off	>8.8	>12.75
Likelihood ratio	2.62	5.63
Sensitivity	61.6	82.3
Specificity	76.5	85.3
PPV	93.8	97.0
NPV	25.5	45.3

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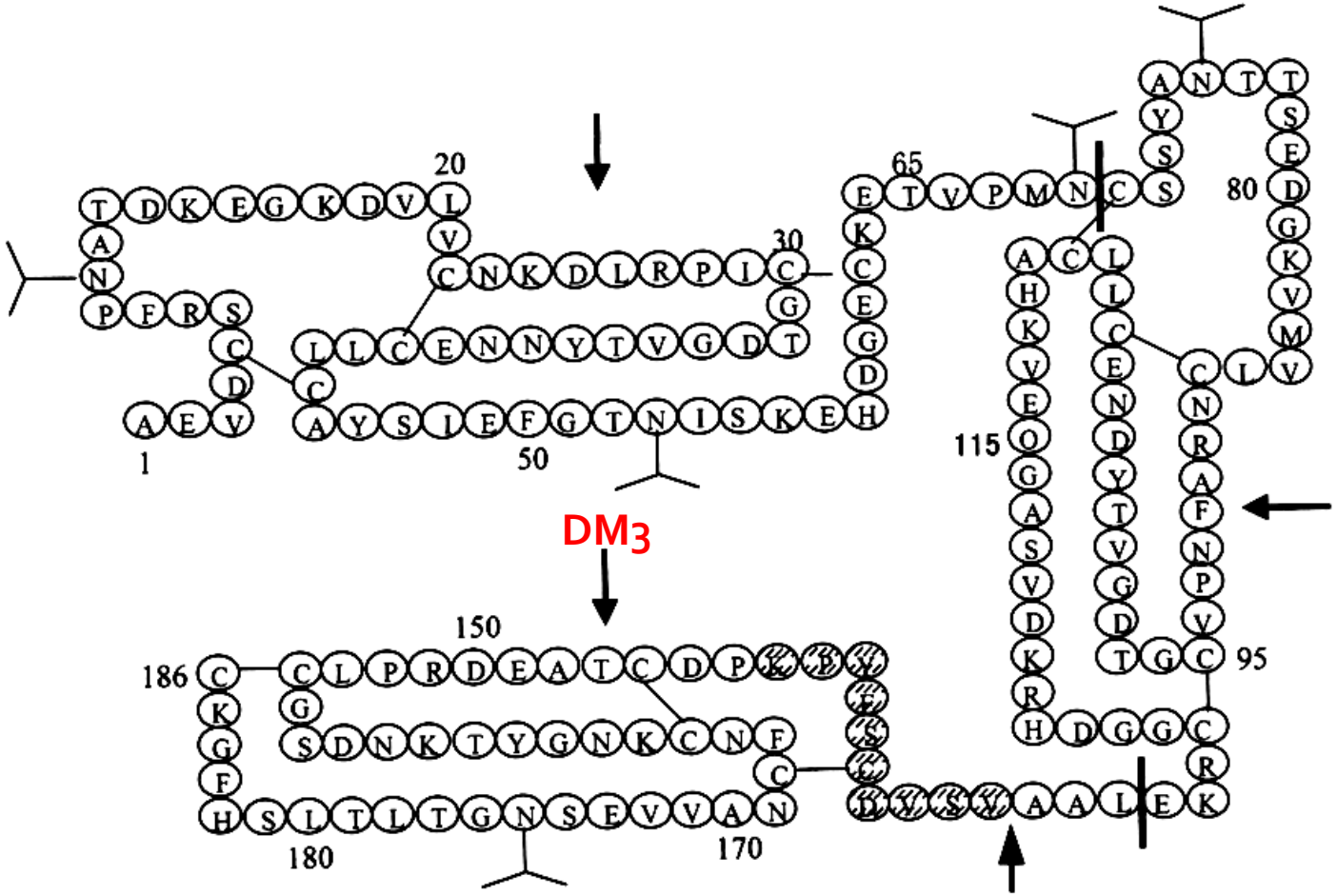
Egg allergen components

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

Ovomucoid (Gal d 1)

