

Rare events in Flow Cytometry: Basophil Activation Test by whole blood expression of CD203c in pediatric Food Allergy, Cow's milk, Egg white and Peanut.

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Basophils: short history

- 1902 Richet and Portier, discovery of Anaphylaxis
- 1962 Shelley, Microscopic studies
- 1970 Leynadier, Benveniste: Basophils Degranulation
- 1981 DeWeck, Basophiles in Flow cytometry with Alcyan Blue
- 1991 Knol, CD63 activation in Flow Cytometry
- 1997 Sainte Laudy, Sabbah, CD63 developement in Clinical Allergy
- 1999 Abuaf, Problems of non specific activation in FCM
- 2000 Buehring, Blood 1999 94 2343-56,, HLDA7, CD203c 97A6
- 2001 Crockard, Editorial Clin Exp Allergy:
Laboratory Based allergy diagnosis: Should we go with the flow ?
- 2006 D.Ebo Allergy BAT Review



BASOPHILE ACTIVATION TEST (BAT)

- ◆ **B**iological diagnostic approach of cellular mechanism in allergic diseases.
- ◆ **Q**uantitative study, by flow cytometry, of in vitro activation of basophils, to evaluate **IgE dependent response** specific antigen.
- ◆ Based on modifications of expression of surface markers of the basophil membranes.

The Basophil Activation Test, by flow cytometry, as basic biological analysis, begins with pre-analytique phase.

- Samples of venous blood are drawn « bedside patients »
- allowing whole blood technique with lysis of red cells.
- enrichment of White Blood Cells is also a possibility.

- The basophils must be alive with good viability !
- The time to proceed for labeling for FCM is only few hours and no more than 24h.

Samples with AntiCoagulant

Blood is collected in standard vacutainer tubes with either Citrat, or EDTA, or Heparin.

Heparin for CD203c

EDTA for Kit Allergenicity^o

K-EDTA, ACD for Flow-Cast^o

Next, some choices are required for the analytical phase.

- The use of enrichment in WBC by gravity sedimentation, may be done,
- by simple "buffy coat" or use of Dextran.
- The whole blood method is often used, followed by lysis of red cells.

- Lysis methods are numerous;
- Some may be inadequate for basophils expression.
- Comparative trials must be performed.

Flacon de lyse préconisé.

Référence : PN 6602764 ou PN 6603152

Whole blood lysing reagent kit

Lysis Methods for CD203c.

Lyses utilisables disponibles dans le laboratoire:

Ref: IM2402 Lysing Solution NH4Cl 10X Concentrate, 20 ml; Part of StemKit
To be initially used

Ref PN IM 3514 IOTEST3 Lysing Solution 10X, 20 ml.
To be used in second stage.

Products preserved at 2-8°C, not frozen.

1 X solution should be prepared for daily use: 1 vol 10X in 9 vol of water.

Fix & Lyse in Allergenicity Kit

A minimum of 3 tubes are required to perform a BAT:

- **Negative Test**, basic reference for expression of asleep basophiles,
- **Positive Test**, with anti-IgE,
reference of maximal expression capacity by Basophils,
- **Allergen Test**. with specific liquid allergen,
- **One additional for each allergen.**

The choice, the origin, and dilution of the allergen are very important for BAT preparation.

Allergens for BAT.

- Home made by laboratory
- Liquid drugs for venous injections
- Liquid Allergens
 - Flow Cast Buhlmann
 - Stallergènes
 - Allerbio
 - Other

Allergens and dilutions

- **Choice of allergens** : for example in our study

Allergenic Extracts of Egg White, Peanuts ,(Stallergènes®)

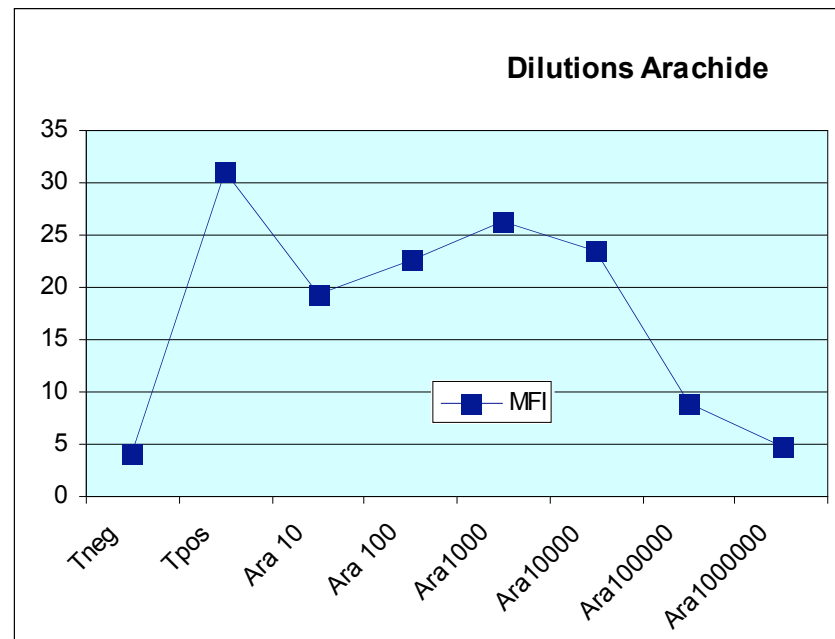
for prick tests

Native food Allergen for Cow's Milk (Nactalia $\frac{1}{2}$ non fat) because soluble extract not possible (Prion risk).

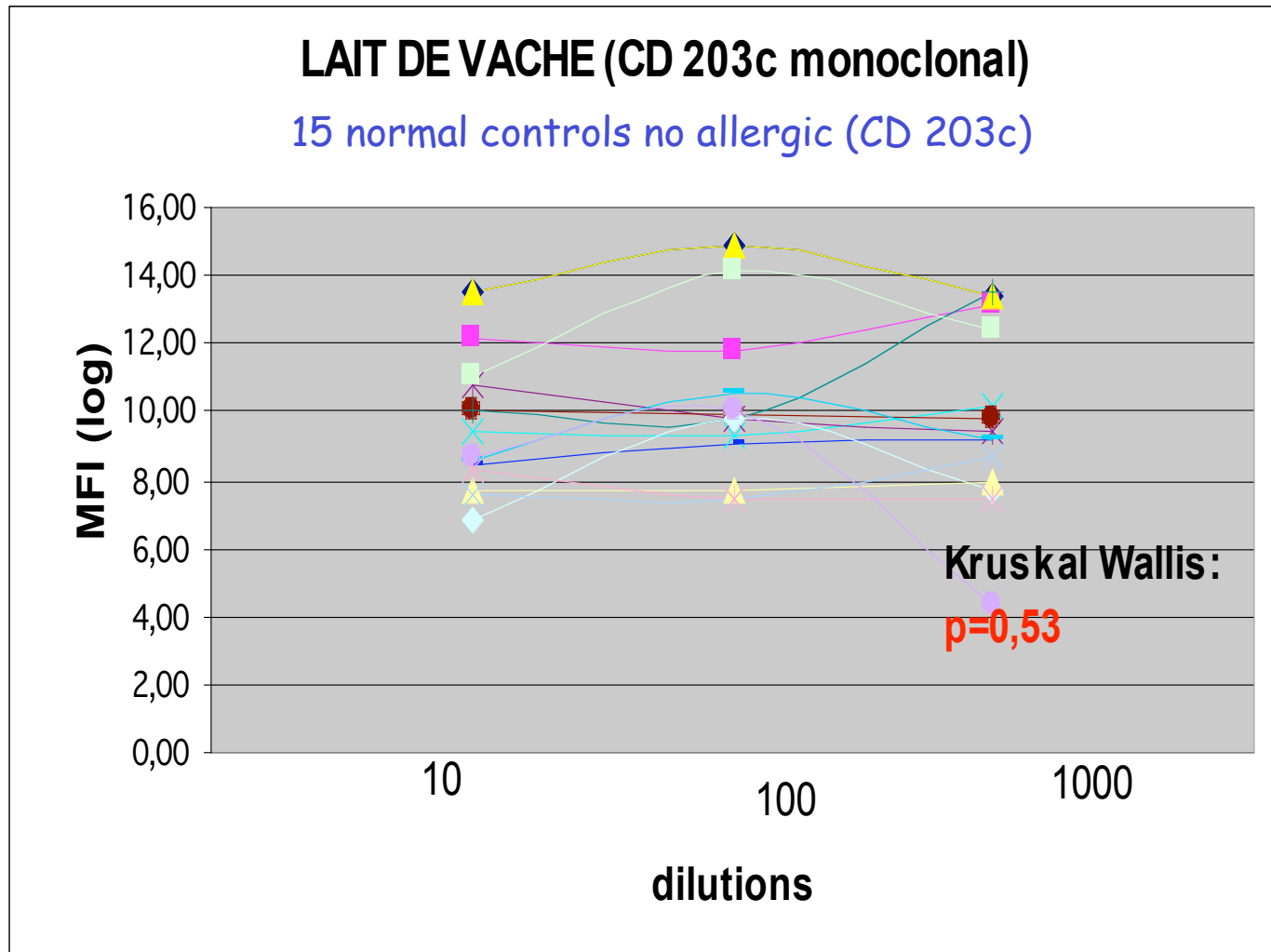
- **Establish the dilution of Allergens to use.**

Dilution de $1/10^0$ until $1/1\ 000\ 000$

Dilution	MFI	IndexMFI
Tneg	4,07	
Tpos	31,1	7,64
Ara 10	19,4	4,77
Ara 100	22,7	5,58
Ara1000	26,4	6,49
Ara10000	23,5	5,77
Ara100000	8,9	2,19
Ara1000000	4,79	1,18



Validation of dilutions for allergens



- No False Positives.
- Absence of direct toxicity by allergens
- MFI stable at the 3 dilutions assessed

CD and Basophils in Flow Cytometry

Mastocytes and Basophils express, the **CD45** of Leucocytes, and some other Human Leucocyte Differentiation Antigens – HLDA :

CD63, CD117 SCFR , CD123-IL3r, CD125-IL5ra, CRTH2, CCR3.

Mast cells of umbilical cord express:.

CD92, CD97, CD98-4F2, CD99-E2, CD108, HLA-I, CD147-Neurothelin, CD151-PETA3.

Unfortunately, none of these CD are restricted to this lineage of cells.

This is mainly by use of **antibodies such as anti-IgE, CD45, CD63 et CD203c** that basophils are identified by Flow Cytometry in Allergy.

At the HLDA workshop held in June 2000, the new **CD203c (97A6/NPP3)** was recognised to be specific for basophils and mastocytes and their precursors.

During presentation of allergens Basophils increase the surface expression of CD45 (160%), CD47 (90%), CD63 (200%) and CD203c (more).

Finally we can observe a very strong increase of the expression for the surface marker, and in Flow Cytometry the fact appears by an **increase of Mean Fluorescence Intensity = MFI.**

Basophils and CD in Flow Cytometry HLDA CD63

PROW and IWHLDA present the GUIDE on: **CD63**

Authors: Masja de Haas; David O. Azorsa

Reviewer: Martin E. Hemler

ALTERNATE NAMES FOR CD63

- LIMP, MLA, PTLGP40
- granulophysin
- lysosomal-membrane-associated glycoprotein 3, LAMP-3
- melanoma-associated antigen, ME491
- neuroglandular antigen, NGA

REAGENTS

CD63-SPECIFIC MABS NEWLY ASSIGNED AT SIXTH INTERNATIONAL WORKSHOP

NAME(Workshop)	SOURCE or REFERENCE
TEA 3/18	Sanchez-Madrid, Madrid
LP9	Partridge, Sheffield

SELECTION OF OTHER CD63-SPECIFIC REFERENCE MAB

CLB-gran/12	CLB, Amsterdam
RUU-Sp2.28	Nieuwenhuis, Utrecht

Basophils and CD in Flow Cytometry HLDA CD63

CELLULAR EXPRESSION OF CD63

- Intracellular lysosomal/endosomal/granule protein, in Weibel-Palade bodies of vascular endothelium**
 - Translocated to the surface of activated platelets, endothelium**
- Degranulated neutrophils, monocytes, macrophages, and endothelium
- Immunohistochemistry showed staining of fibroblasts, osteoclasts, smooth muscle, neural tissue (brain white matter and peripheral nerves) and synovial lining cells

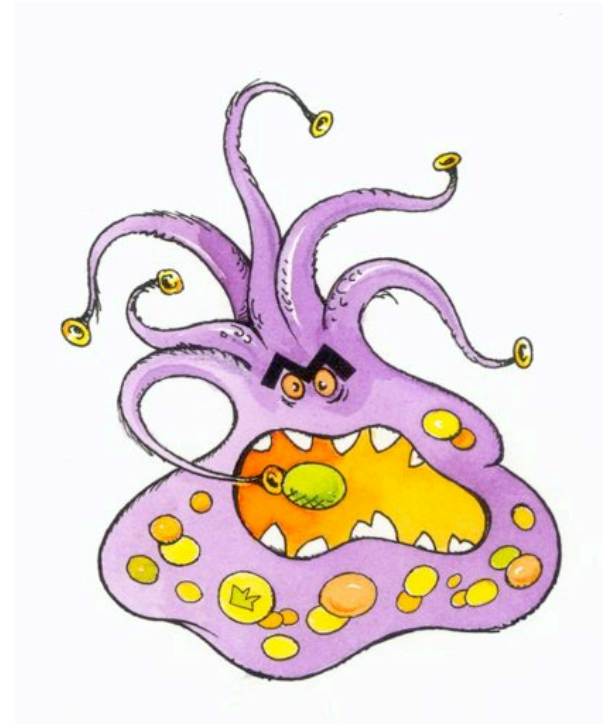
CELLULAR FUNCTION OF CD63

- CD63 transfection reduced melanoma cell motility on fibronectin, collagen and laminin, and reduced the growth and metastasis of melanoma cells in nude mice (Radford et al. 1995,1997)**
- CD63 has been used as a marker for late endosomes**

HLDA7, June 2000, Harrogate UK

MYELOID Lineage :

- 34 Ab proposed, 17 Ab defined
- 20 laboratories.
- 5 new CD establised:



CD111	PRR1/Nectin1 PolioVirus Related Receptor
CD112	PRR2Nectin 2 Receptor Herpes Virus
CD177	NB1 found in neonatal Neutropenia
CD203c	NPP3/PDNP3 97A6 : Restricted Baso, Masto
CD204	Macrophage Scavenger Receptor role in defense against infections (listeria).

