Use of Basophils from Clinically Documented Allergic Patients

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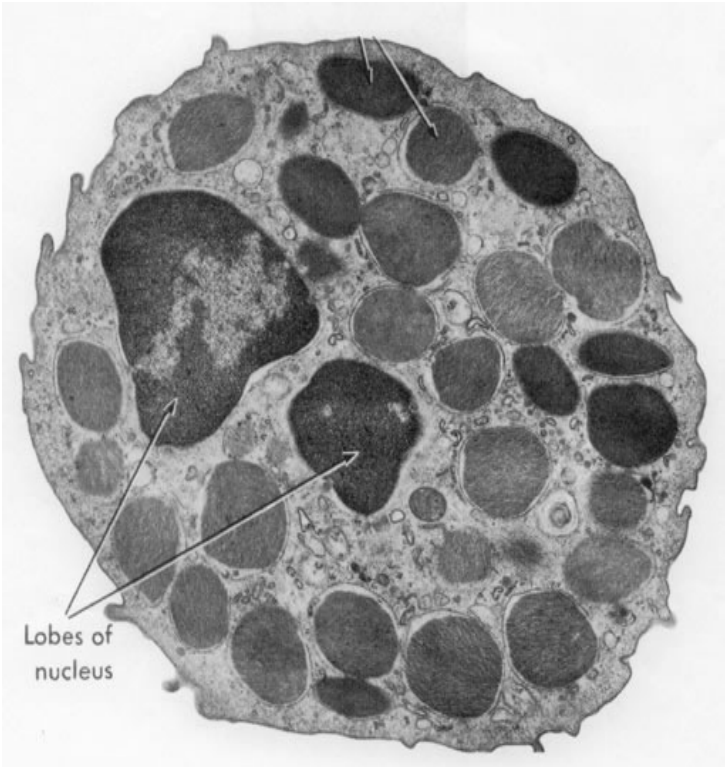
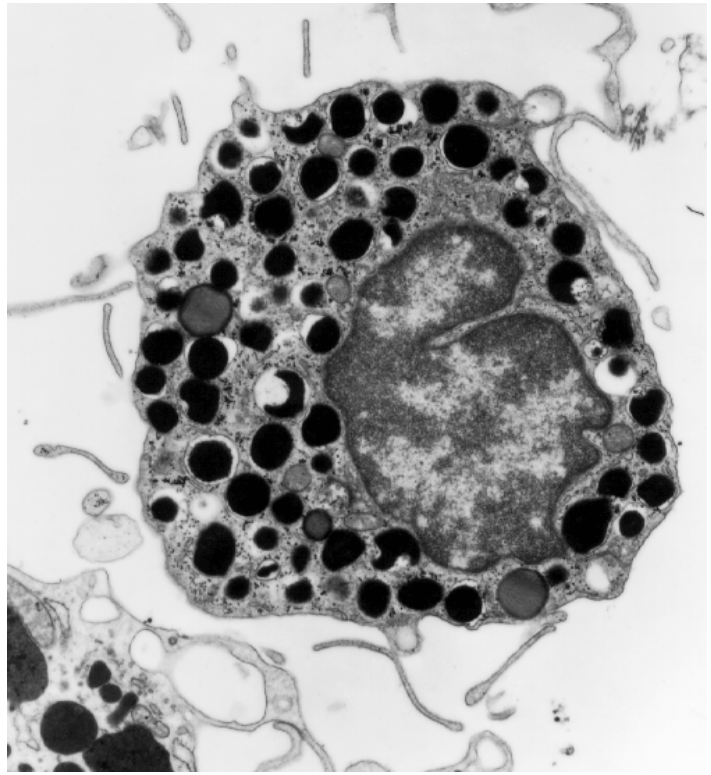
Basophils

- Least studied and understood types of leucocytes.
- Comprise less than 1% of nucleated blood cells in humans.
- Basophils are found throughout the animal kingdom. They are found in most vertebrates. This conservation suggests a non-redundant role in immunity.