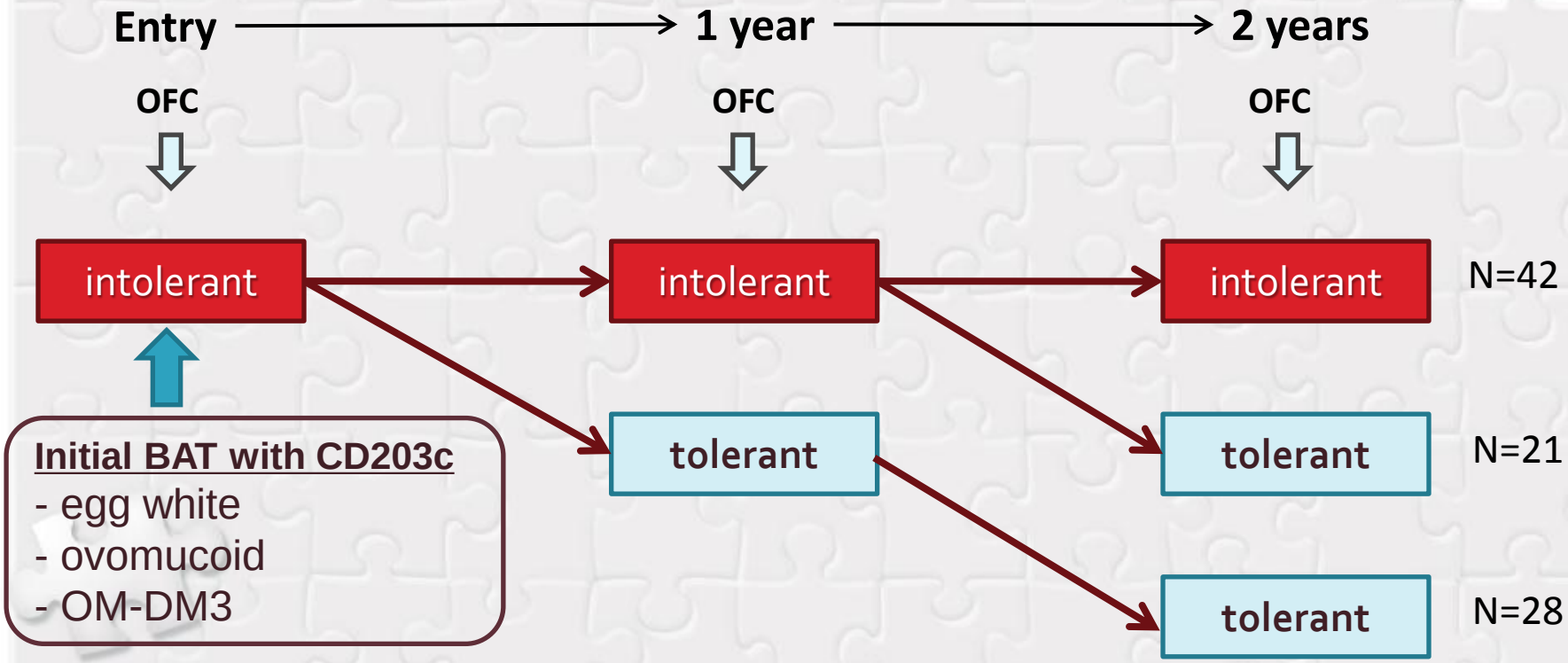
- Heat/digestion-stable protein
- Allergenic in small amount
- Higher ovomucoid IgE indicates intolerance to both raw and heated eggs.
  - Ando H, Borres MP, Urisu A: Utility of ovomucoid-specific IgEconcentrations in predicting symptomatic egg allergy. J Allergy ClinImmunol2008;122:583-588.
- Absence or lower ovomucoid IgE may predict tolerance development.
  - JarvinenKM, Sampson HA: Specificity of IgEantibodies to sequential epitopesof hen's egg ovomucoidas a marker for persistence of egg allergy. Allergy 2007;62:758-765.

The Third domain of ovomucoid (OM-DM3)



N-terminal amino acid of ovomucoid sequence and primary structure （Kato et al., 1978）

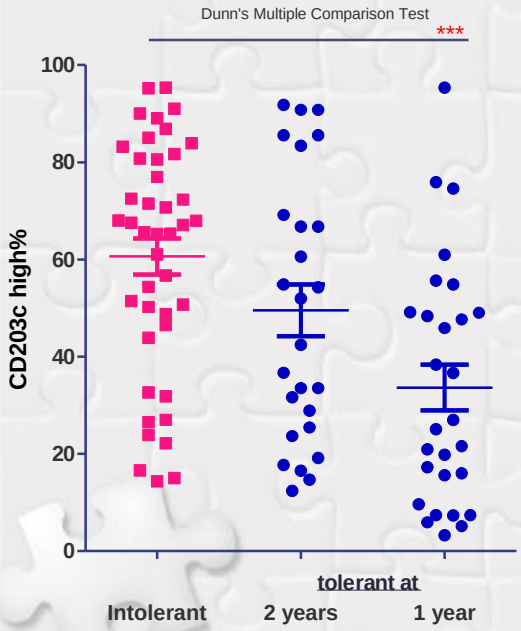
Natural history of egg allergy and Basophil activation test at initial challenge test