Basophils and CD in Flow Cytometry **HLDA CD203c**

E-NPP3 (CD203c)

Author: Hans-Joerg Buehring

Reviewer: Kimihiko Sano

PROW 1:47-49(2000)

MOLECULAR Families

- Families in which ENPP3 is a member
 - ENPP3-->type II transmembrane protein
 - ENPP3-->Ecto-nucleotide pyrophosphatase/phosphodiesterase family
>ectoenzyme
- CD203C belongs to a series of ectoenzymes that are involved in hydrolysis of extracellular nucleotides. The corresponding protein is also detected in pancreas, small intestine, colon, and liver. Human E-NPP3 mRNA is expressed in glioma cells, prostate, and uterus.
- Expression of human CD203C protein is detected in uterus, basophils, and mast cells

The two main surface Antigens of Basophils used for BAT

CD 63 (gp 53):

- * expressed on **baso, mastocytes, macrophages, platelets** in non restricted way (Myeloid line).
- * weakly expressed at the surface of quiescent basophil.
- * The test requires a pre-stimulation by IL3, and gating with Anti-IgE fluo.

CD 203c: E-NPP3

- * transmb protin \in ectonucleotide pyrophosphatase /phosphodiesterase (E-NPPS), preexistent and in stock in granules but \neq of CD63
- * **restricted** expression on **basophils, mastocyt**s and **precursors** among hematopoietic cells.
- * **fast up regulation ++** (5min) by activation **Fc ϵ RI** (IgE mb-Ag) or (IgE mb-anti IgE) and Allergens.

Whether or not to pre-stimulate by IL3 ?.

- Necessary with CD63, used in kits.
-
- Not useful and in fact harmful with CD203c
 - Thanks Annick O.

The validity of the positive and allergen tubes,
as compared to the negative tube still remains debatable.

Basophils in F C M: the problems to be discuss

CD63: Myeloid Specificity not restricted to Baso.

CD63: Gating of Baso by anti-IgE => stimulus ?

CD63: Stimulation by IL3 => not physiologic

CD63: Used in routine. No problems with lysis

CD63: Good correlations to others clinical tests.

CD63: Results percentages positive and negative cells

CD203c: HLDA7 2000 identified as specific for Baso.

CD203c: Delicate to use; soft Lysis necessary

CD203c: Results: percentages, MFI, Index MFI

Correlations with other Biologic and clinical tests

Sensivity – Specificity – to be established

Predictive values VPP, VPN.

Dilution of substances to check for each molecule

Selection des Basophiles = « gating »

Selection of positive and negative windows :

Anti-IgE, CRTH2, CD3, CD123, CCR3, HLA-DR....

Are at the center of the debate.

BAT Interpretation.

Histograms :

Evaluated in % of activated basophils; the problem: choice of limit for positive.

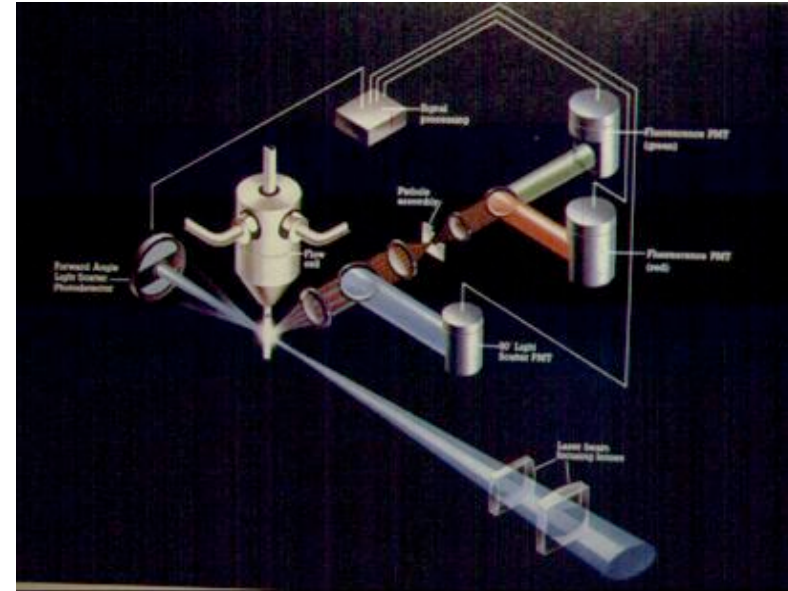
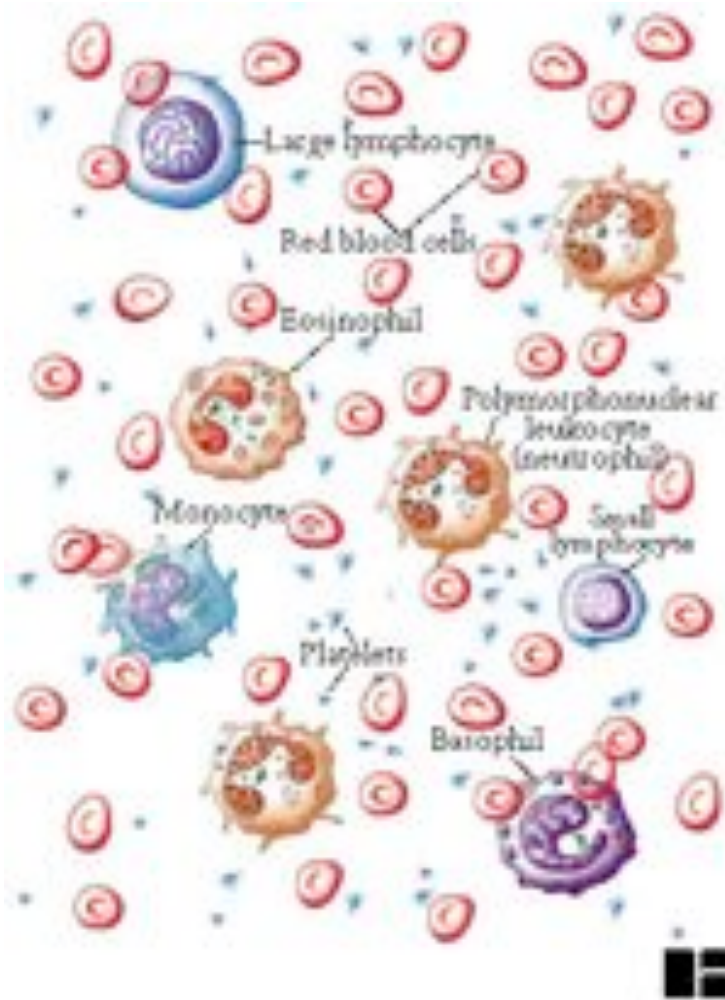
or

results in Mean Fluorescence Intensity (MFI)

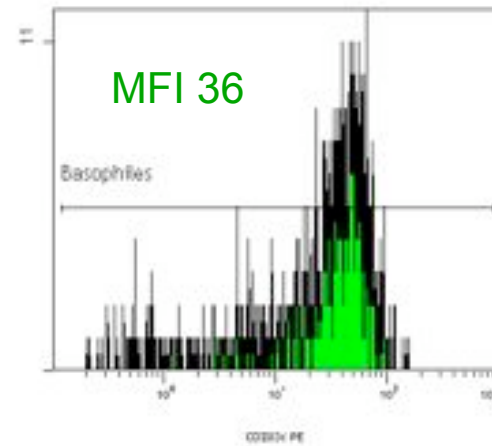
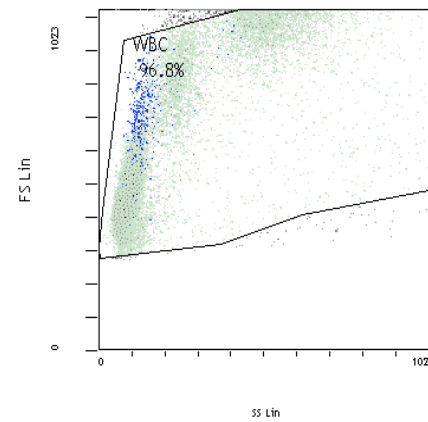
Interpretation for TAB results :

- MFI index : Allergen Test upon Negative Tube

Exploring the WBC by Flow Cytometry



Principle of cytometer



Histograms (TAB CD 203c)

Range of interest for BAT.

- Inhalant allergens
- Food
- Hymenoptera venoms (wasp, bee..)
- Hevea Latex
- Drugs
 - Myorelaxants
 - Curares
 - AINS
 - Antibiotics
 - Opioids
- Imagery contrast products

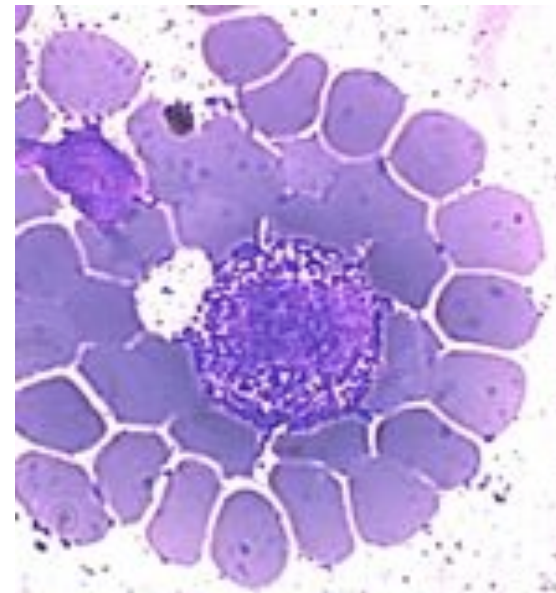
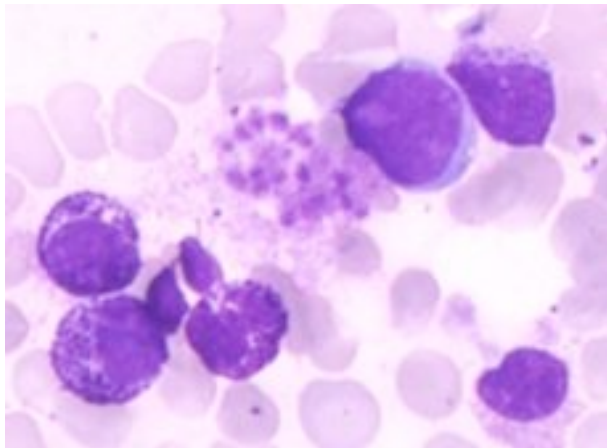
How Rare events become Frequent : a Clinical Case.

Request in Hematology Lab , a sample Basophils rich :
To localisate Basophils with CD203c

Case of Madame Leca.. 82 years followed up for **Thrombocythemia** 2.265 G/L
hospitalised for digestive **bleeding**.

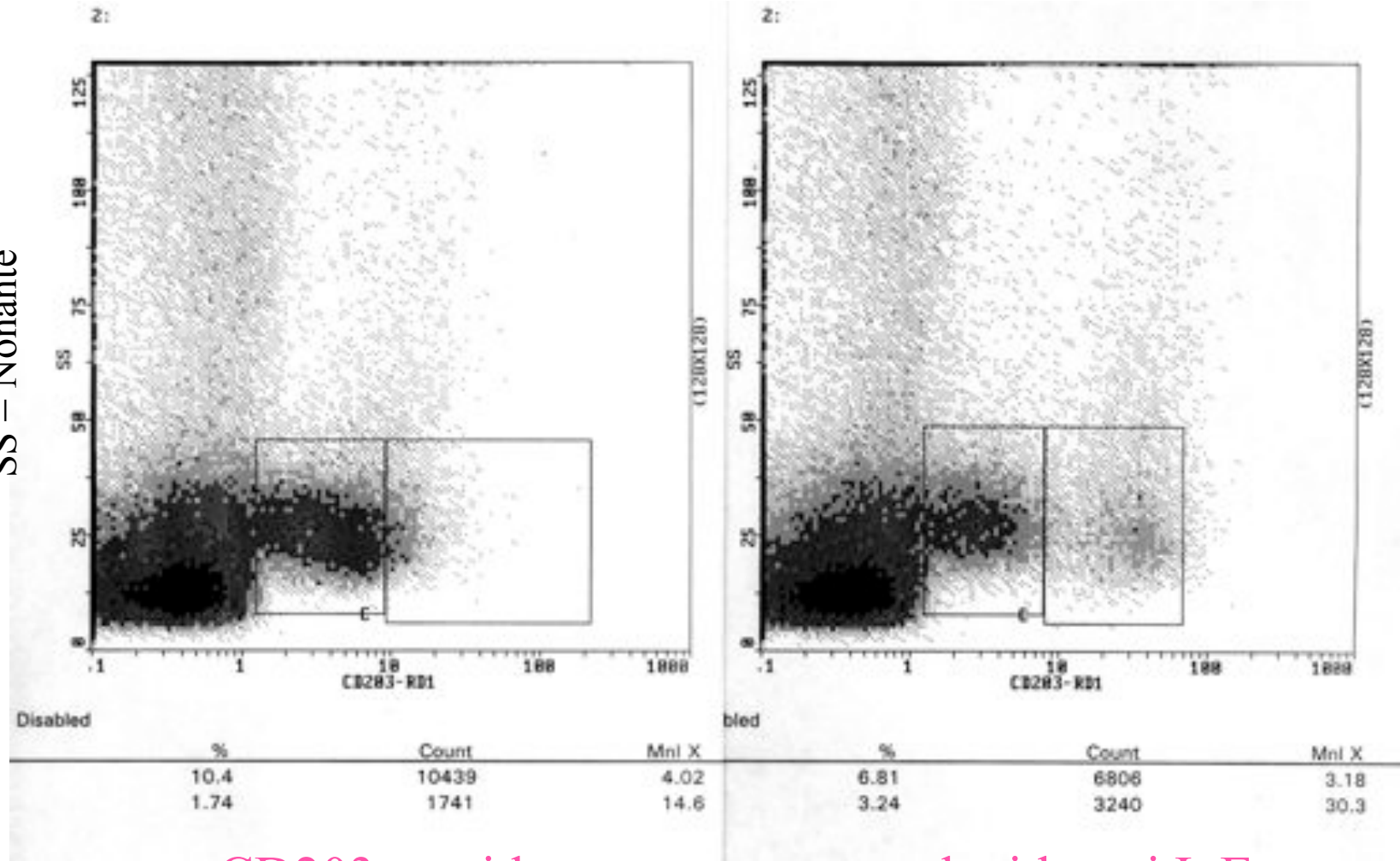
NF: **Basophils 34 %** **7% Blasts**
Myelogram **Basophiles 38%** **and 22% of Blasts.**
Caryotype: **Translocation (3;22) chromosome Phi complex. Transcript BCR-ABL +**