Basophil

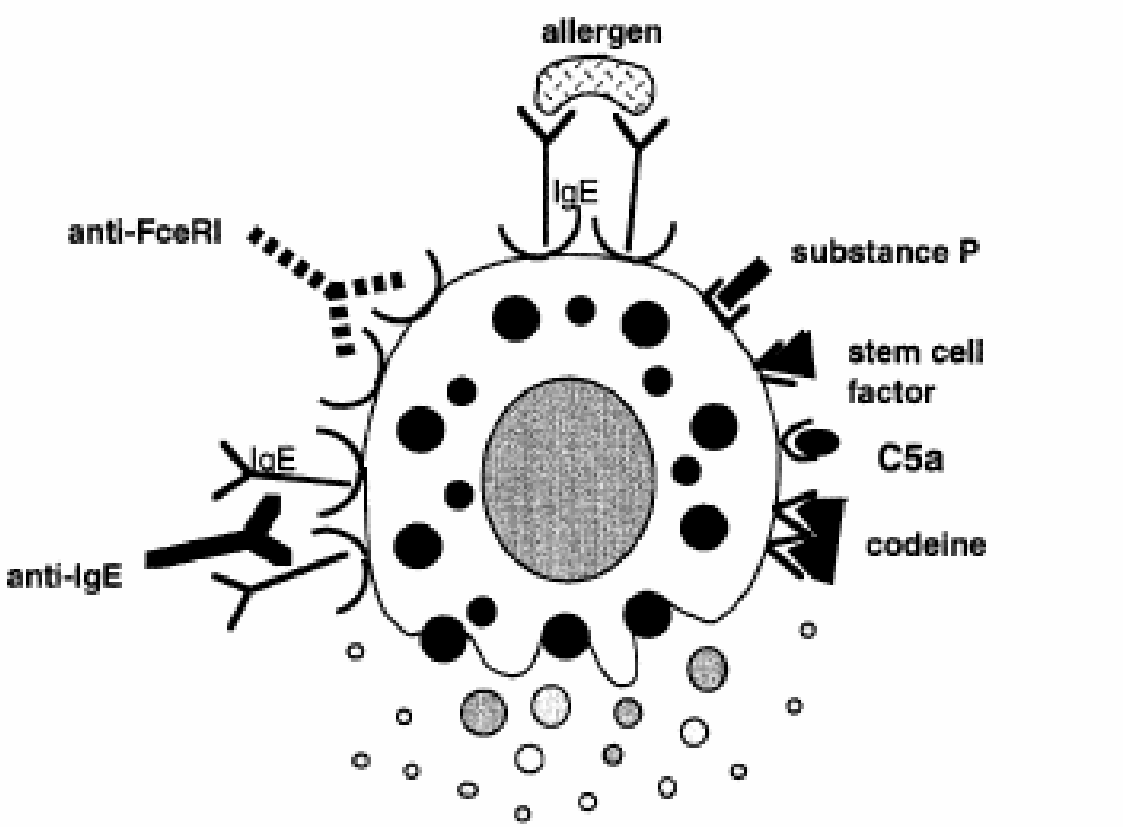


Mast cell

Similarities Between Mast Cells and Basophils

- Both cell types express the high-affinity IgE receptor, FcεRI.
- Both release mediators by 5 different mechanisms.
- Both are highly mobile and can readily infiltrate tissues at sites of inflammation.
- Mast cells and basophils are endowed with a wide set of chemotactic receptors.
- Both synthesize and release histamine.

Basophil and Mast Cell Activation



Fifth Mechanism: Stimuli That Cause Basophil Cytokine Release Without the Need for Prior Basophil Sensitization with Allergen-specific IgE

- *Helicobacter pylori* antigens
- HIV-1 envelope gp41 peptides
- HIV-1 gp120 induces IL-4 and IL-13
- Bacterial peptidoglycans can activate basophils by stimulation through the TLR2 receptors resulting in IL-4 and IL-13 release.

Basophils in Allergic Reactions

- Basophil recruitment to the skin has long been known to occur in contact dermatitis (Jones-Mote reaction).
- Basophils:
  - have been demonstrated in bronchial biopsies from patients with asthma
  - detected in nasal lavage after allergen challenge in patients with allergic rhinitis
- Skin biopsies of patients with atopic dermatitis.
- In contrast to mast cells cytokine synthesis in basophils is primarily restricted to IL-4 and IL-13.
- IL-4 is released rapidly from basophils and there is evidence that the basophils contain preformed IL-4.

Mechanism of activation

FcεRI-mediated

Allergens

Autoantibodies
- to IgE
- to FcεRI

Non-FcεRI-mediated

Receptors for endogenous substances:

Chemokine receptors
Cytokine receptors
Complement receptors
FcγR
Receptors for neuropeptides
Glucocorticoid receptors
β_2-Adrenergic receptors
Histamine receptors

Autoantibodies to the above
Pharmacological agents interacting with the above

Receptors for exogenous substances:

Toll-like receptors
Leukocyte Ig-like receptors ?
fMLP receptors (FPRL-1 and FPRL-2)
Formyl peptide receptors

Biological effects

(readout)

Surface activation markers
CD63
CD203c

Degranulation
Histamine release
Degranulation tests

Secretion of
Lipid mediators
Chemokines
Cytokines

Signaling events
Intracellular Ca^{2+}
Kinase phosphorylation
Second messengers: cAMP, IP_3
mRNA for inflammatory products

Other biological effects
Adhesion
Chemotaxis
Apoptosis

From: Kleine-Tebbe J, Erdmann S, Edward EF, et al. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. Int Arch Allergy Immunol 2006;141:79-90.

Diagnosis of Allergy

Based on:

- Evocative clinical history
- Positive skin tests (considered gold standard)
- Detection of allergen specific IgE

Disadvantages of Classical Diagnosis

- Clinical history can be unreliable.
- In some cases skin testing can cause an adverse reaction and often can not be done in patients with certain skin disorders.
- The level of IgE present does not always correlate to the severity of an allergic reaction, and someone who has “outgrown” an allergy may have a positive IgE for many years afterward.
- In these cases as well as for the understanding functional allergen epitopes it is useful to have a functional in vitro assay.

In vitro Functional Assays: Basophil Mediator Release Assays

- Allergen-induced basophil histamine release assays have been described in the literature since 1961.
- Have been used with whole blood, leukocyte preparations or isolated basophils.
- Most common mediator to measure is histamine however leukotrienes and IL-4 and IL-13 have also been measured (plant lectins).
- Wide range of sensitivities and specificities when compared to “gold standards”, e.g., skin testing, nasal or bronchoprovocation, etc.

Flow Cytometry Based Assays for Measurement of Basophil Activation

- Developed in the early 1990s with the discovery of the basophil activation marker, CD63.
- Can be done on whole blood – there is no need to isolate basophils, thus more physiological.
- Very little blood is required from allergic patient.
- Very rapid test.