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

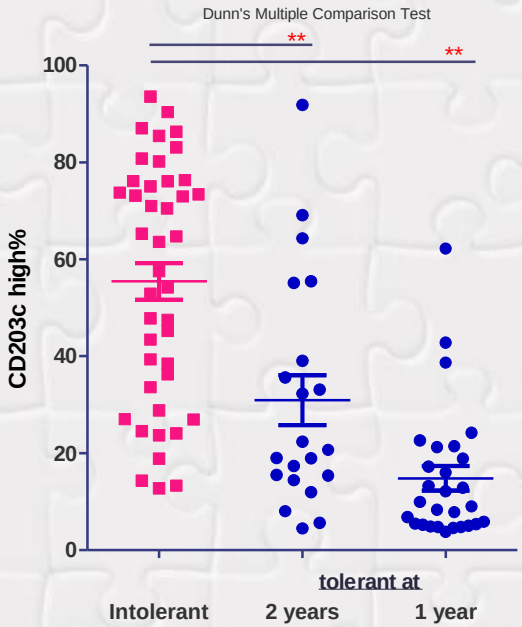
Low response to OM-D3 may predict future tolerance

Egg white



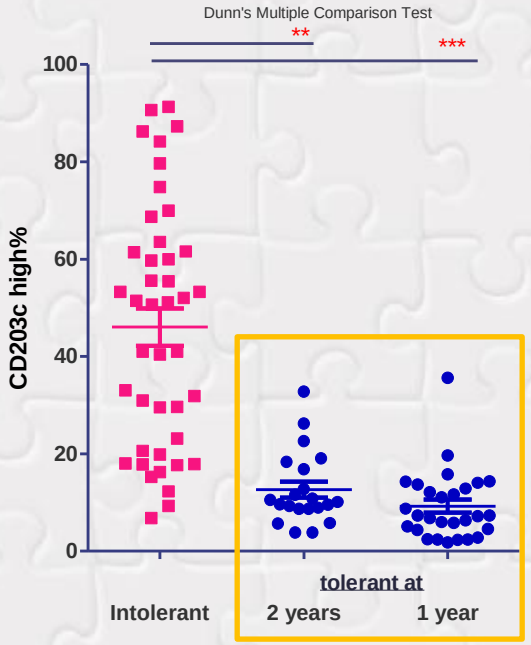
Kruskal-Wallis test P value	0.0003

Ovomucoid



Kruskal-Wallis test P value	< 0.0001

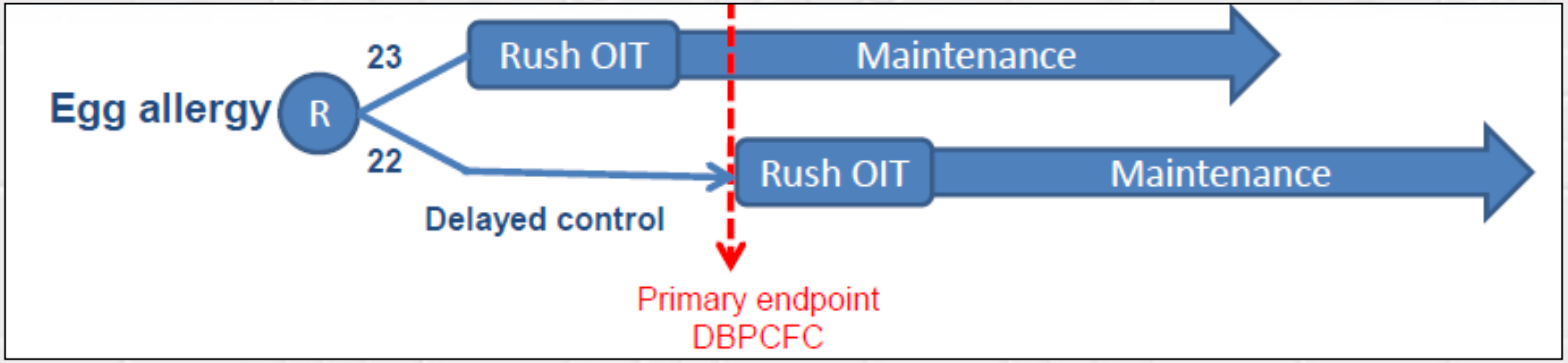
OM-D3



Kruskal-Wallis test P value	< 0.0001

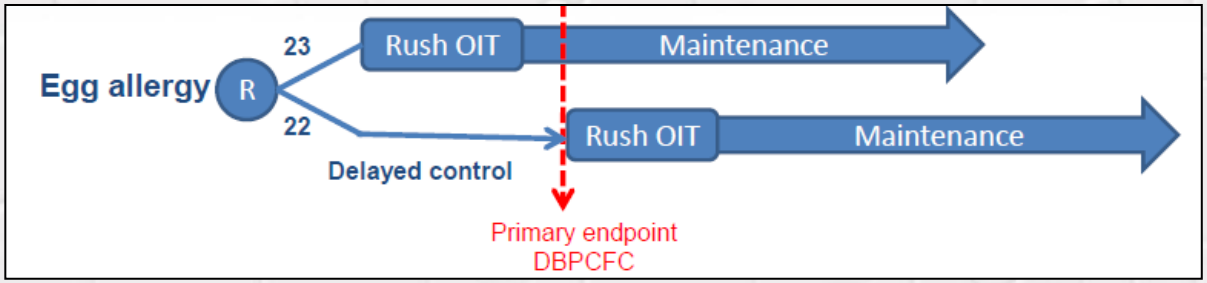
Oral immunotherapy for severe egg allergy : a randomized controlled trial