Conclusion:
Acutisation of Myeloid Leukemia
BASOBLASTS ???.



a Clinical Case : Blood Cells in FCM

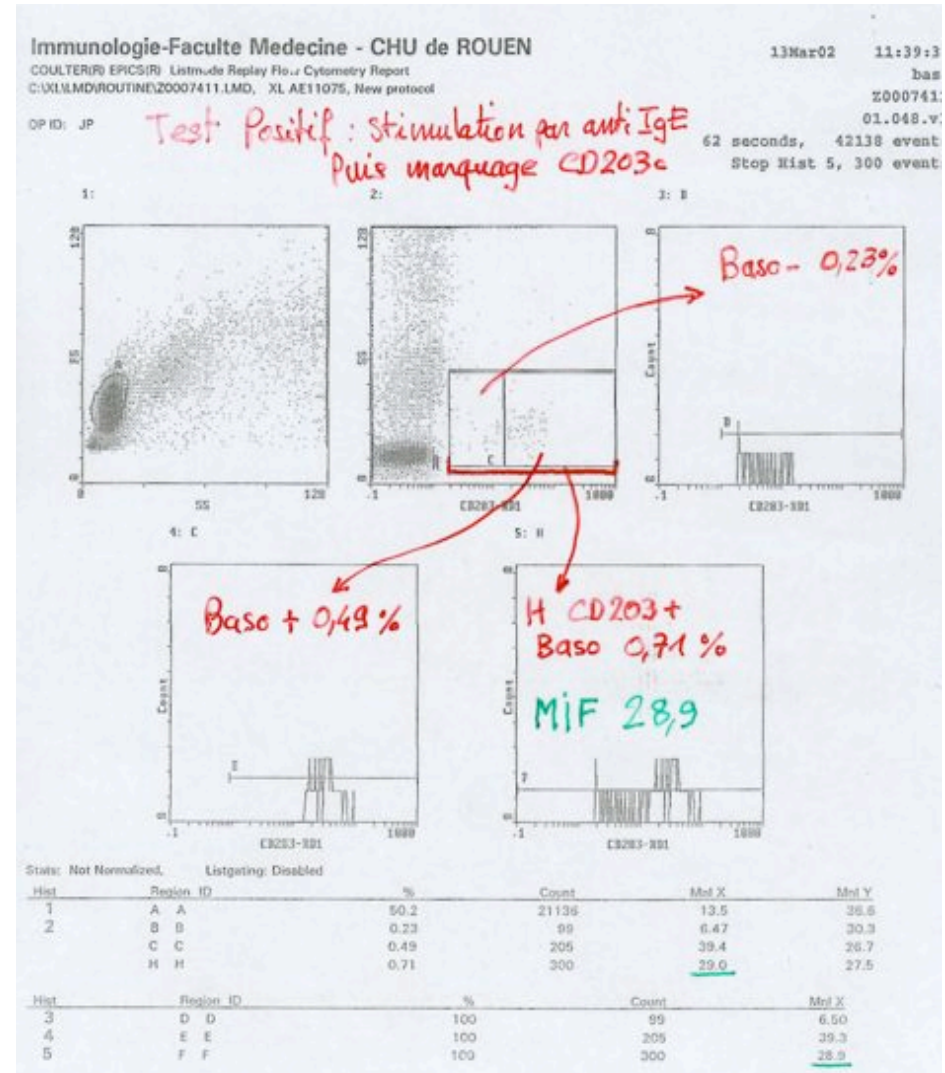
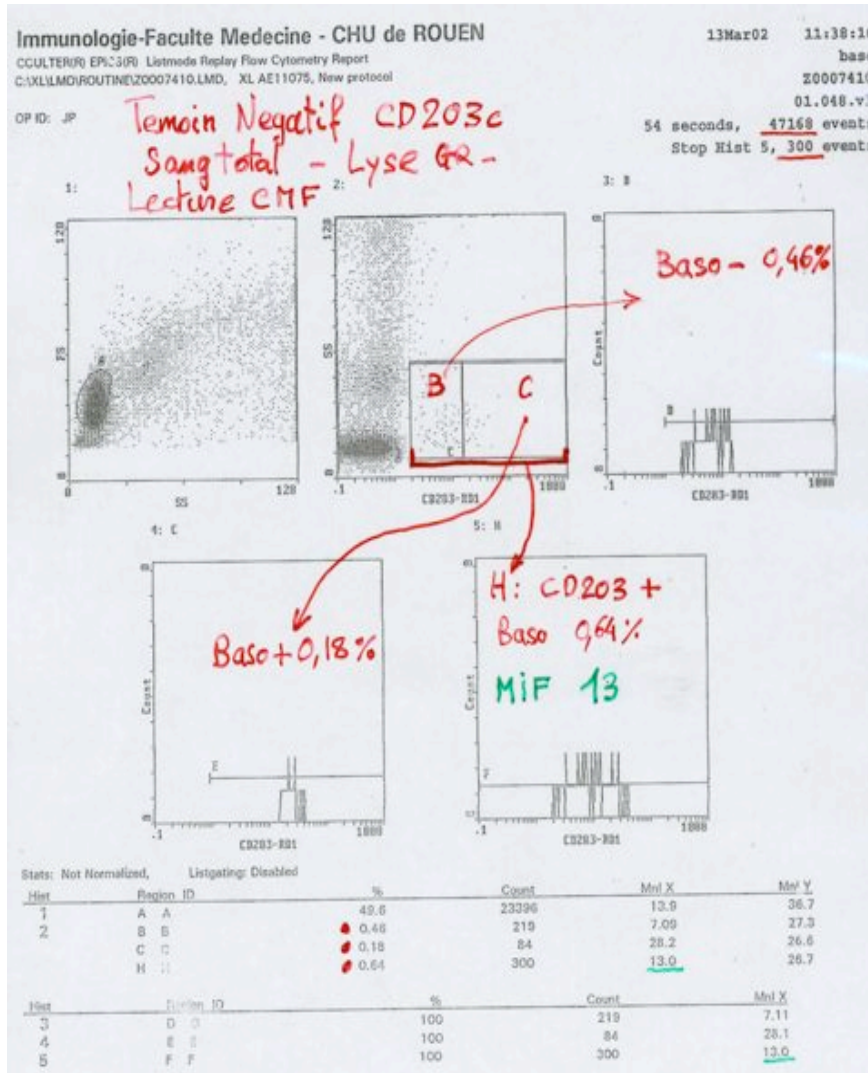
SS = Nonante



CD203c without

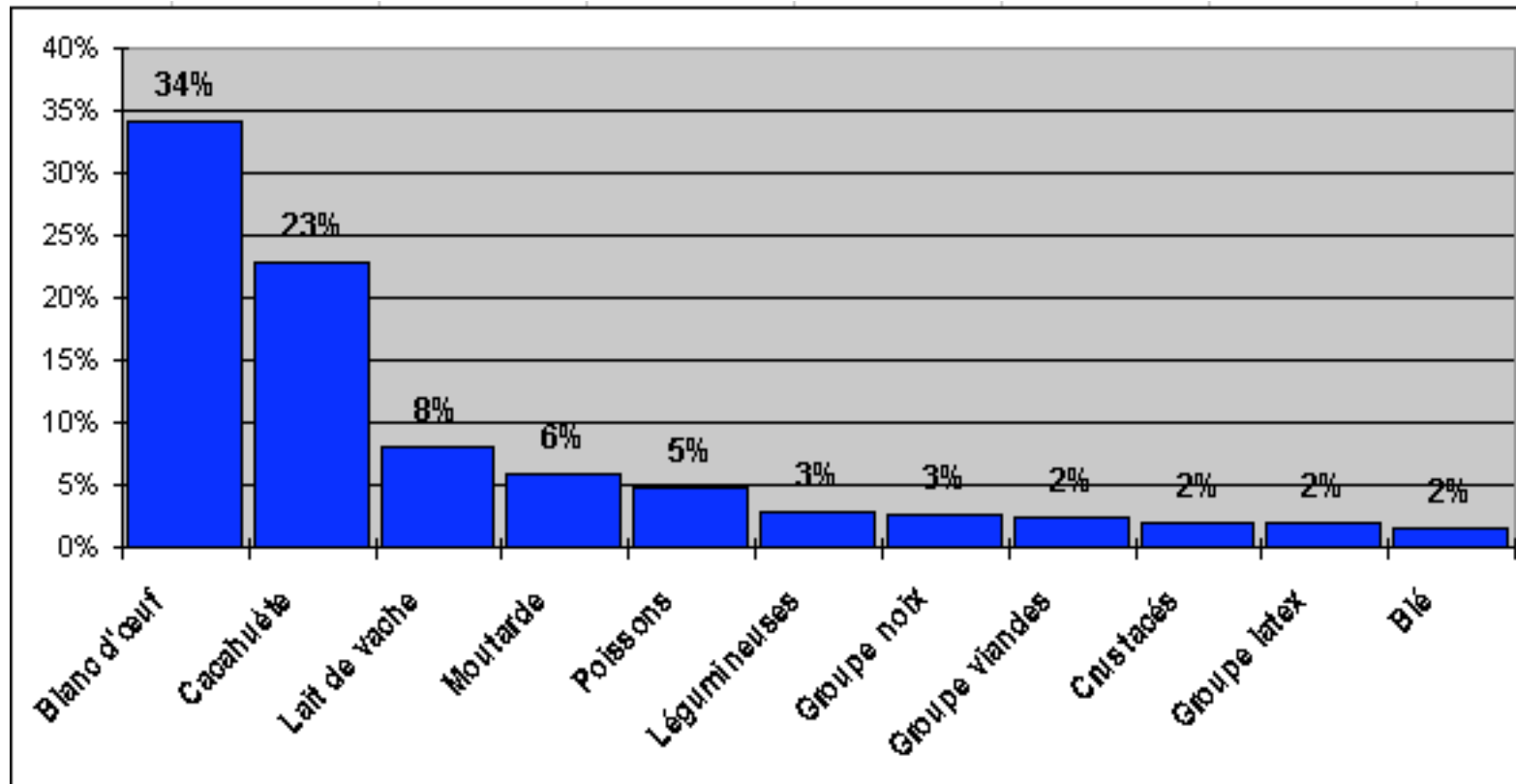
and with anti IgE

Basophils with CD203c by flow cytometry. The initial Protocol.



PROJECT of OUR STUDY :

- 1- Use BAT with CD203c and « kit Allergenicity » for food allergy in children : Cow'sMilk, Egg White, and Peanut.
- 2- Study **sensitivity and specificity** by comparison with clinical results, spécifique IgE for allergens and Oral Provocation Tests (OPT).
- 3- Define a **predictive test for ill safe or persistence of allergic disease.**



Subjects studied

- **Control Population** : - non allergic.
- **Allergic:**
 - **Children with clinical or biological allergy** :
=> specific IgE for food allergens.
=> Clinical : cutaneous (eczema, urticaria, angio-oedema) digestive, anaphylactic shock.
 - **New borns** 4 month old included in **protocol Nestec**, not allergic to milk but with genetic susceptibility to develop an allergy.