Flow Cytometry

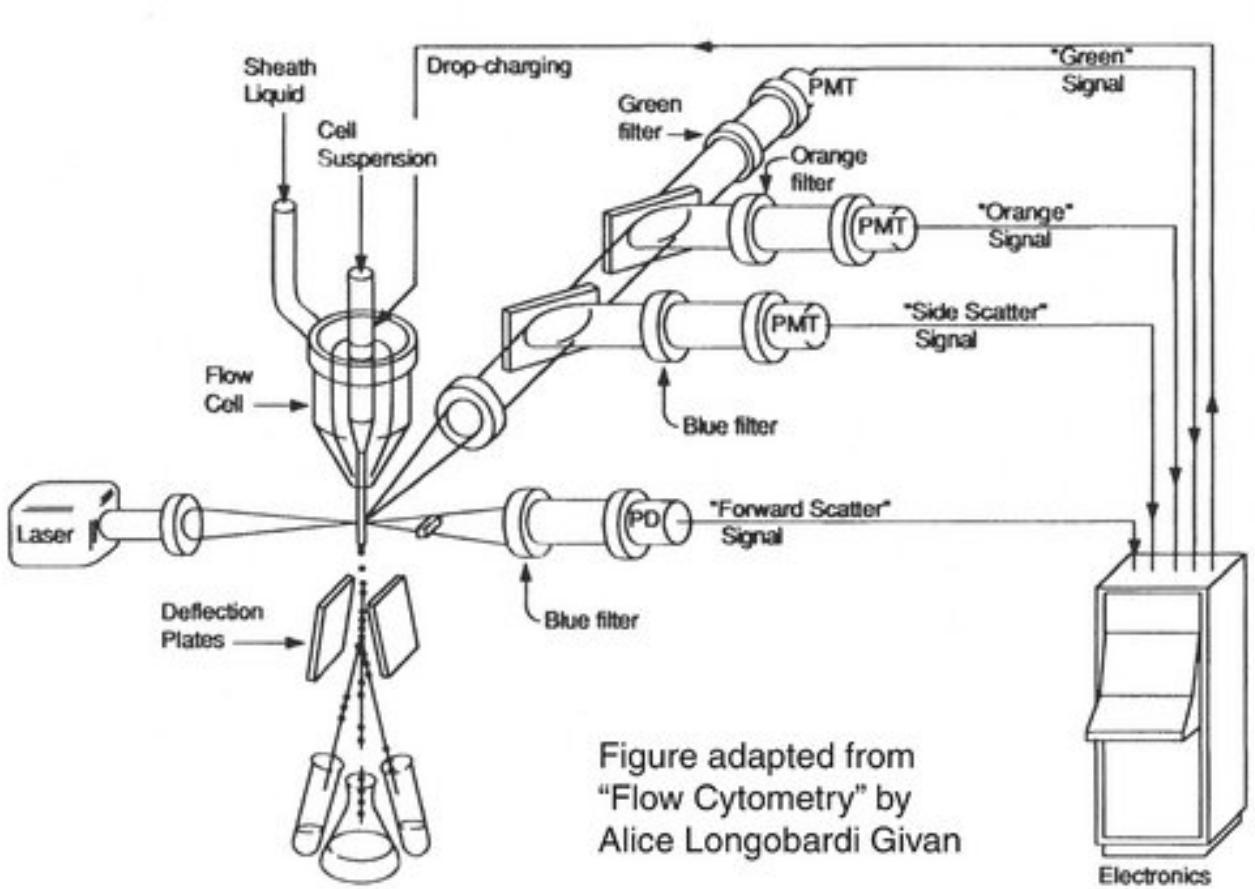
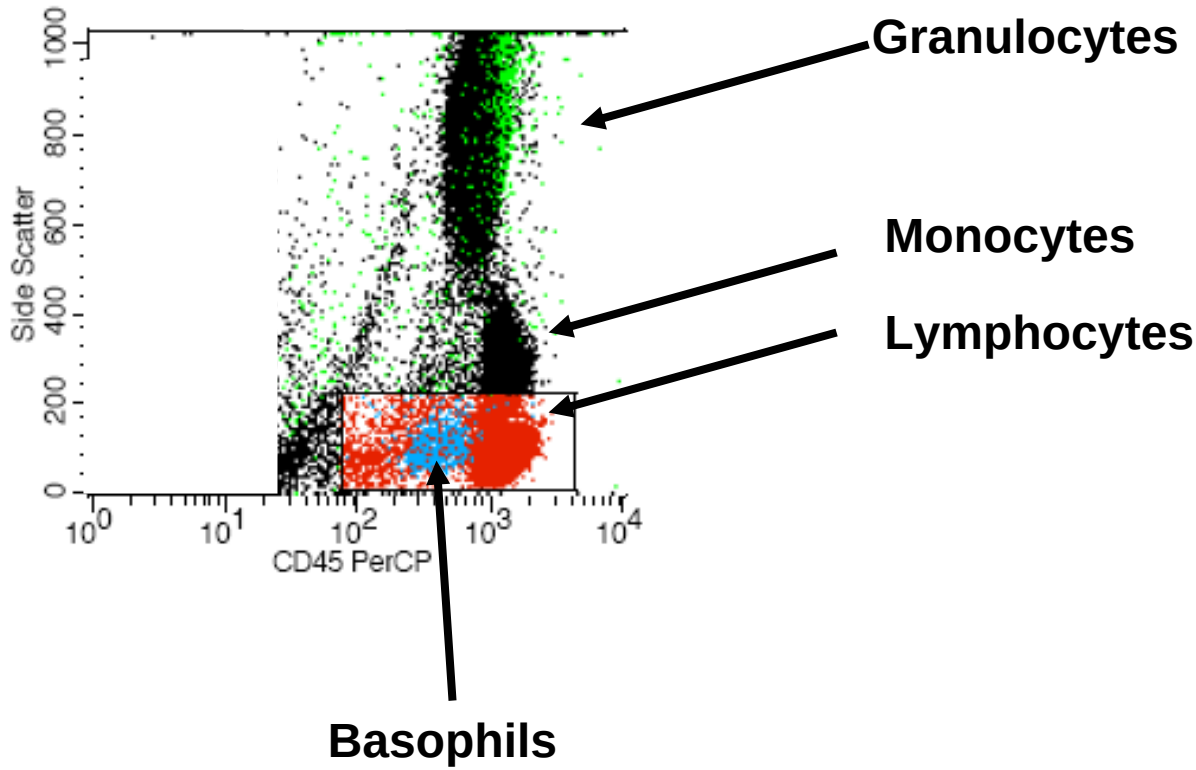