Study design

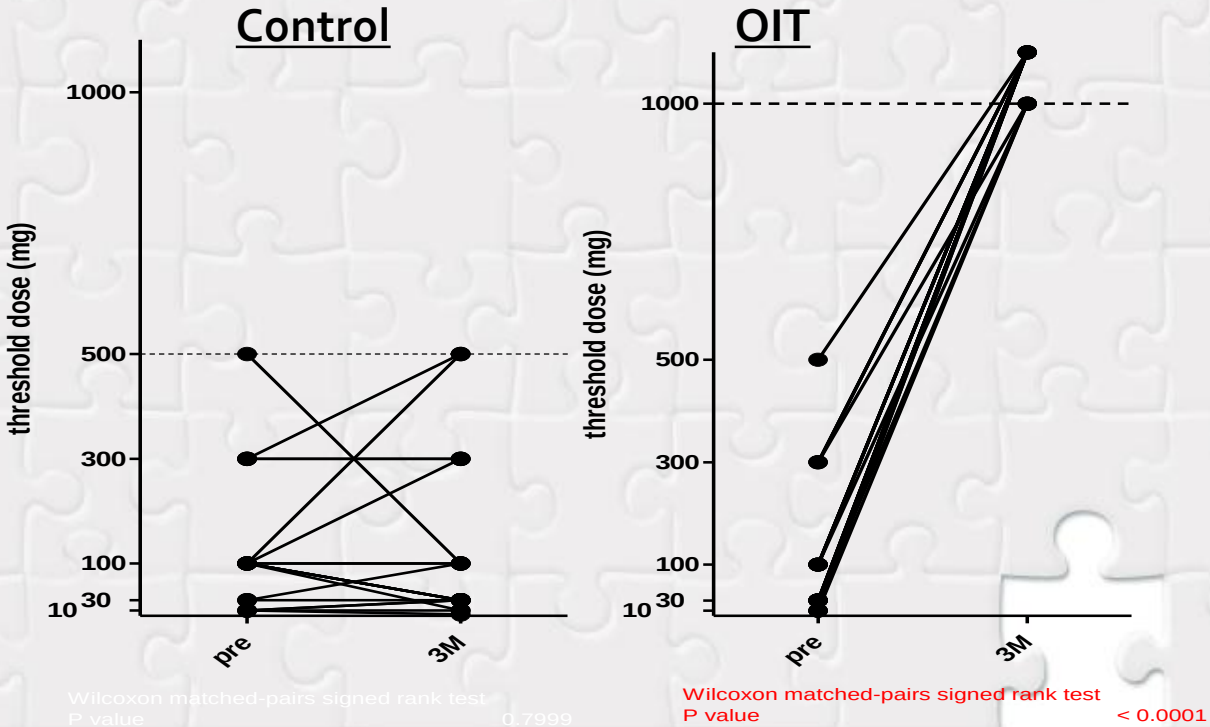


Ito N, Fujisawa T, ShimojoN, Iwata T, manuscript in preparation

Oral immunotherapy for severe egg allergy : a randomized controlled trial



Threshold In DBPCFC



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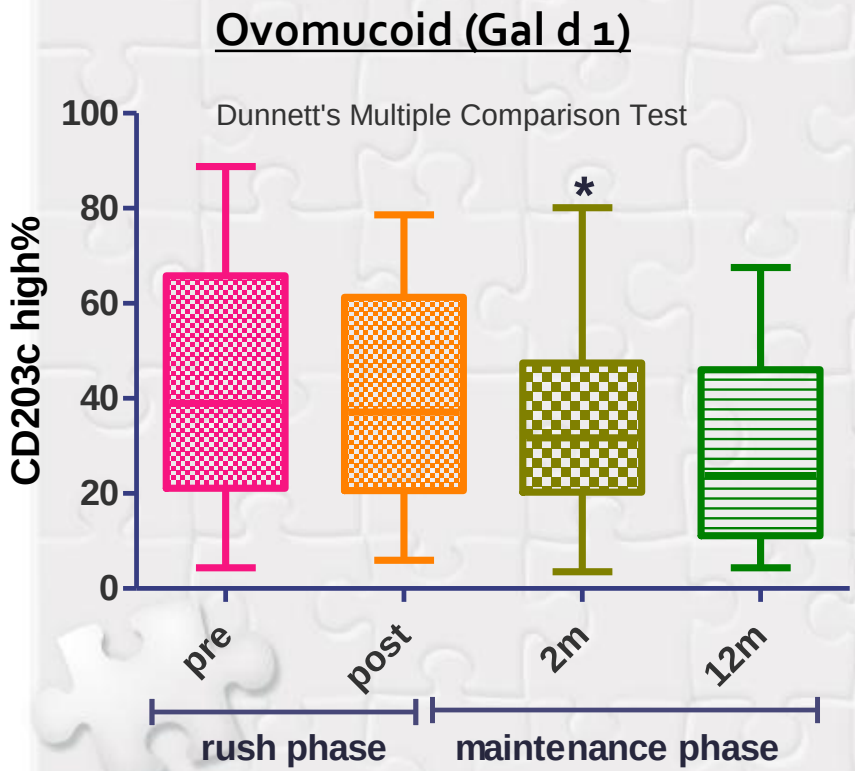
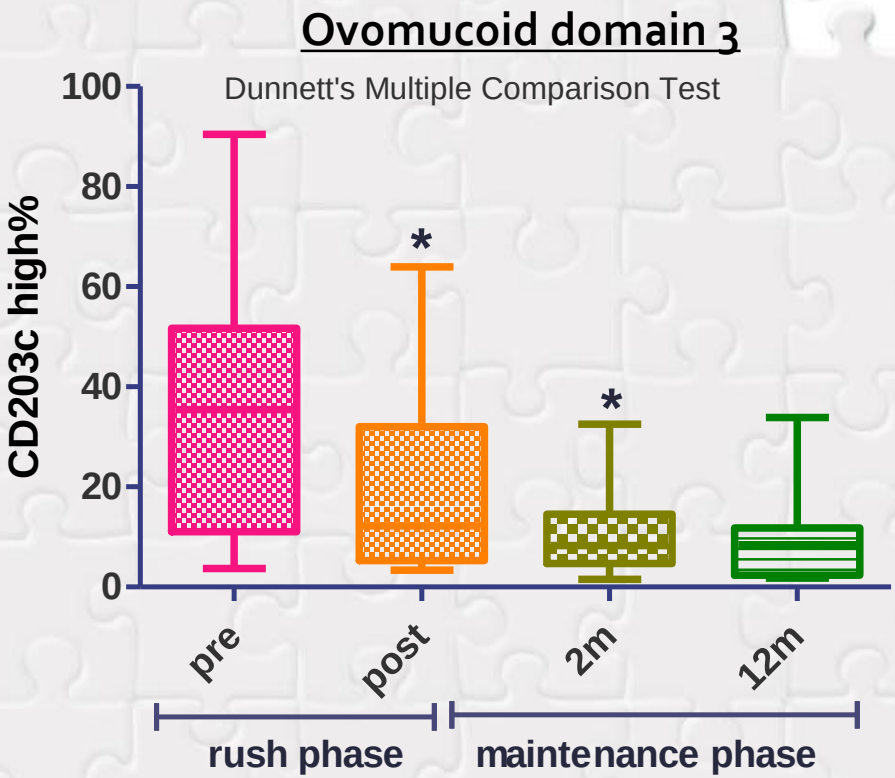
BAT with CD203c
Ovomucoid domain 3



Repeated Measures ANOVA
P value 0.0169



Repeated Measures ANOVA
P value < 0.0001

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Epidemic of wheat-dependent exercise-induced anaphylaxis due to hydrolyzed wheat-containing facial soap