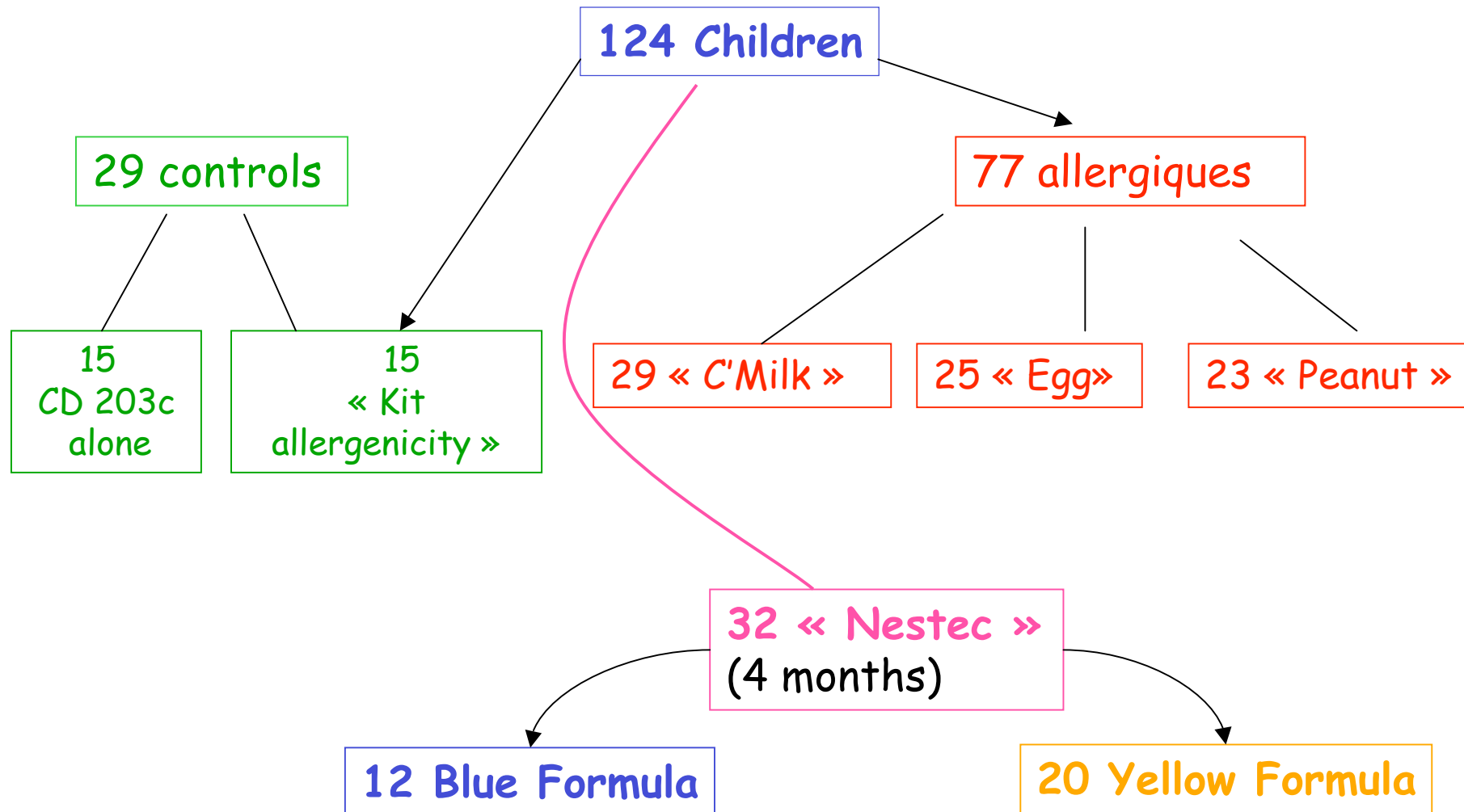
PATIENTS ET METHODS

M

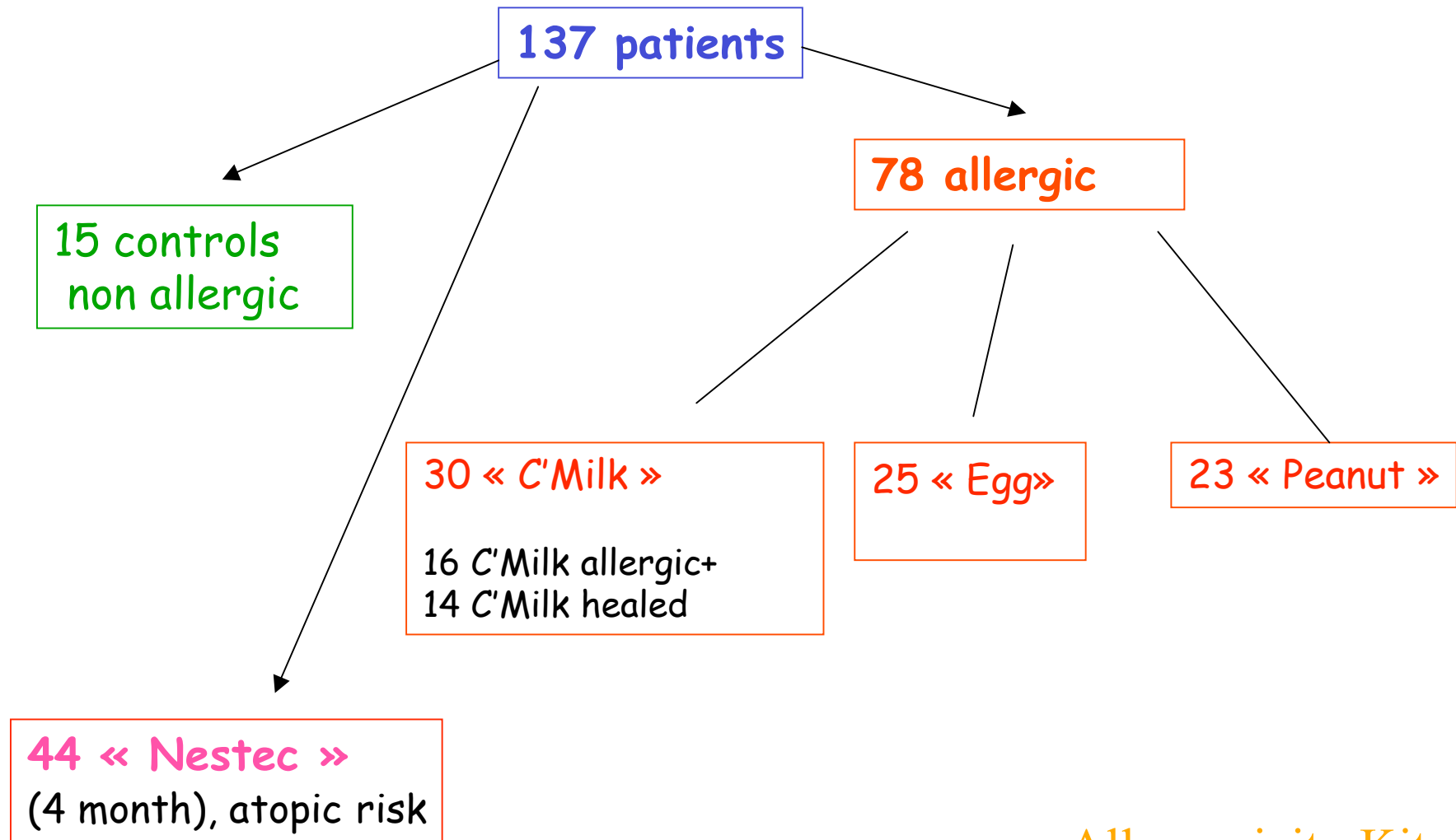
Réalisation du TAB par 2 procédures :

1. **Marquage CD 203c seul** sur population témoin afin de déterminer les dilutions optimales d'allergènes et vérifier l'absence de fausse positivité.
 2. Application de la procédure technique du **Kit Allergenicity** sur population allergique et contrôle négatif.
- **Lecture au cytomètre (XL ou FC 500) immédiate**
 - **Interprétation des histogrammes** : résultat exprimé en moyenne d'intensité de fluorescence en log (MFI)
 - **Interprétation des résultats du TAB :**
 - index de MFI par rapport au Tube Négatif
 - et % de basophiles activés (CD 203c bright).

Food Allergies : Groups followed by BAT



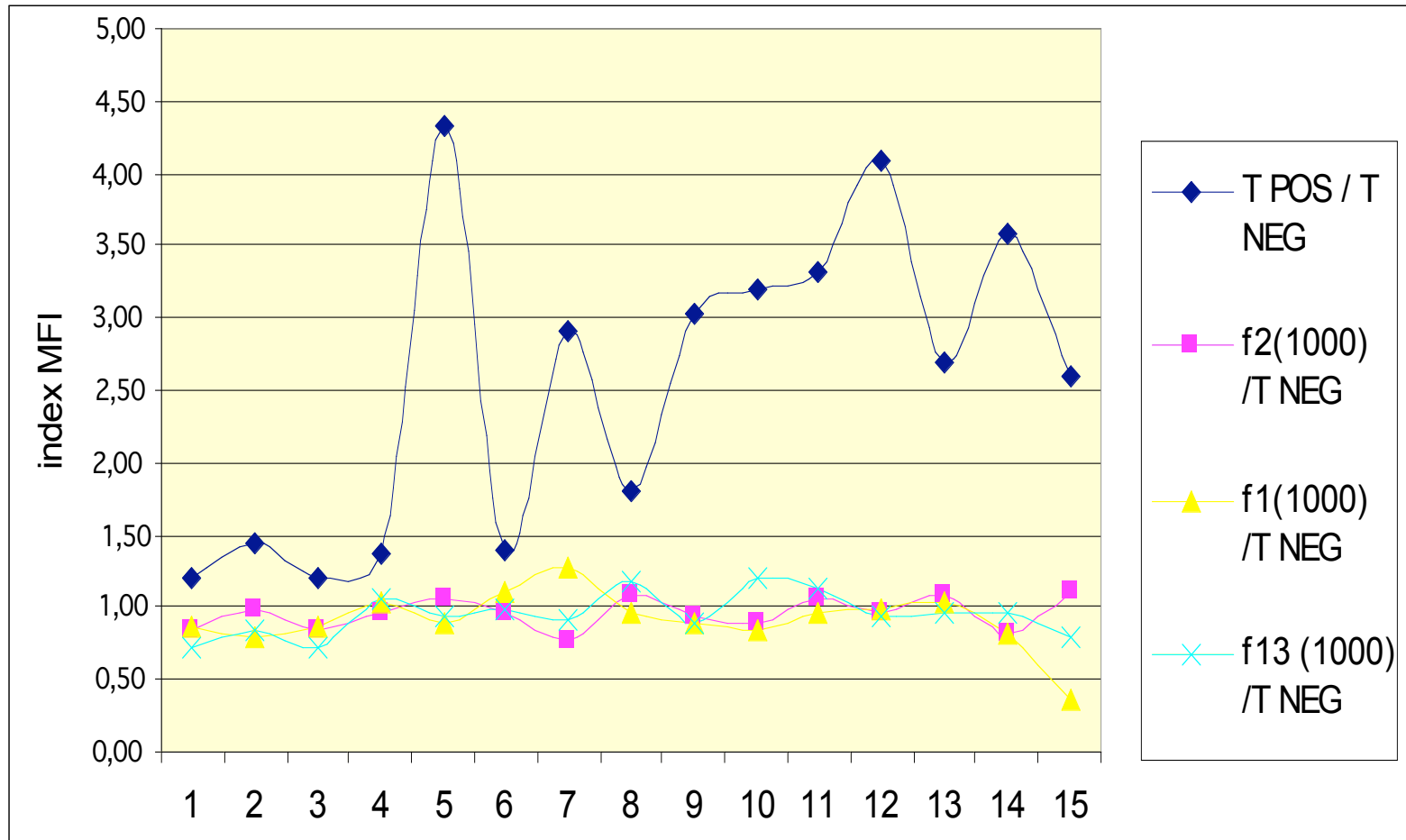
Food Allergies : Groups followed by BAT update



Allergenicity Kit
July 2006

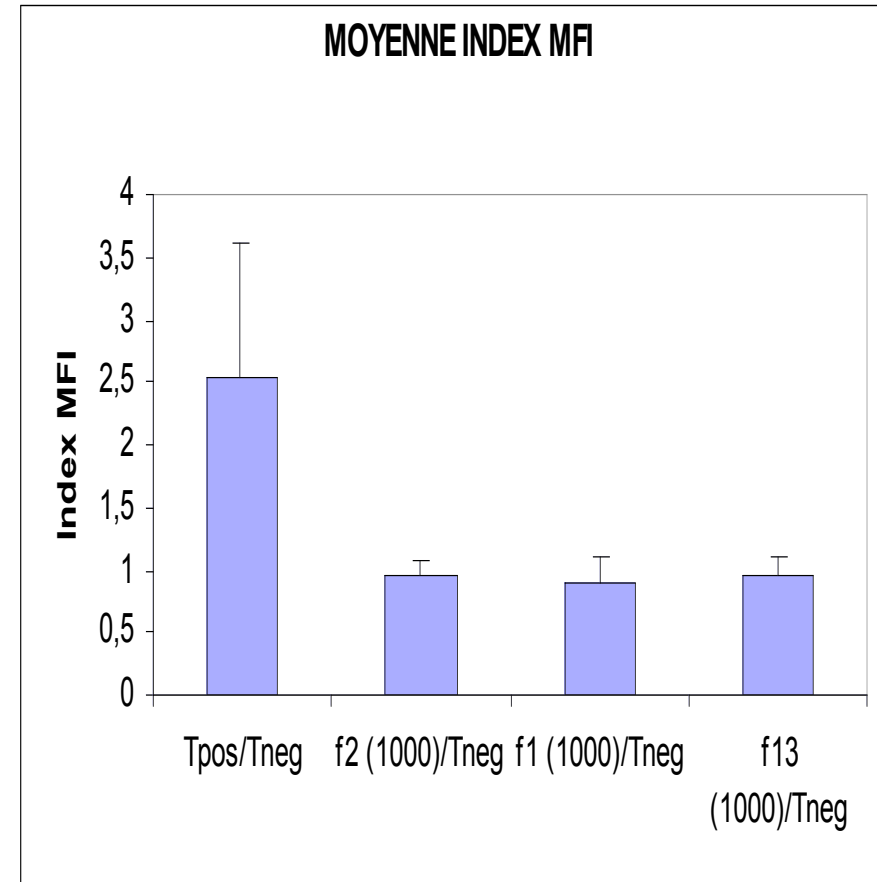
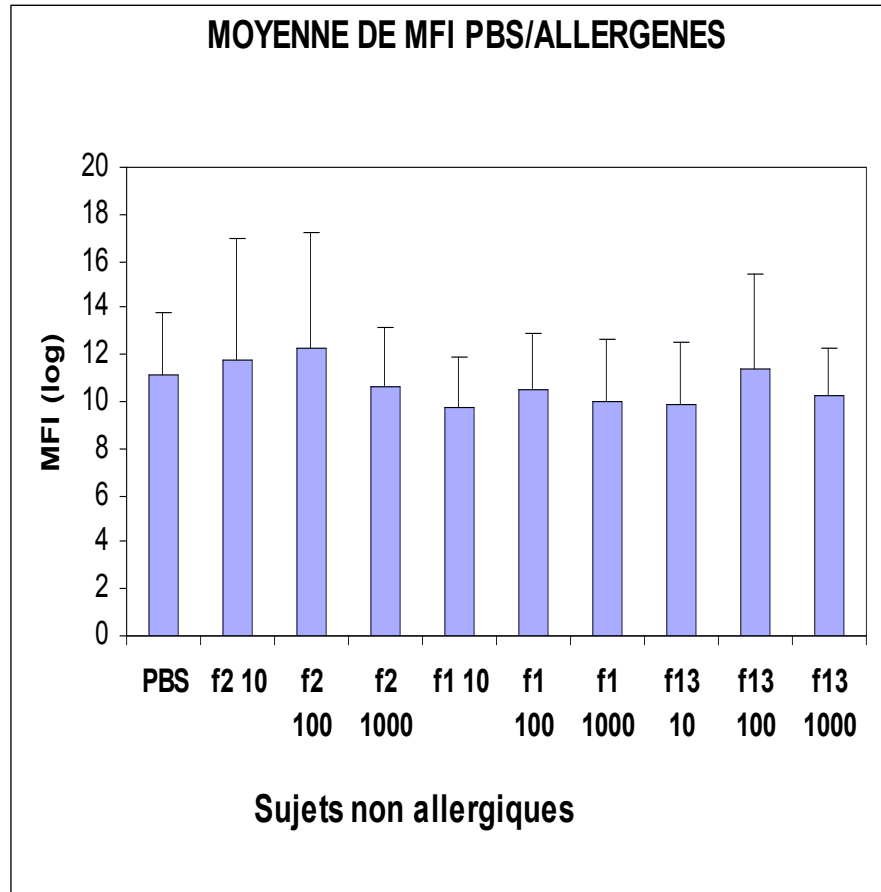
Allergens and dilutions : Validation

15 control subjects non allergic (CD 203c alone)



Allergens and dilutions : results

15 control non allergic subjects (CD 203c alone)



APPLICATIONS of BAT

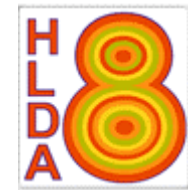
- Diagnosis of allergic diseases.
- BAT **CD 63** used for curares, myorelaxants, venoms, antibiotics, pollens, latex...
- Sensivity from 75 to 100%, and specificity 90 to 100%.
- **CD 203c** more sensitive, and more specific than CD 63 (Boumiza et al, Clin Exp Allergy 2003)
- Developement by **Beckman Coulter®** of **Allergenicity Kit** available from Sept 2004.