Figure adapted from "Flow Cytometry" by Alice Longobardi Givan

CD45 by Side Scatter of Peripheral Blood Leukocytes



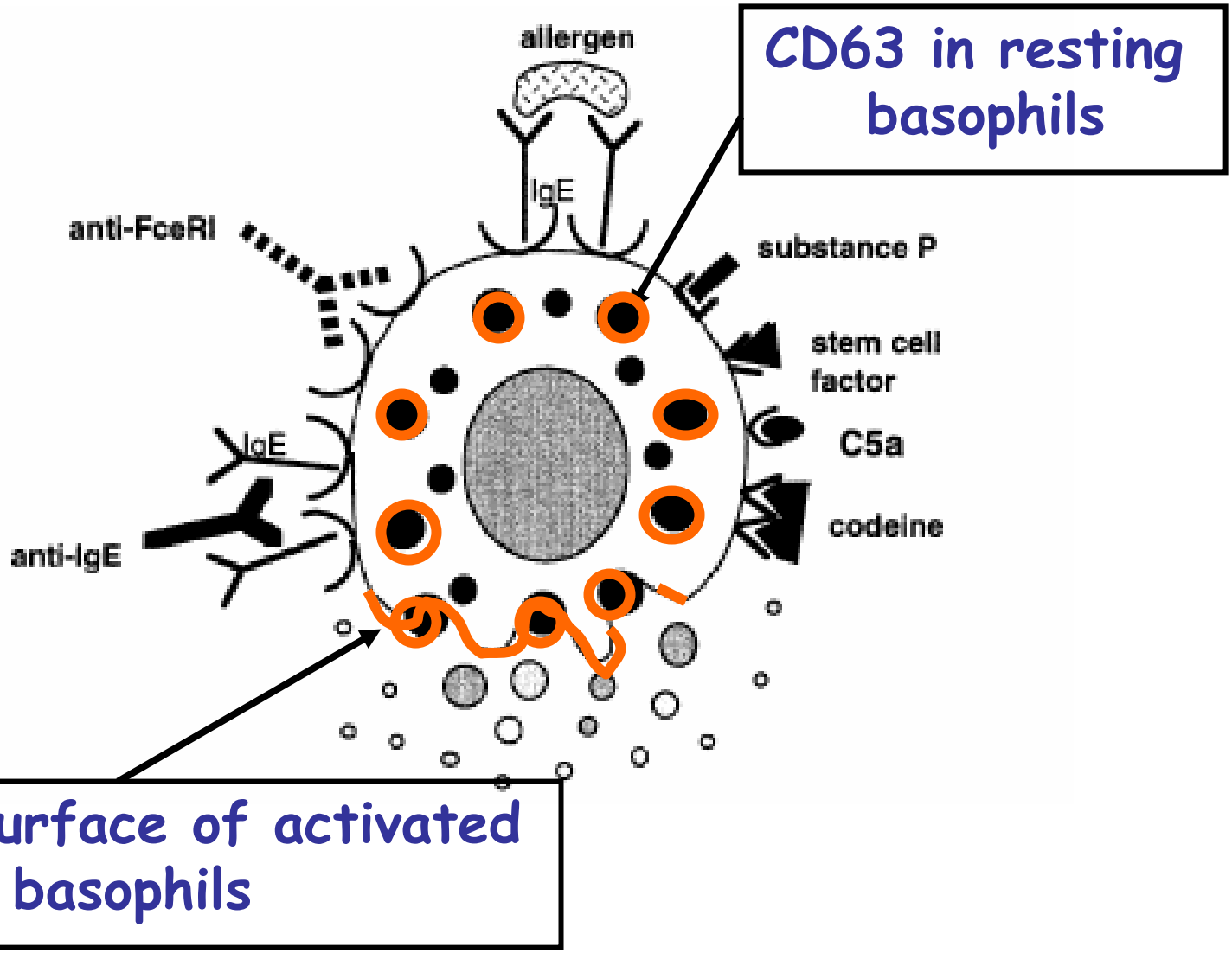
Flow Cytometry and CD63

Has been used in allergy diagnosis to:

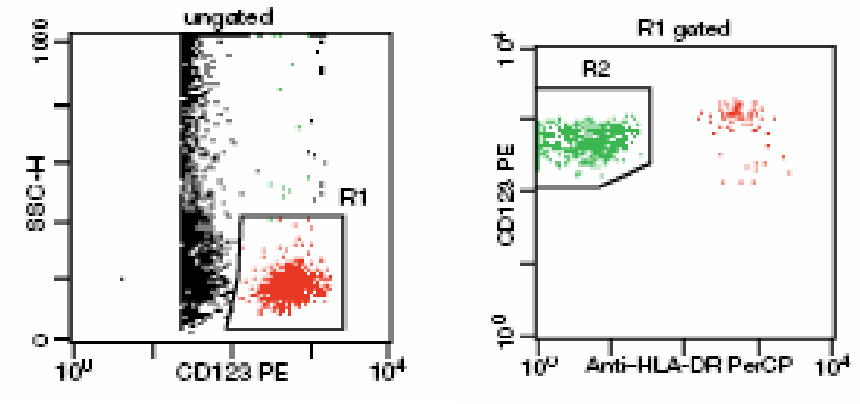
- Latex
- Pollen
- Primary food allergies, e.g., sesame,papaya, guar gum and others.
- Secondary food allergies resulting in cross-reactivity, e.g., birch pollen.
- Venoms
- Beta-lactam antibiotics
- Dust mites
- Assess the allergenicity of chemically modified and recombinant allergens.