Hydrolyzed wheat protein (HWP)-containing soap

▲これが「茶のしずく石鹸」のもっちり泡。肌を傷つけず、美肌へ導く。

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

Anaphylaxis

Generalized urticaria
Abdominal cramp
Hypotension

Epidemic of wheat-dependent exercise-induced anaphylaxis due to hydrolyzed wheat-containing facial soap

FukutomiY: Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol2011;127:531-533 e531-533.

Nealy 1000 cases in 3 years have been reported!!

Spaghetti
at lunch with friends
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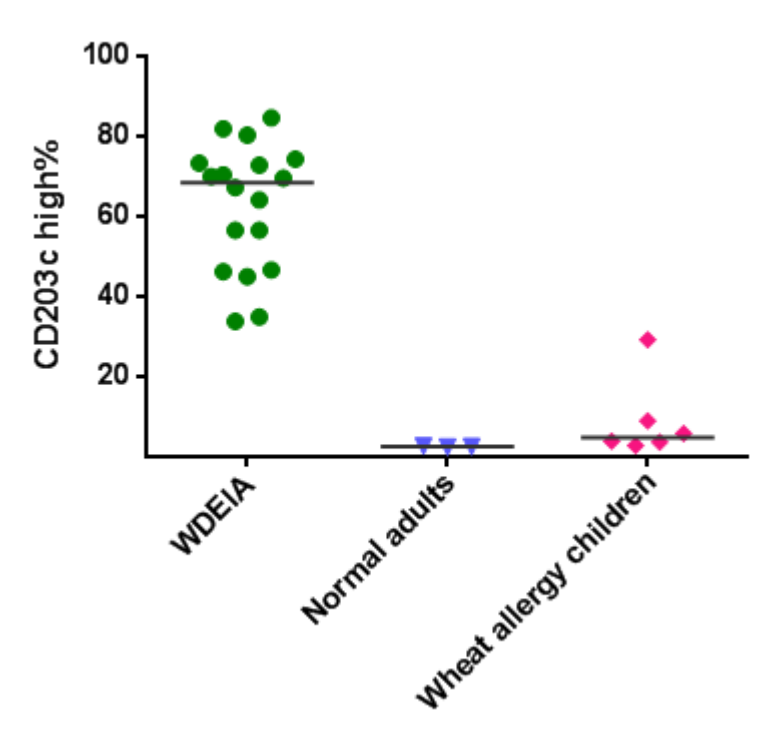
Aerobics exercise at gym