Contain and use for ALLERGENICITY KIT



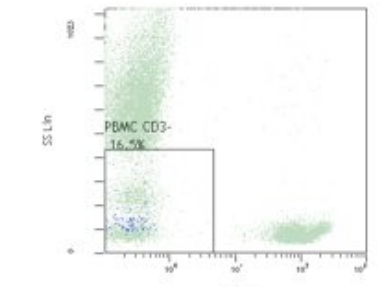
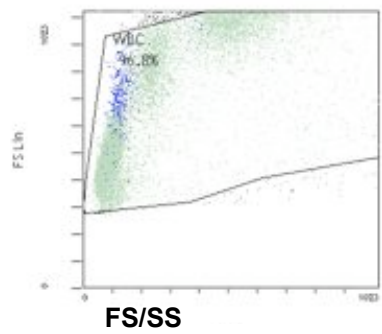
- Mix of 3 fluorescent monoclonal antibodies :
 1. **97A6** ⇒ **CD203c-PE** HLDA7, Harrogate 2000
 2. **UCHT1** ⇒ **CD3-PC7**, Paris 1981 (« negative gate » for T Lymphocytes)
 3. **BM16** ⇒ **CRTH2-FITC CD294**, HLDA8 Adelaide 2004 Prostaglandin family expressed on basophils, eosinophils but also on Th2 and Tc2.

<http://mpr.nci.nih.gov/prow/>
www.hlda8.org

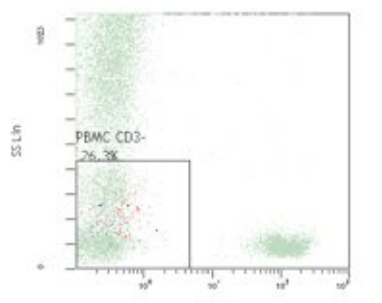


- **Activation Solution** (Buffer calcium ⇒ activation in vitro of basophils for EDTA)
- **Positive Control** (anti IgE, Immunotech® Marseille)
- **Stop solution** (rich EDTA ⇒ stop basophils activation)
- **Lysis solution for Red Cells**
(lysis by cyclic amine interacting with internal carbonic anhydrase)

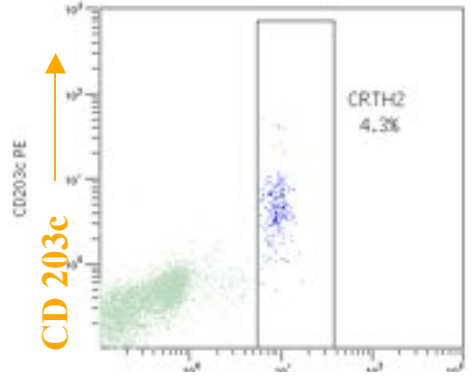
Histograms with / FC 500 (Allergenicity Kit)



SS/CD3 pc7 (Tneg)

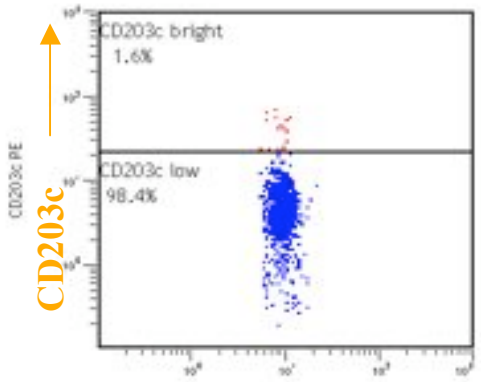


SS/CD3 pc7 (Tpos)



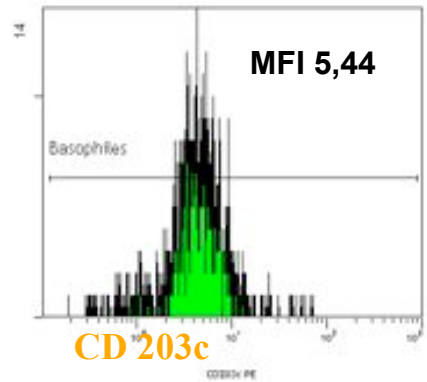
CD 203c

CRTH2



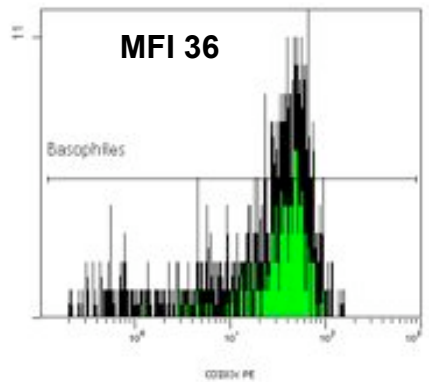
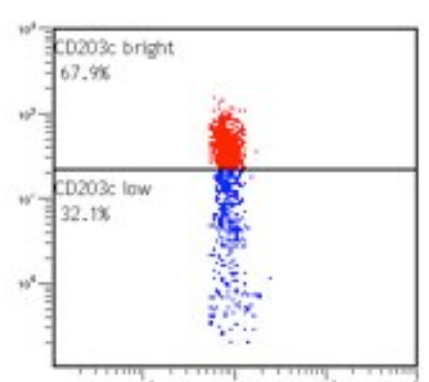
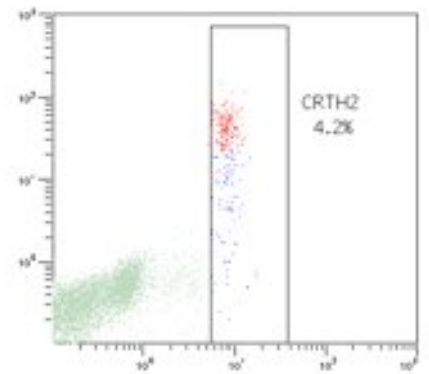
CD203c

CRTH2

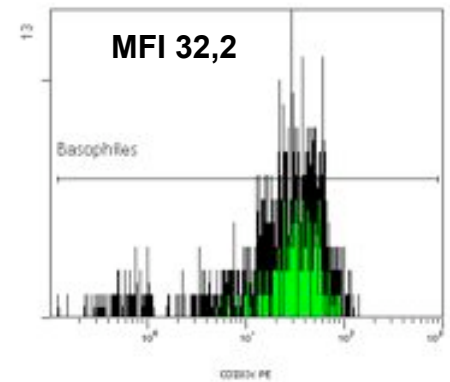
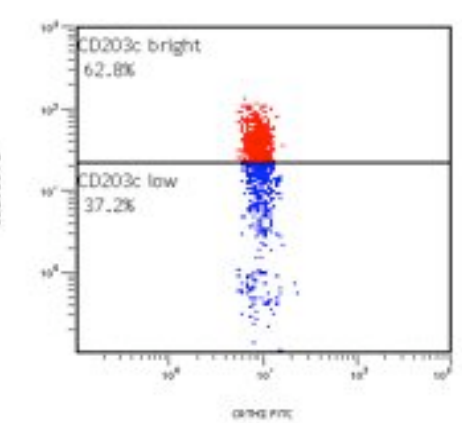
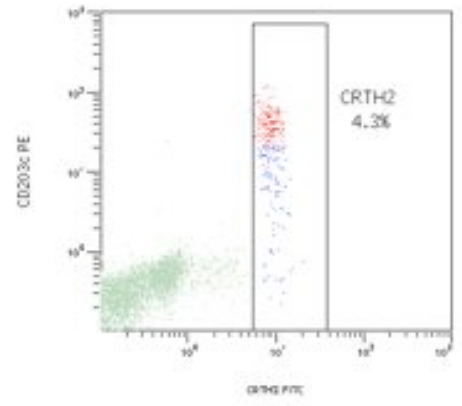


CD 203c

Tpos (anti IgE)



Egg 1/1000

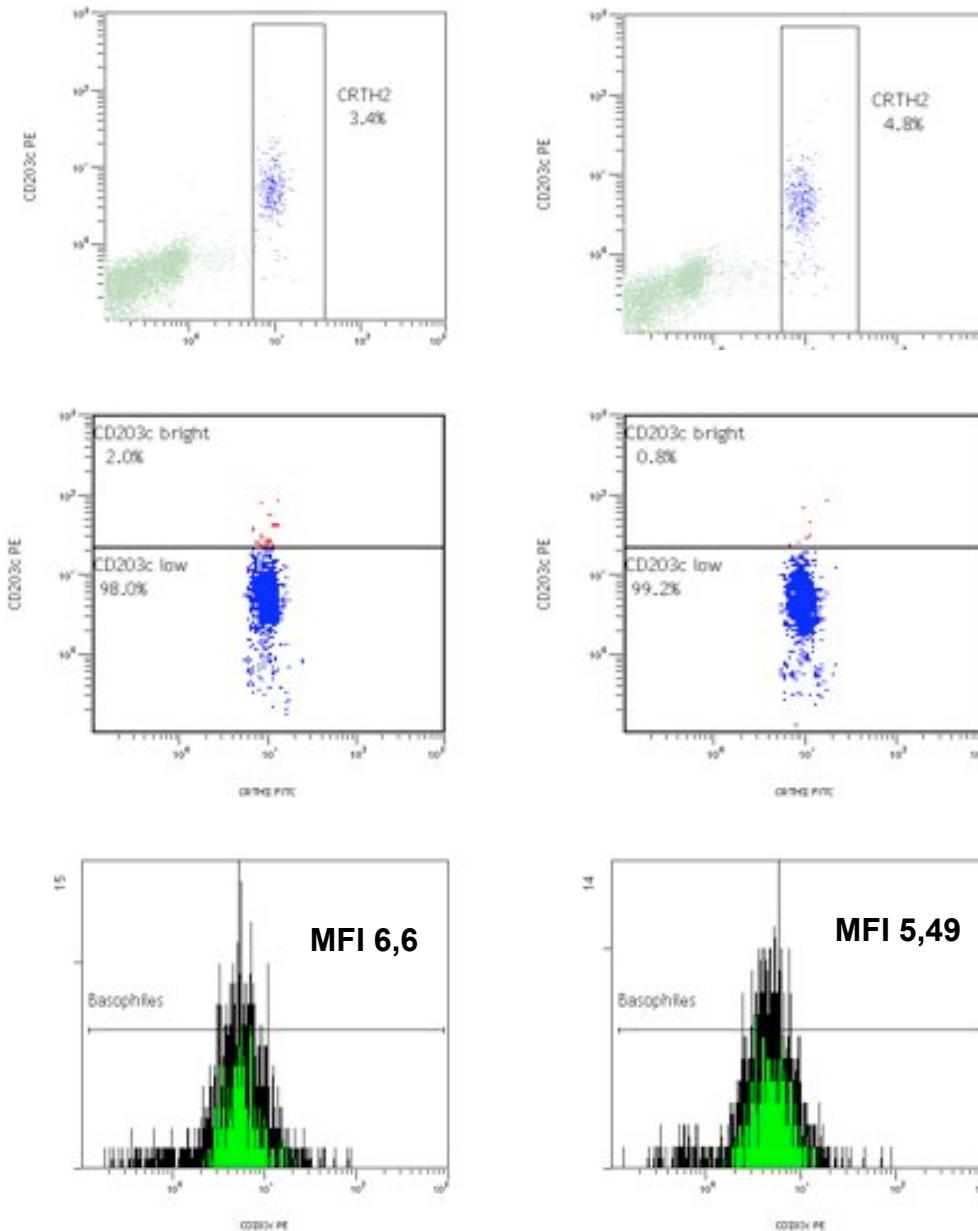


Lucie B., 11,5 years

Histograms / FC 500 (Allergenicity Kit)

Milk 1/1000

Peanut 1/1000



Lucie B, 11,5 years old

History :

Familial Atopia

Quincke's Œdema at 10 month after introduction of milk.

C'Milk healed at 3 years:

Was Sensitised to Peanut

(TCutaneous +), at 4 years then succes of reintroduction

Remains Allergic to Egg White: œdème of face,

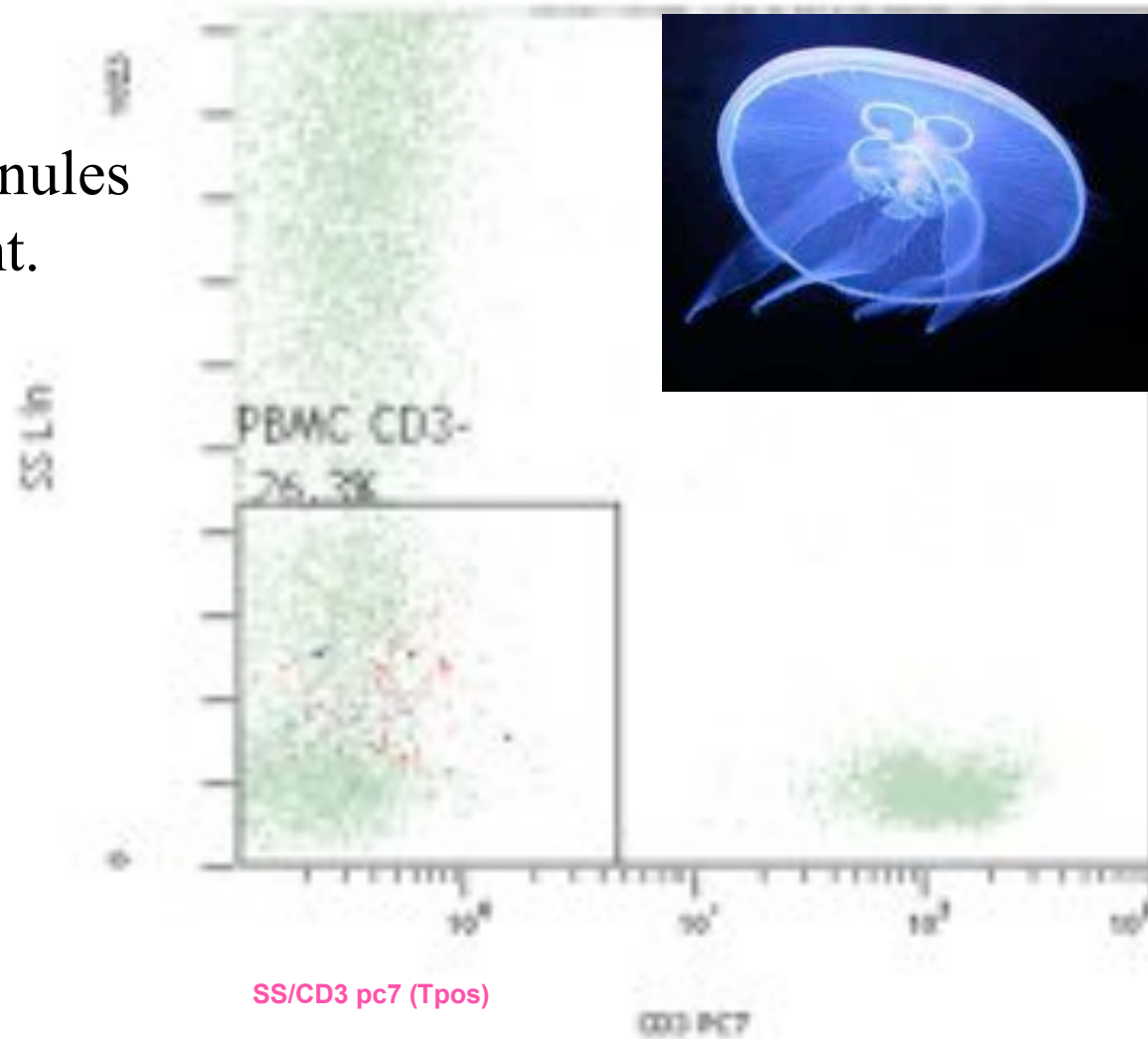
TC +, IgE White Egg classe 2.