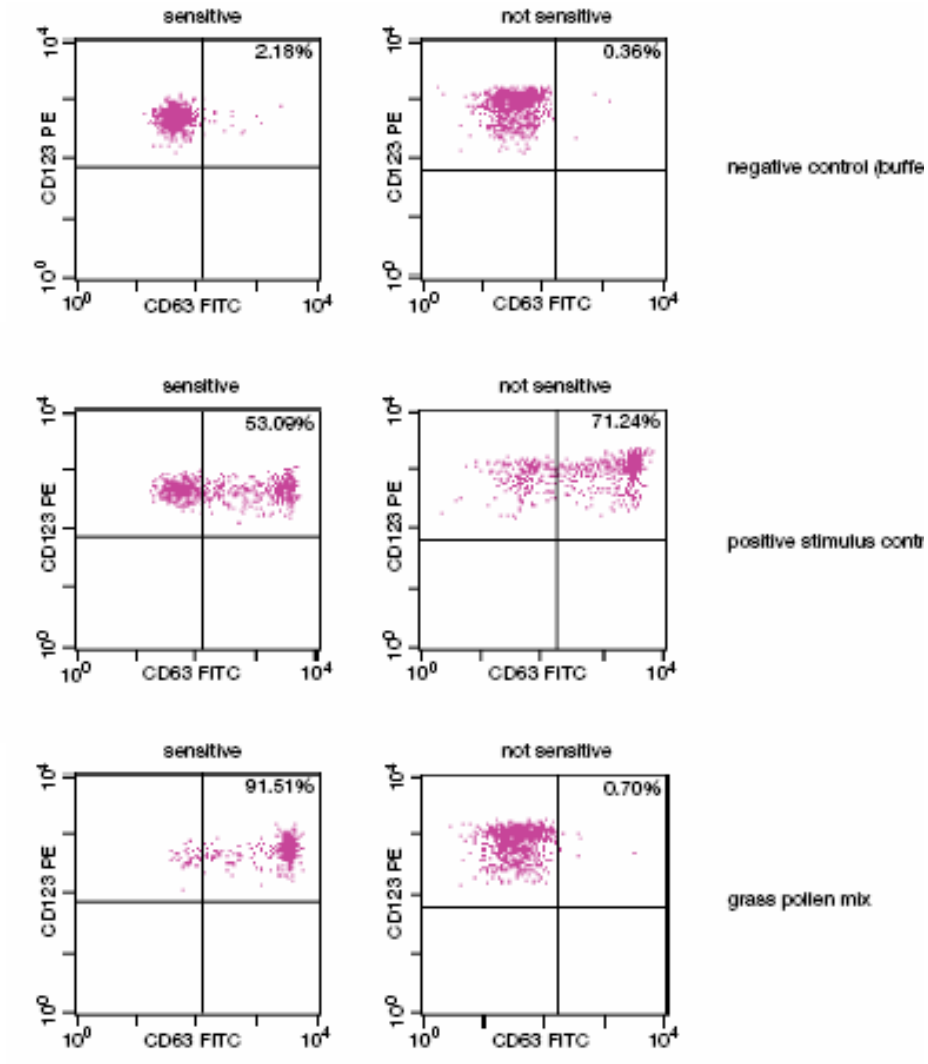
Measurement of CD63 on Basophils

- Basophils are generally identified as CD123+ cells (on basophils, eosinophils, monocytes, and a subset of peripheral blood dendritic cells) and HLA-DR- cells.


CD63 in resting basophils
allergen
IgE
anti-FceRI
substance P
stem cell factor
C5a
codeine
IgE
anti-IgE
CD63 on surface of activated basophils