Anaphylaxis

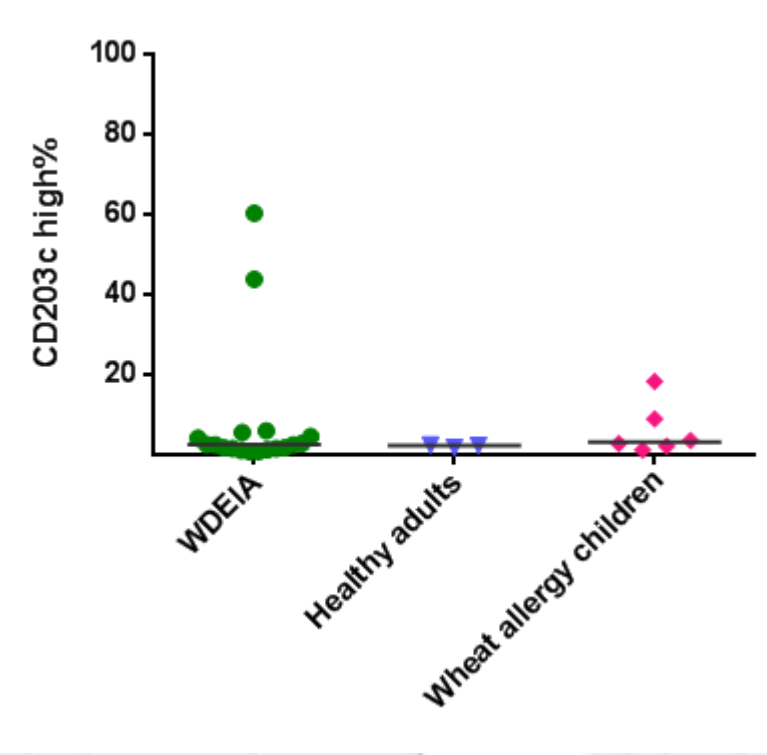
Generalized urticaria
Abdominal cramp
Hypotension