Échec du TPO en 2003, et 2005 (apres prelevement pour TAB !)

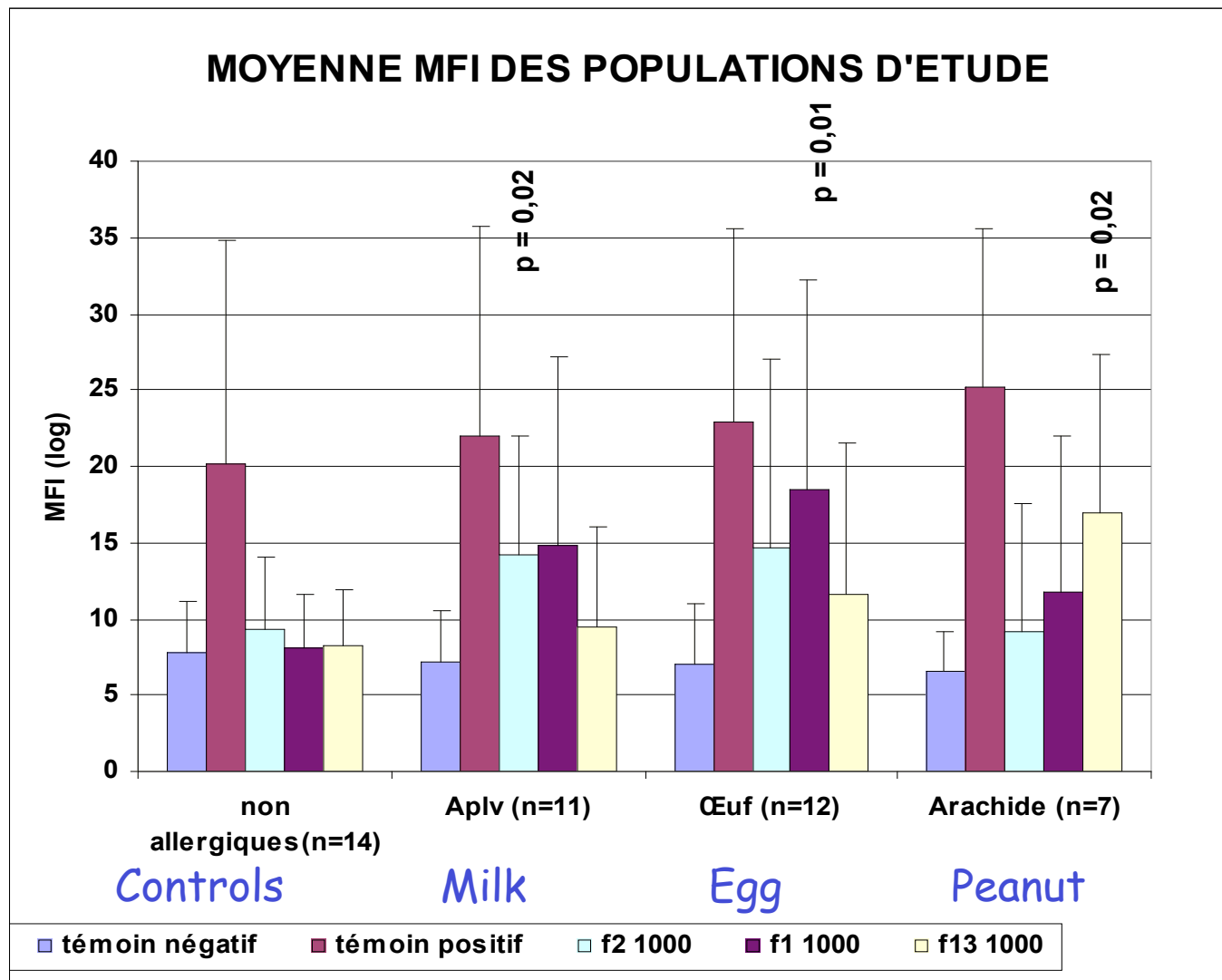
Localisate rare events: BASOPHILS **neg** and **pos**

Basophils granules are transparent.

90LS between Lympho and Monocytes



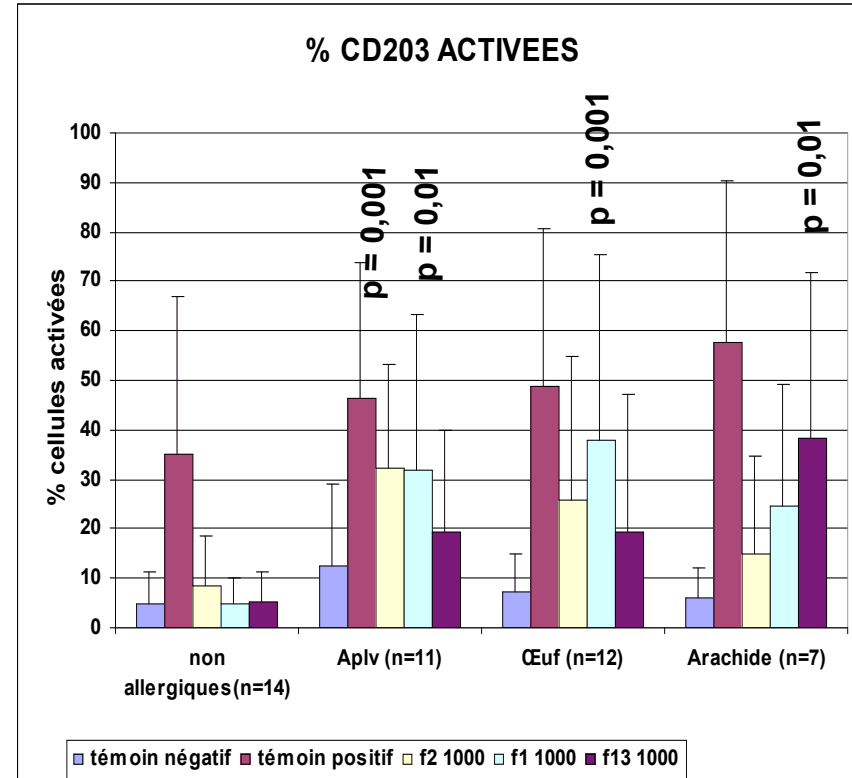
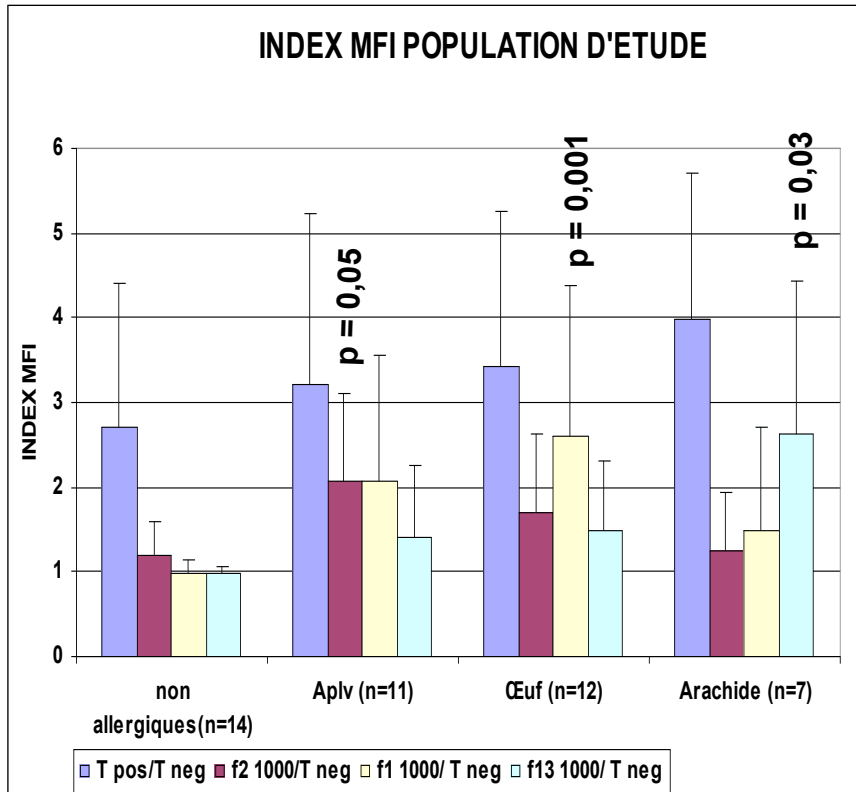
RESULTS « Allergenicity Kit » Means of MFI



RESULTS

Index MFI

and % activated positive cells



Results Index MFI

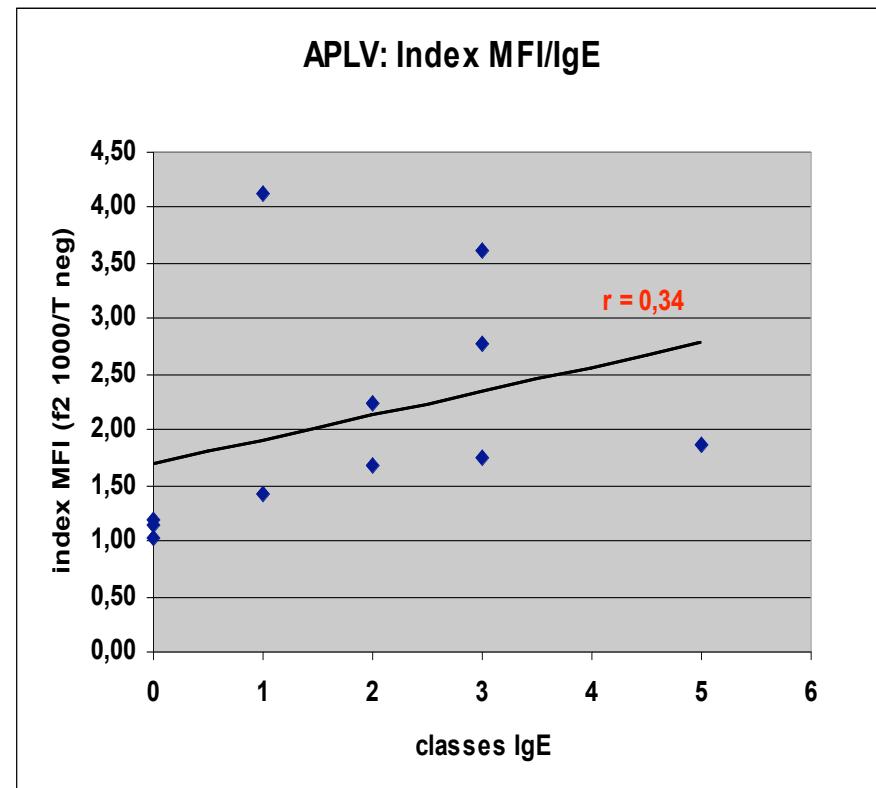
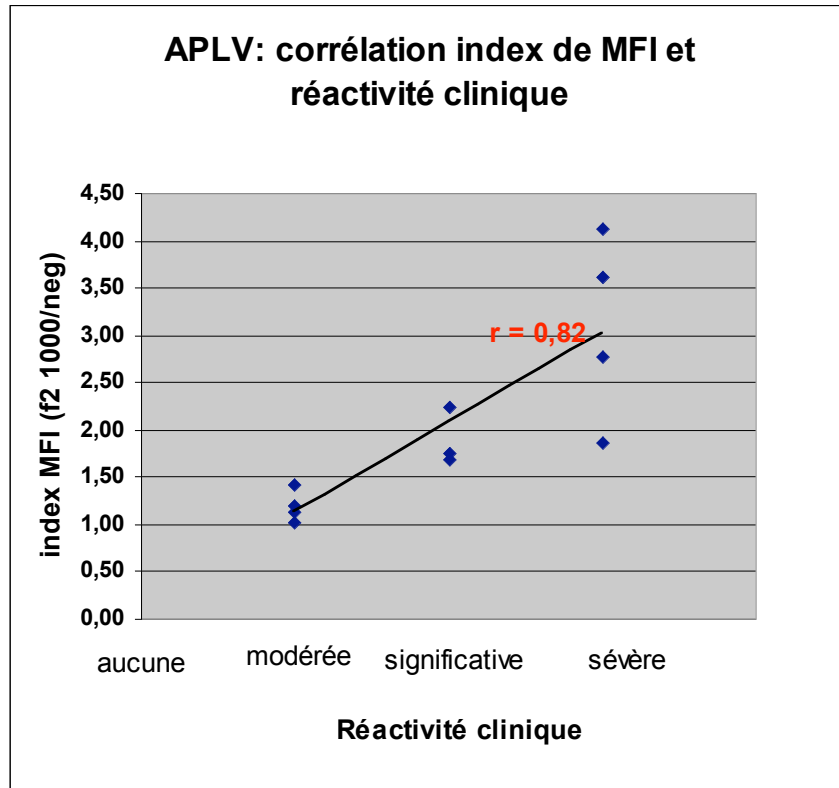
MOYENNE D'INDEX DE MFI POUR LES DIFFERENTES POPULATIONS ETUDIEES												
	T pos/T neg											
	moy	sem	min	max								
Non allergiques(n= 15)	2,61	0,59	1,68	6,96								
Aplv (n= 16)	2,44	0,50	0,72	7,77								
Œuf (n= 23)	3,19	0,35	1,03	6,63								
Arachide (n= 19)	3,54	0,42	0,87	7,39								
Nestec (n=44)	2,38	0,16	1	6,33								
Aplv guéris (n= 14)	2,24	0,32	0,93	4,22								
	f2 Lait 1000/T neg				f1 BlancŒuf 1000/ T neg				f13 Arachide1000/ T neg			
	moy	sem	min	max	moy	sem	min	max	moy	sem	min	max
Non allergiques(n= 15)	1,18	0,10	0,91	2,45	0,99	0,04	0,83	1,35	0,84	0,2505	0	1,12
Aplv (n= 16)	2,05	0,24	0,78	4,12	1,85	0,33	0,82	5,65	1,6	0,225	0,66	3,27
Œuf (n= 23)	1,52	0,14	0,91	3,61	2,63	0,34	0,9	6,67	1,89	0,2315	0,9	4,49
Arachide (n= 19)	1,48	0,22	0,84	4,84	1,83	0,35	0,36	6,67	2,77	0,3441	1,04	6,28
Nestec (n=44)	1,14	0,12	0,89	6,12	1,19	0,12	0,89	6,28	1,17	0,1176	0,81	5,8
Aplv guéris (n= 14)	1,08	0,03	0,91	1,25	1,2	0,22	0,32	3,94	1,31	0,2165	0,83	3,9