Commercially Available Test for Basophil Activation

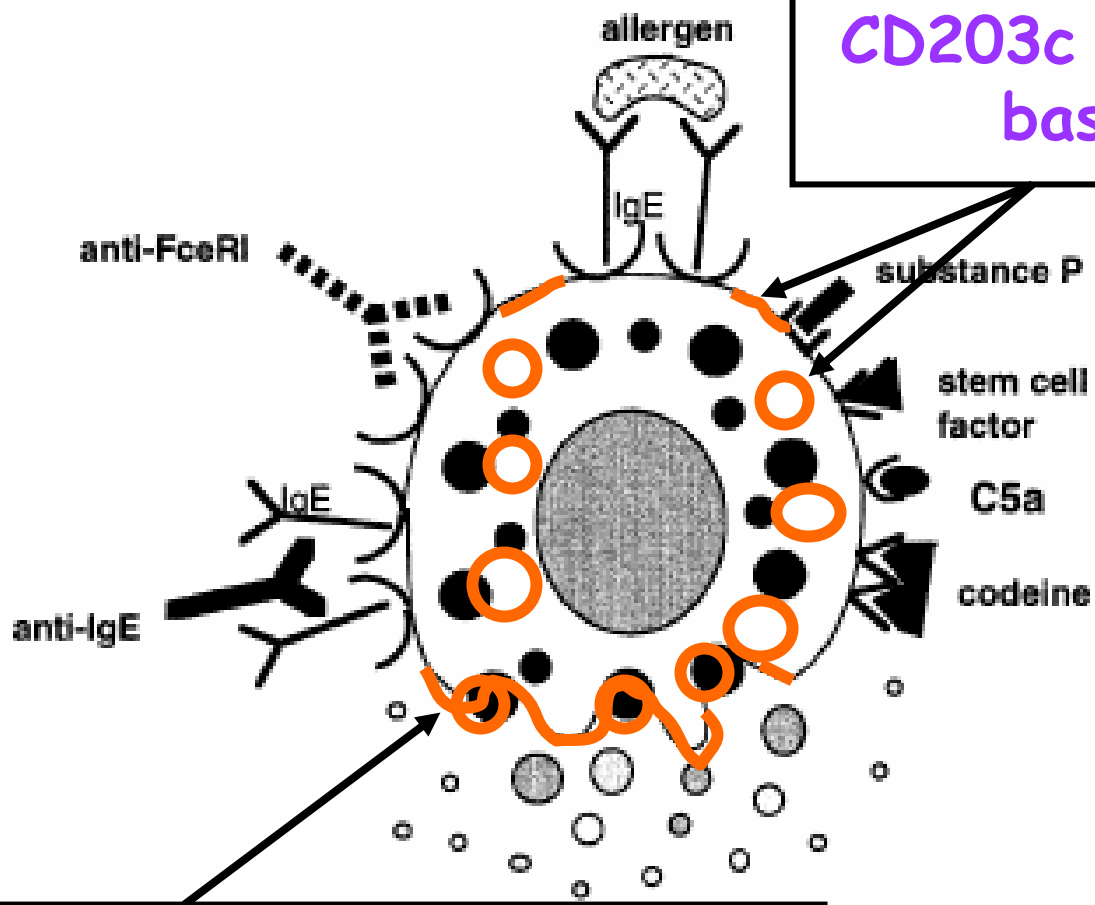




CD203c

- **CD203c (E-NPP3) is a type II transmembrane molecule and belongs to a family of ecto-nucleotidepyrophosphatase/phosphodiest-erase (E-NPP) enzymes that catalyze the hydrolysis of oligonucleotides, nucleoside phosphates and NAD.**
- **Expressed on basophils, mast cells and their CD34+ progenitors. As opposed to CD63 basophils are the only cell in the which expresses CD203c.**
- **Upon degranulation of basophils, CD203c expression is upregulated.**

CD203c on resting and activated basophils



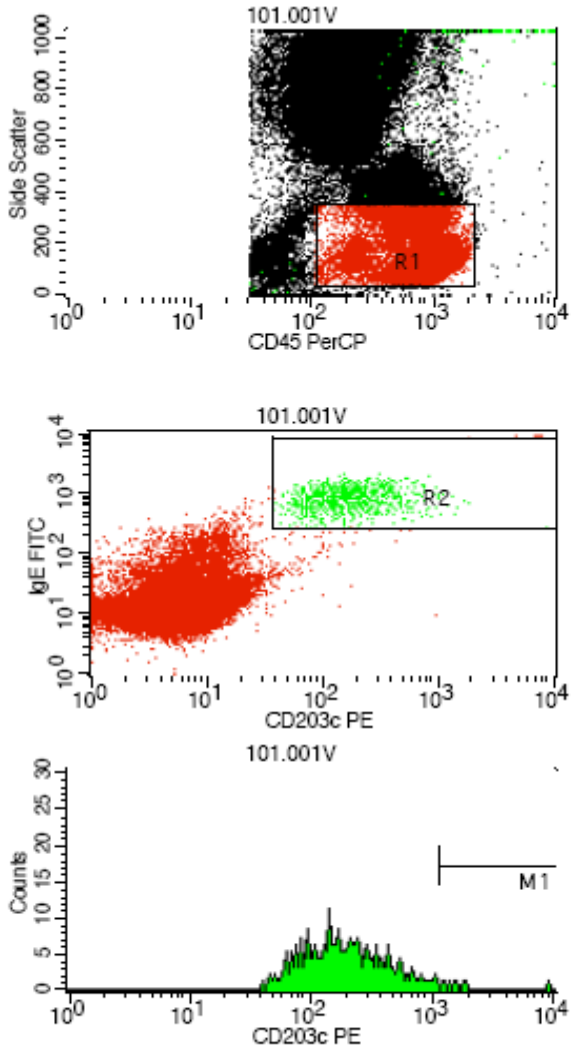
CD203c on resting basophils

CD203c on surface of activated basophils is upregulated

CD203c Surface Expression

1. 100 μL heparinized whole blood from an appropriate basophil donor.
2. Incubate with controls or allergen for 10 minutes.
3. Stop reaction by placing tube on ice.
4. Stain cells with:
    1. PE-anti-human CD203c
    2. PerCP-anti-human CD45
    3. FITC-anti-human IgE

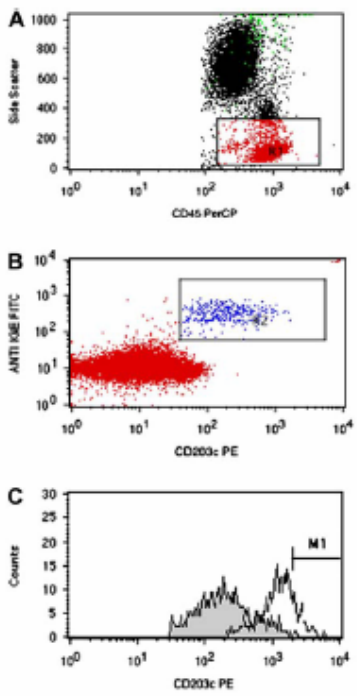
Identification of basophil CD203c expression by flow cytometry



Chronic “idiopathic” urticaria (CIU)

- Defined as the daily or almost daily occurrence on wheals (hives) for at least 6 weeks in which neither signs of vasculitis nor causative drugs, foods, and/or physical factors can be identified as triggering agents.
- In about 30-40% of cases an IgG antibody has been identified that reacts with the alpha subunit of the high affinity IgE receptor (FcεR1) of basophils and mast cells, or in some cases IgE itself.