Application of "raw" material to BAT

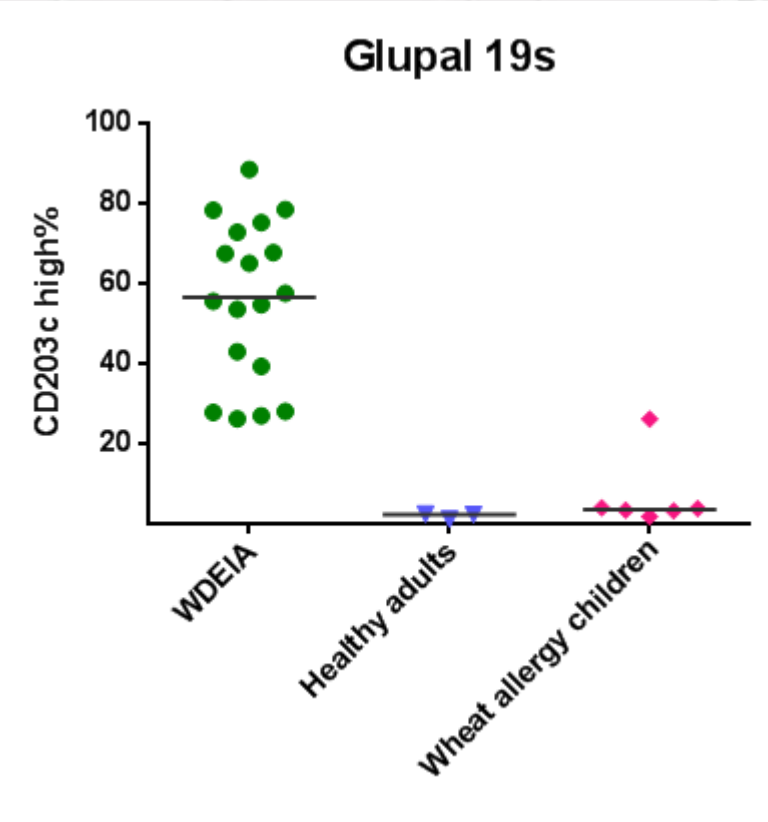
HWP-soap

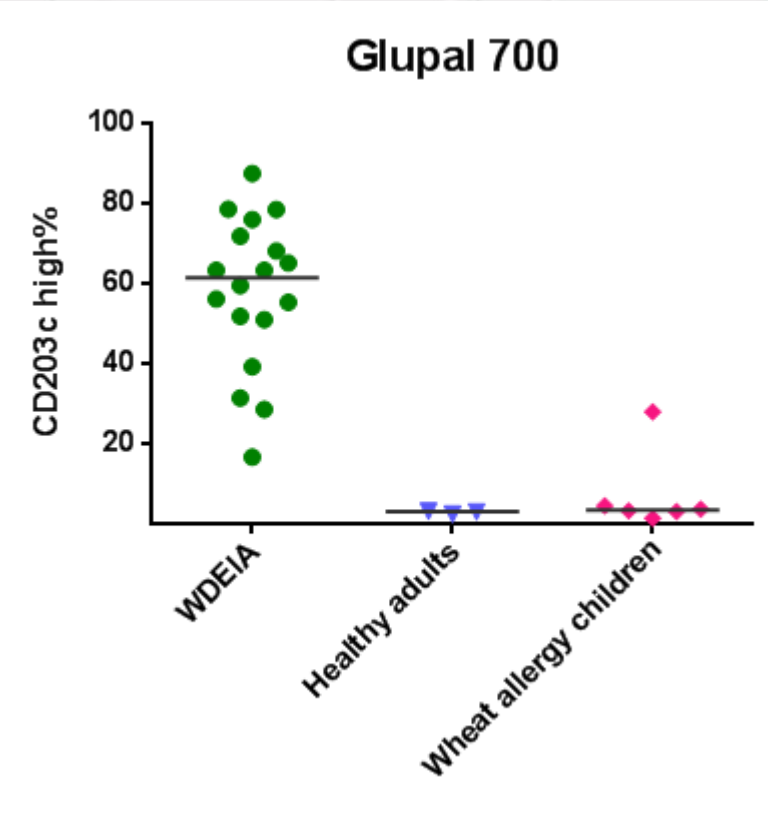


Regular soap

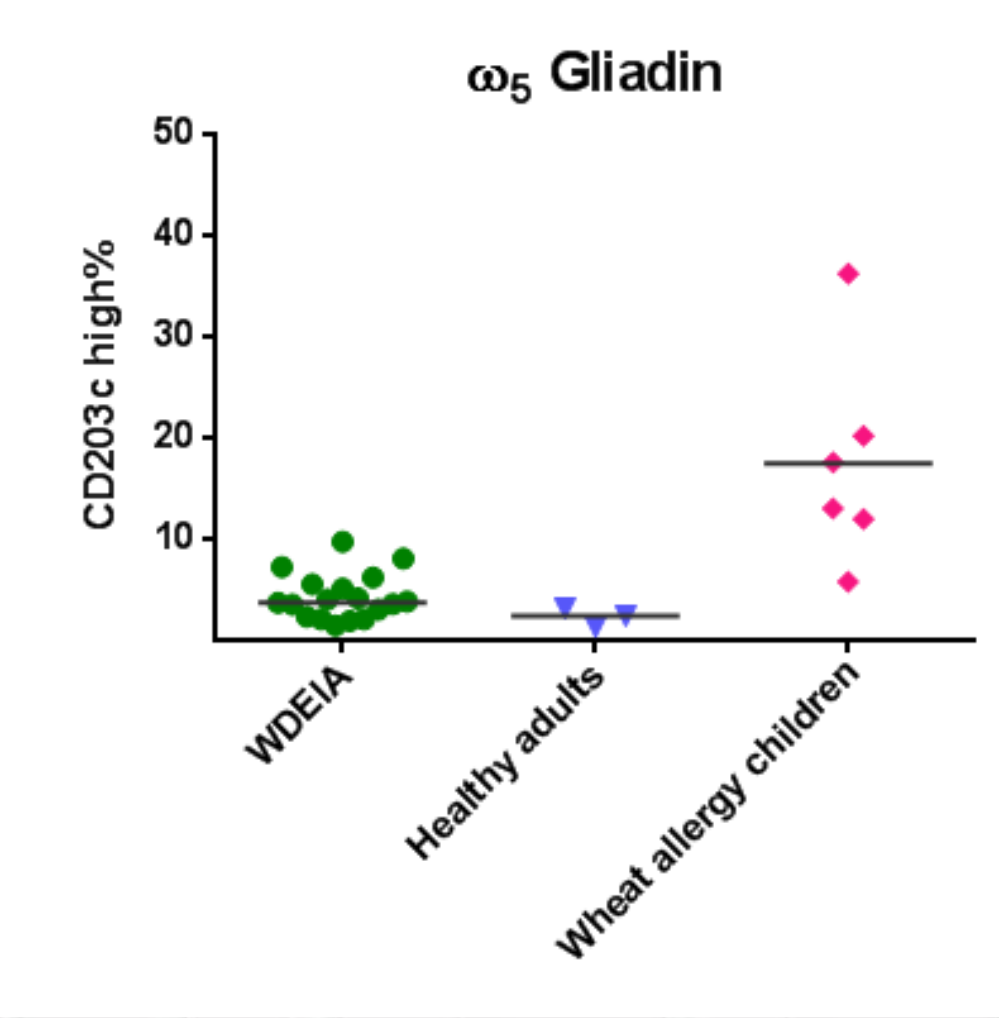


CD203c expression by HWPs

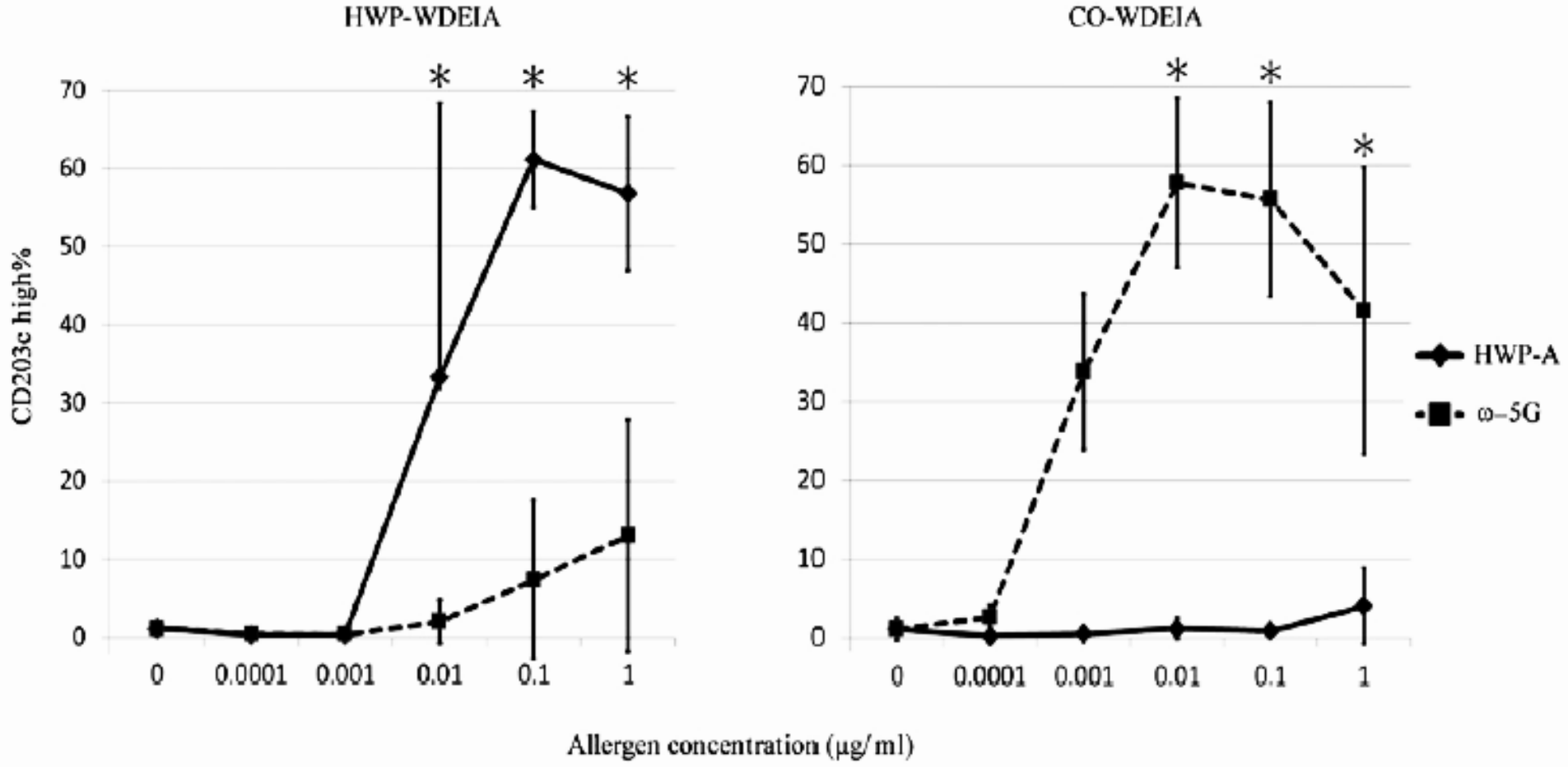




HWP-soap-sensitized patients did not respond to omega-5 gliadin



Two types of wheat-dependent exercise-induced anaphylaxis



Chinuki Y, Kaneko S, Dekio I, Takahashi H, Tokuda R, Nagao M, Fujisawa T, Morita E. CD203c expression-based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol 2012; 129:1404-6.

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.