Résultats in % of positives cells by CD203c

MOYENNE DE % CD203c ACTMEES														
	témoin négatif				témoin positif									
	moy	sem	min	max	moy	sem	min	max						
non allergiques(n= 15)	4,65	0,26	0	22,5	32,61	7,20	0,5	94						
Aplv (n= 16)	12,56	3,72	0	48	33,47	6,07	0,59	88,7						
Œuf (n= 23)	7,82	0,80	3,51	16,3	43,21	5,80	1,2	89						
Arachide (n= 19)	4,26	1,04	0,1	16	44,56	6,53	0,4	93,5						
Nestec (n= 44)	2,93	4,53	0	22,5	25,95	3,05	0,5	70,9						
Aplv guéris (n= 14)	10,15	3,24	0,1	33,9	35,52	6,95	2,9	97,7						
	f2 1000				f1 1000				f13 1000					
	moy	sem	min	max	moy	sem	min	max	moy	sem	min	max		
non allergiques(n= 15)	7,86	2,60	0,21	33,8	4,61	1,38	0,1	17	4,66	0,30	0	19,1		
Aplv (n= 16)	30,47	4,89	1,62	56,2	26,84	6,64	0	77,5	19,4	4,42	0,1	58,9		
Œuf (n= 23)	19,97	4,81	0,1	82,8	35,47	6,35	0,2	87,9	23,5	4,70	0,1	69,9		
Arachide (n= 19)	13,46	4,45	0,2	70,7	20,29	5,65	0,2	67,5	32,9	5,93	0,6	86,4		
Nestec (n= 44)	3,49	0,74	0	17,1	3,52	4,69	0	16	7,96	0,39	3,68	18,9		
Aplv guéris (n= 14)	12,9	4,00	0	46,8	15,34	5,78	0,39	65,1	12,9	3,81	0,28	37,9		

RESULTS

BAT Correlation / clinical / IgE



Jules R. 3,5 years C'Milk+/Egg White+

Clinical data

Quincke's Oedema with
C'Milk.

Allergic to Casein

OPT = failure of labial
test

**ALLERGIC Milk and
EggW.**

Biological results

total IgE : 825 kU/l

Spécific IgE : (kU/l)

f2 Milk Class 3 (3,82)

f1 Egg Class 3 (11,5)

f13 Peanute Class 3 (3,54)

January 2003:

Casein Class 3 (4,72)

α Lactalbumine Négative

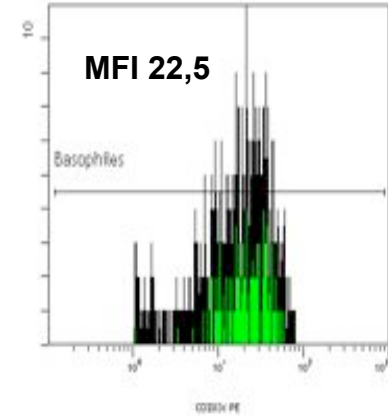
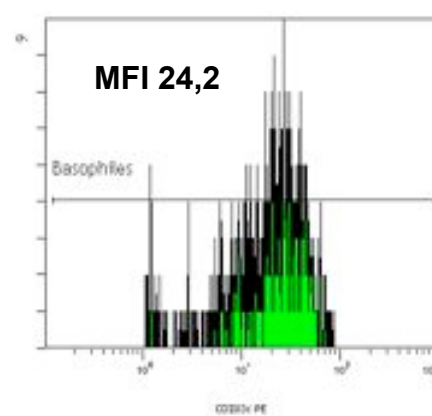
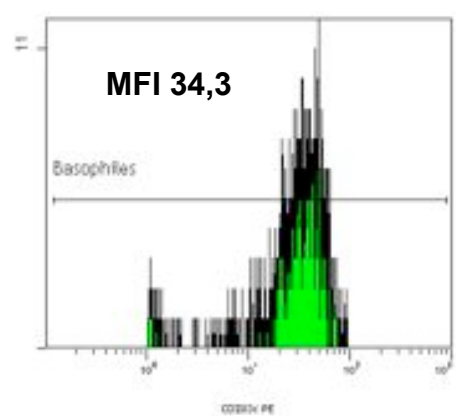
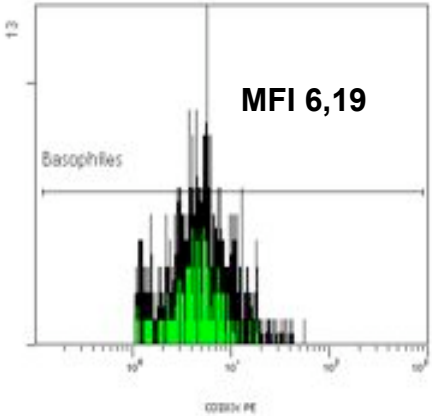
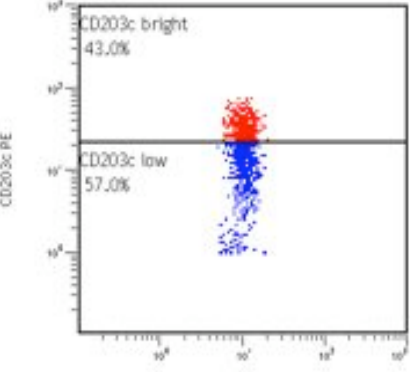
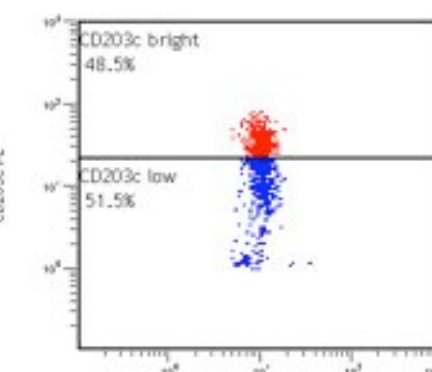
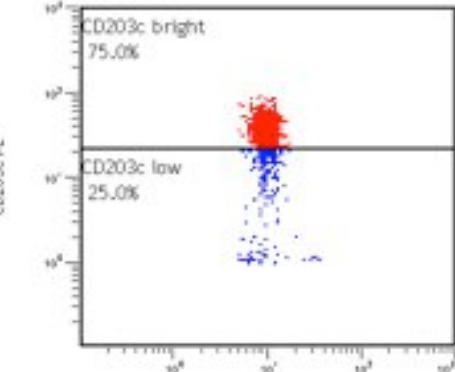
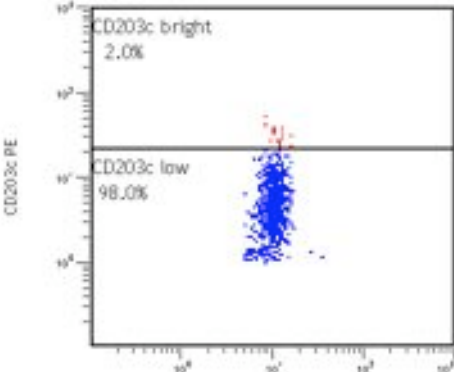
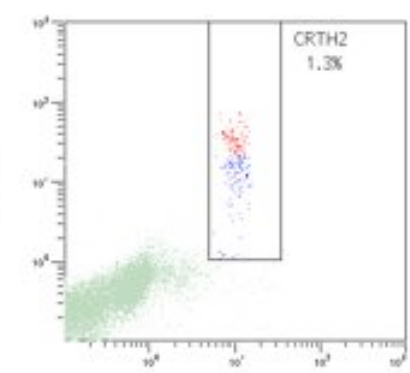
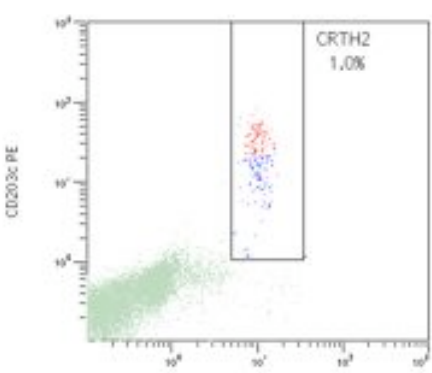
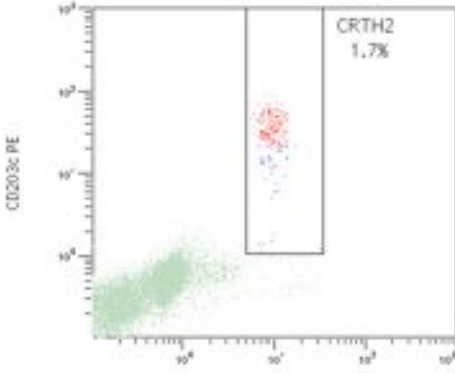
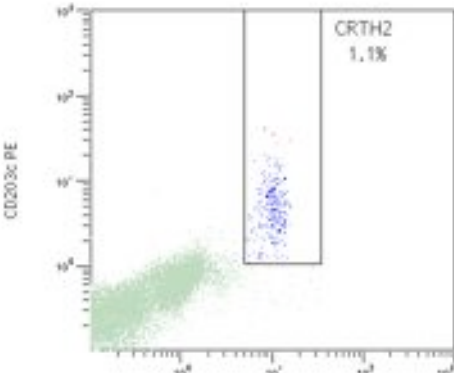
β Lactoglobuline Négative

Wheat, Nuts Négative

Jules R 3,5 years old C'Milk +/- Egg White+ +
T neg T pos

Lait 1/10

Lait 1/1000



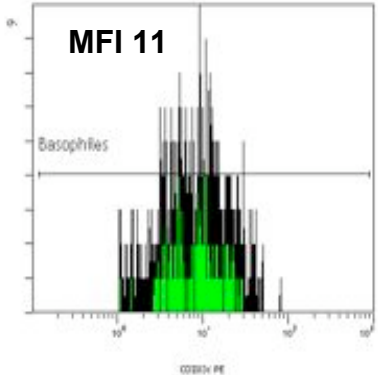
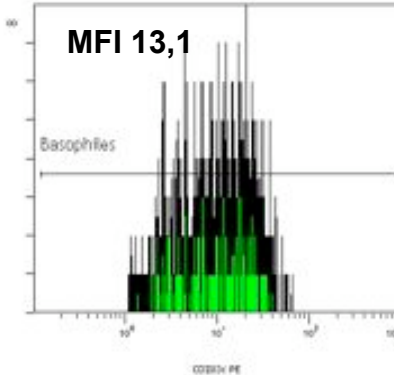
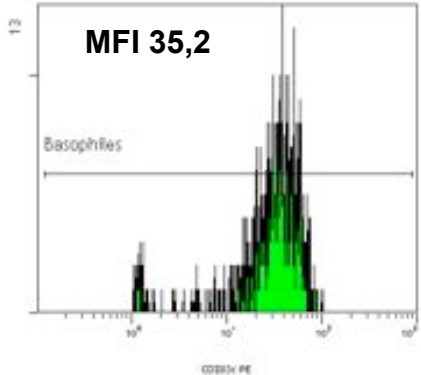
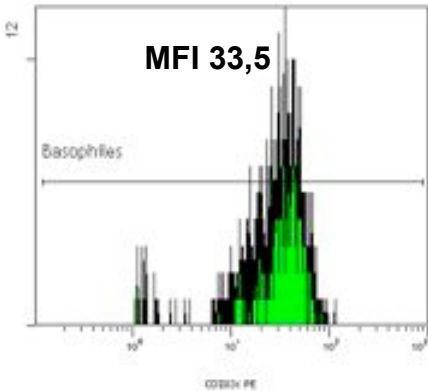
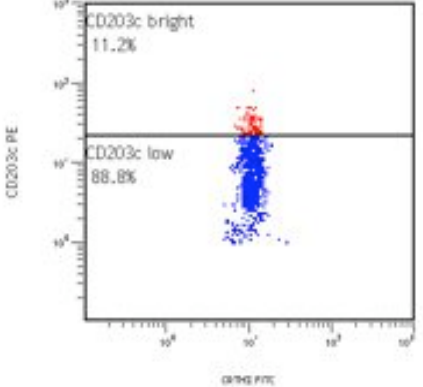
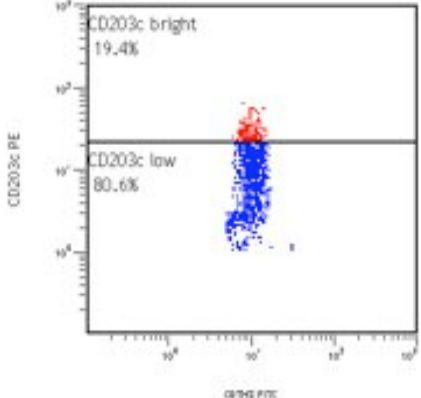
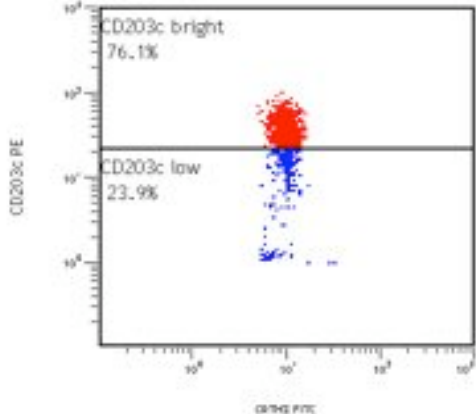
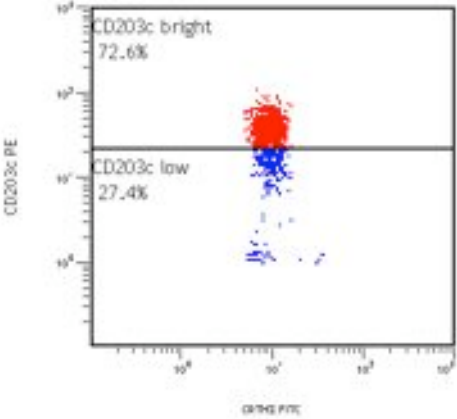
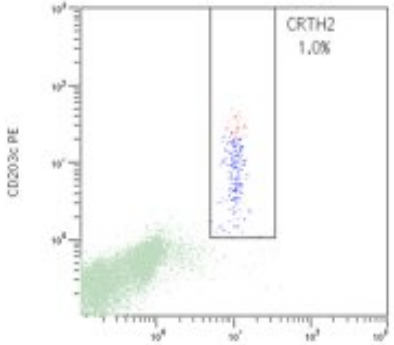
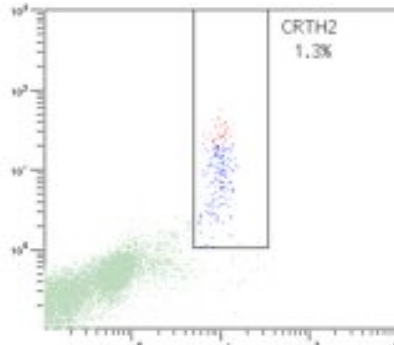
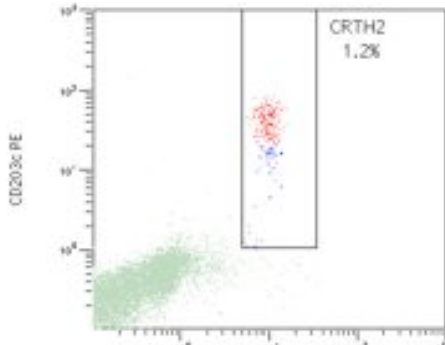
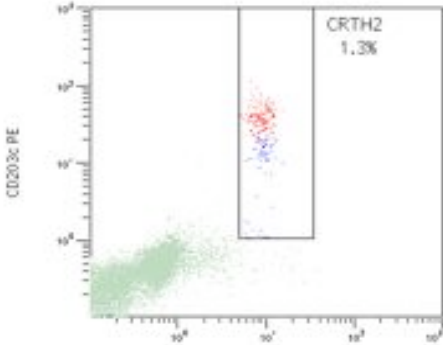
Jules R 3,5 years old C'Milk +/- Egg White +