23 IV 98
- SERUM
- SALINE



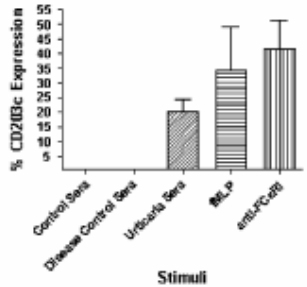


FIG 2. The mean percent (±SEM) CD203c expression above baseline by sera from normal controls (N = 11), disease controls (N = 4), all patients with CU (N = 32), and positive controls, ie, fMLP and anti-FcεRI.

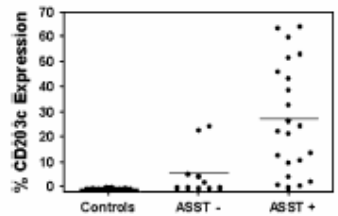
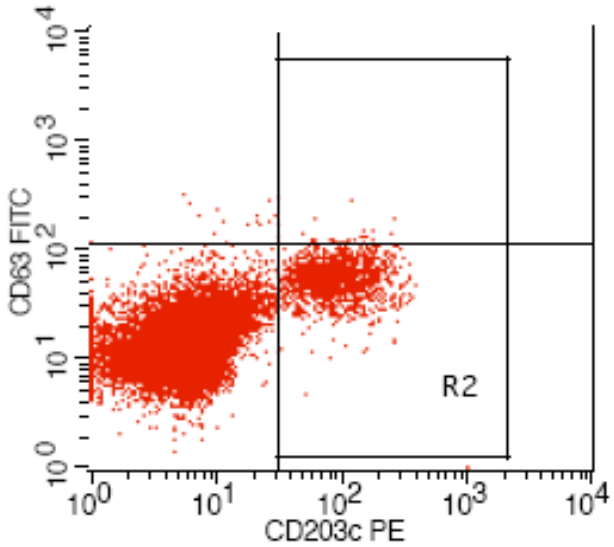
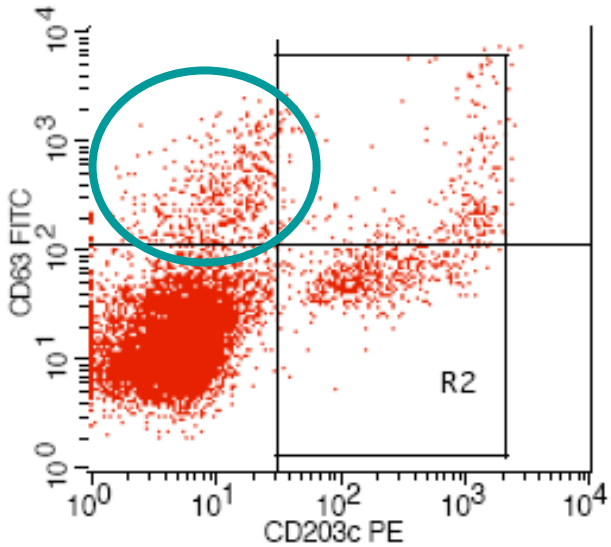


FIG 3. Percent CD203c expression by normal individuals, patients with CU and negative ASST, and patients with CU and positive