Oeuf 1/10

Oeuf 1/1000

Arachide 1/10

Arachide 1/1000



Jules R 3,5 years C'Milk +/- Egg White + RESULTS

Test tube	MFI	Index / T Neg	% Baso activated	IgE spe	OPT
T Neg	6.19		2 %		
T Pos anti-IgE	34.3	5.30	75 %		
Milk 1000	22.5	3.48	43%	3.82	Failure
Egg 1000	35.2	5.51	76,1 %	11.5	Failure
Peanut 1000	11	1.7	11.2 %	3.54	

Asser A, 34 month, A rare case ?

•History: familial atopia (Hay rhinitis father)

Reflux gastro oesophagien depuis la naissance sous allaitement maternel.

Vomissements incoercibles et refus du lait à 9 mois $\frac{1}{2}$ lors du sevrage.
(diversification alimentaire débutée vers 6 mois par fruits et légumes)

⇒Hospitalisation : Dc APLV

IgE spécifiques lait de vache classe 5,

IgE caséine classe 4

β lactoglobuline classe 4

α lactalbumine classe 4

⇒ Régime d'exclusion anallergénique (Néocate®) et éviction totale poursuivie jusqu'à présent.

(A noter un « accident » avec éruption urticarienne 28 mois après ingestion d'un gâteau à la crèche)

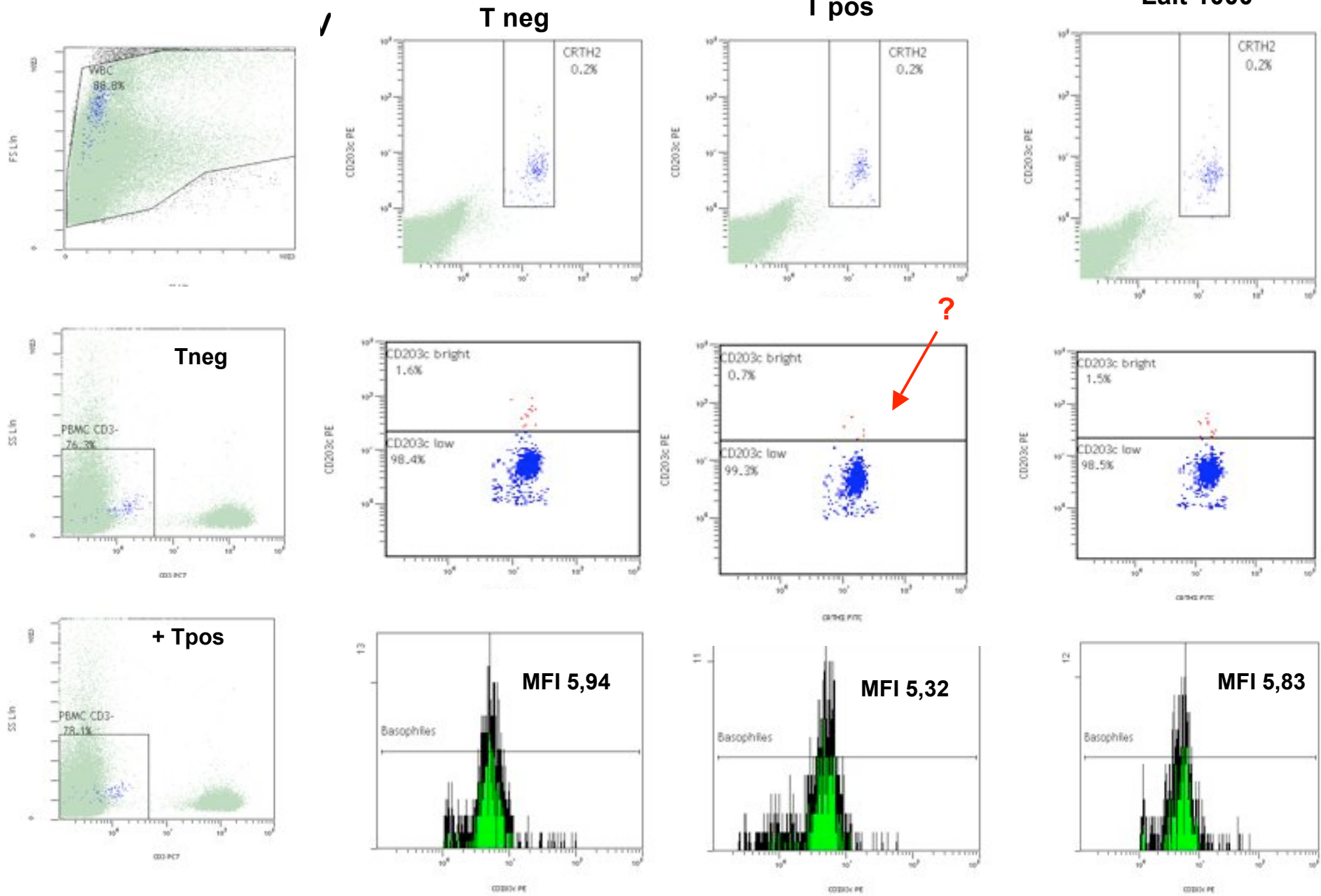
Avril 2005: IgE totales 379 kU/l,

IgE f2 70,7 kU/l (5),

f78 (caséine) 4,39kU/l (3), f77 (β lactoglobuline) 2,78kU/l (2), α lactalbumine 1,99kU/l (2).

Clinically and biologically Highly Allergic

Asser A, 34 month, A rare case ?

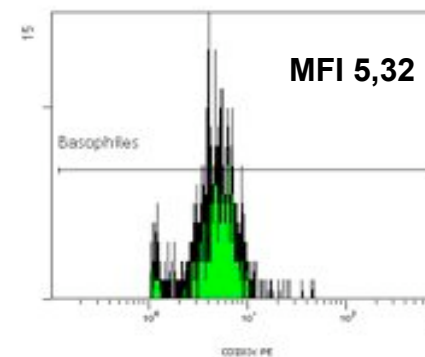
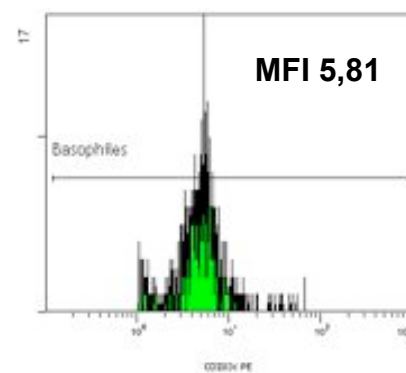
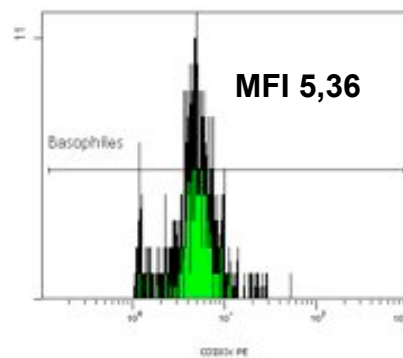
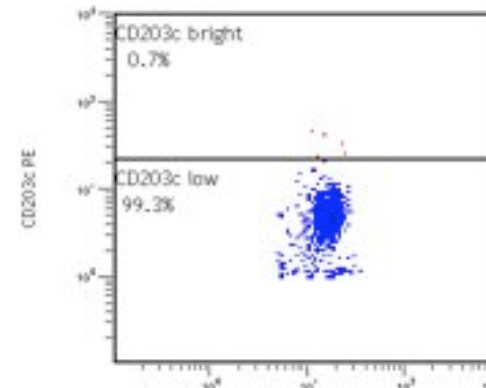
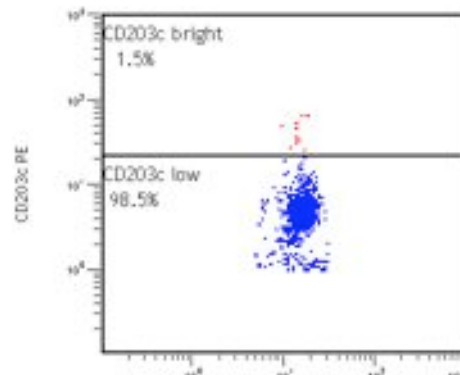
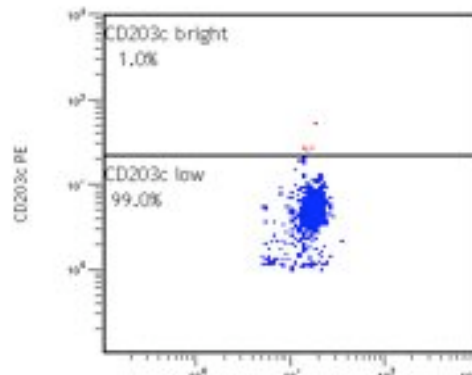
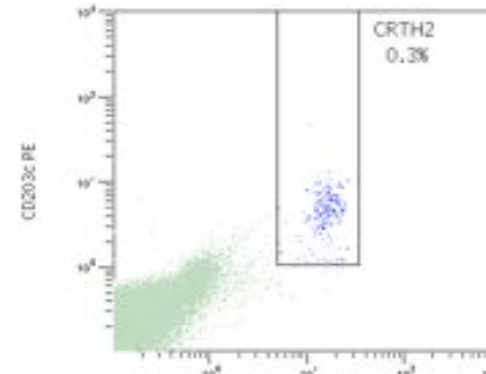
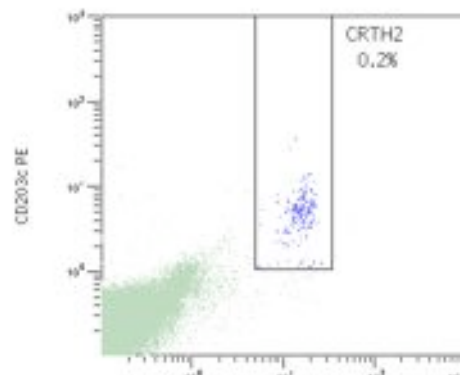
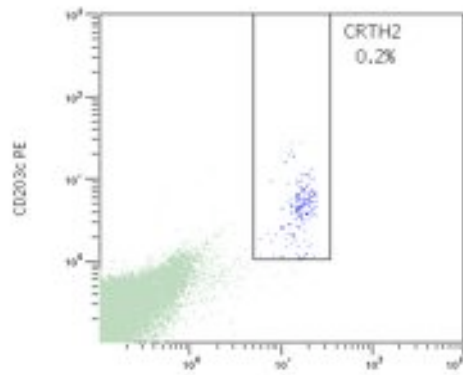


Asser A, C'Milk "non responder"

Lait 1/10

Oeuf 1/1000

Arachide 1/1000



BAT in CMF: CONCLUSIONS

- Interpretation of results :
- Absence of false positivity among the non allergics
positif BAT : **index MFI > 2**
% of activated Cells > 20%
- Sensivity \cong 80 % (n = 32), (100% Milk Healed)
- Specificity 100%

- Triple Labelling : excellent improvement of the detection of basophils in FCM in whole blood technique.
- Good correlation BAT / clinical data for the studied allergens.
- Project: avoid the Oral Provocation Tests (and the related risks) in allergic babies and children.

- Non responders !
 - Some patients (5 to 8 %) do not react to positive control and allergens
hypothesis : deficiency of phosphorylation enzymes Fc ϵ RI (syk tyrosine kinase)
 \Rightarrow Proposal to **incubate** by **IL3** before test (Buhring et al, 2004).

**Immediate Hypersensitivity IgE dependent,
or Intolerance to Cows' Milk proteins?**

Thank you for listening



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