Rapid CD63 Expression on Non-CD203c+ Cells after fMLP Addition to Whole Blood

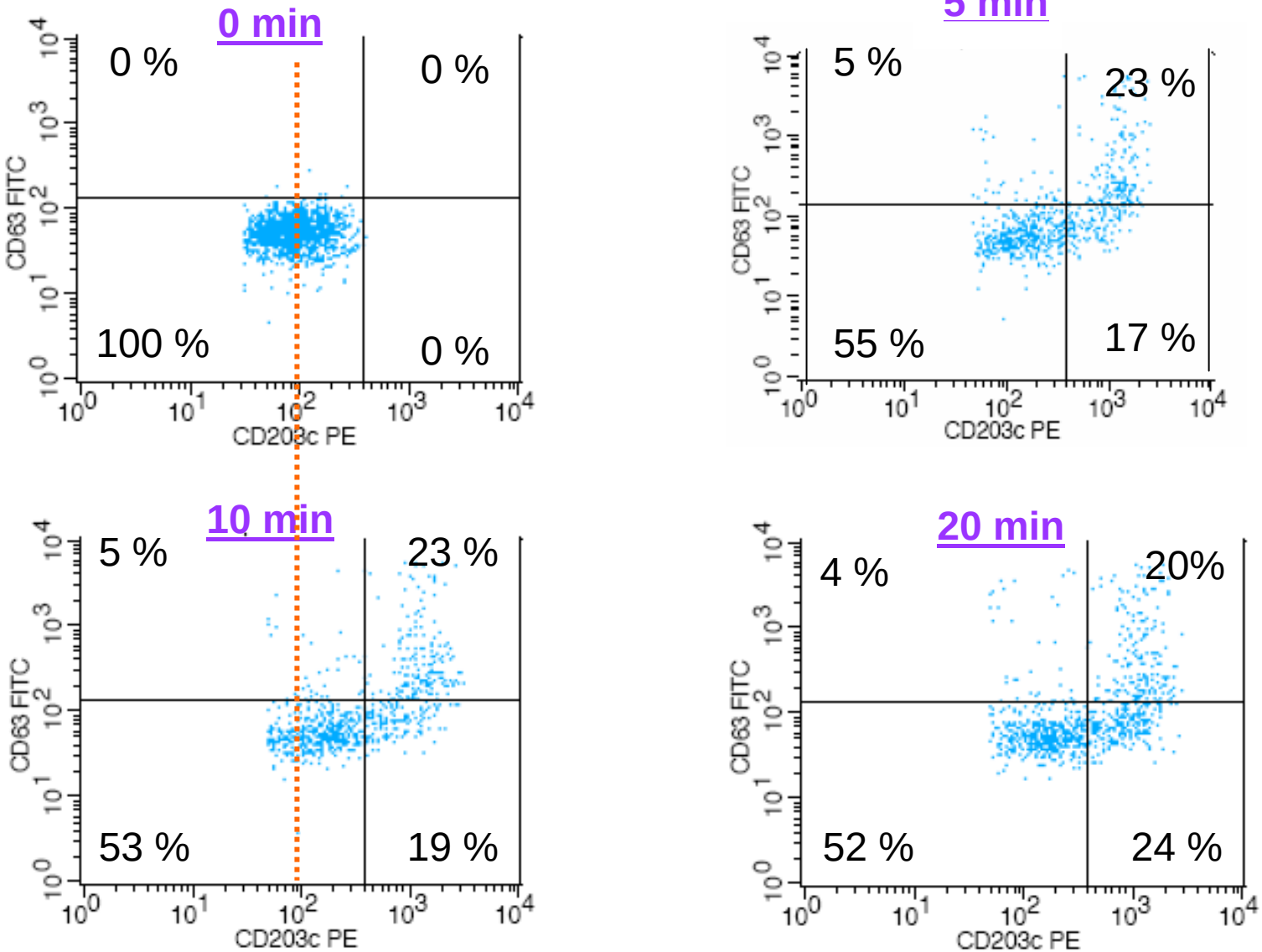


No fMLP

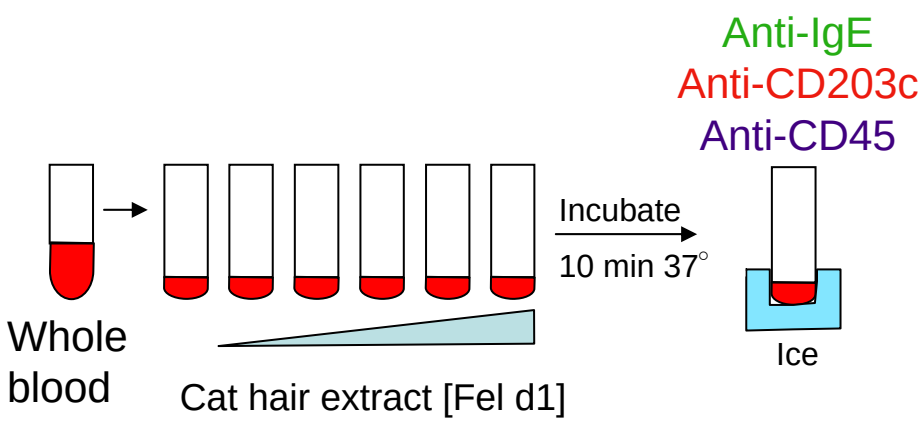


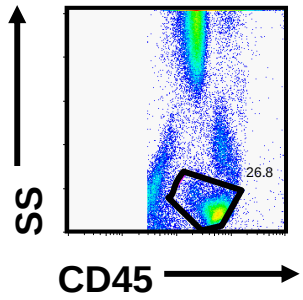
5 min post-fMLP

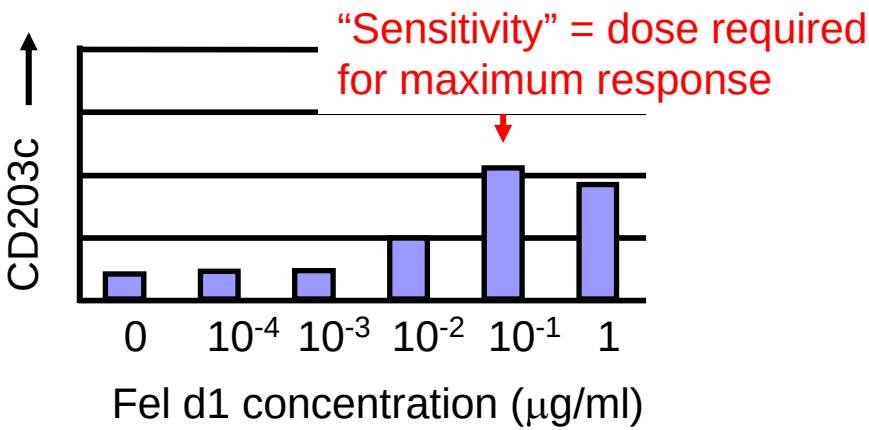
Kinetics of CD203c and CD203+CD63+ Expression on Normal Human Basophils after Stimulation with fmlp

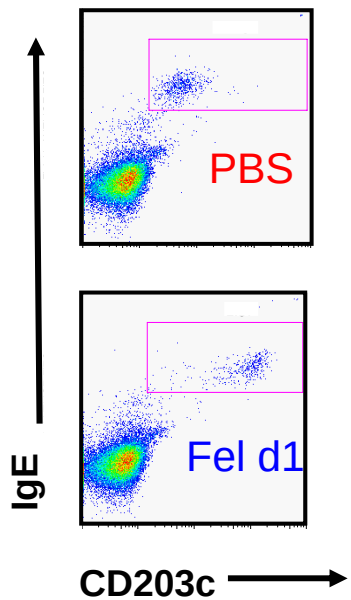


Flow Cytometry Based Assay for *ex-vivo* Basophil Activation from Cat Allergic Individuals

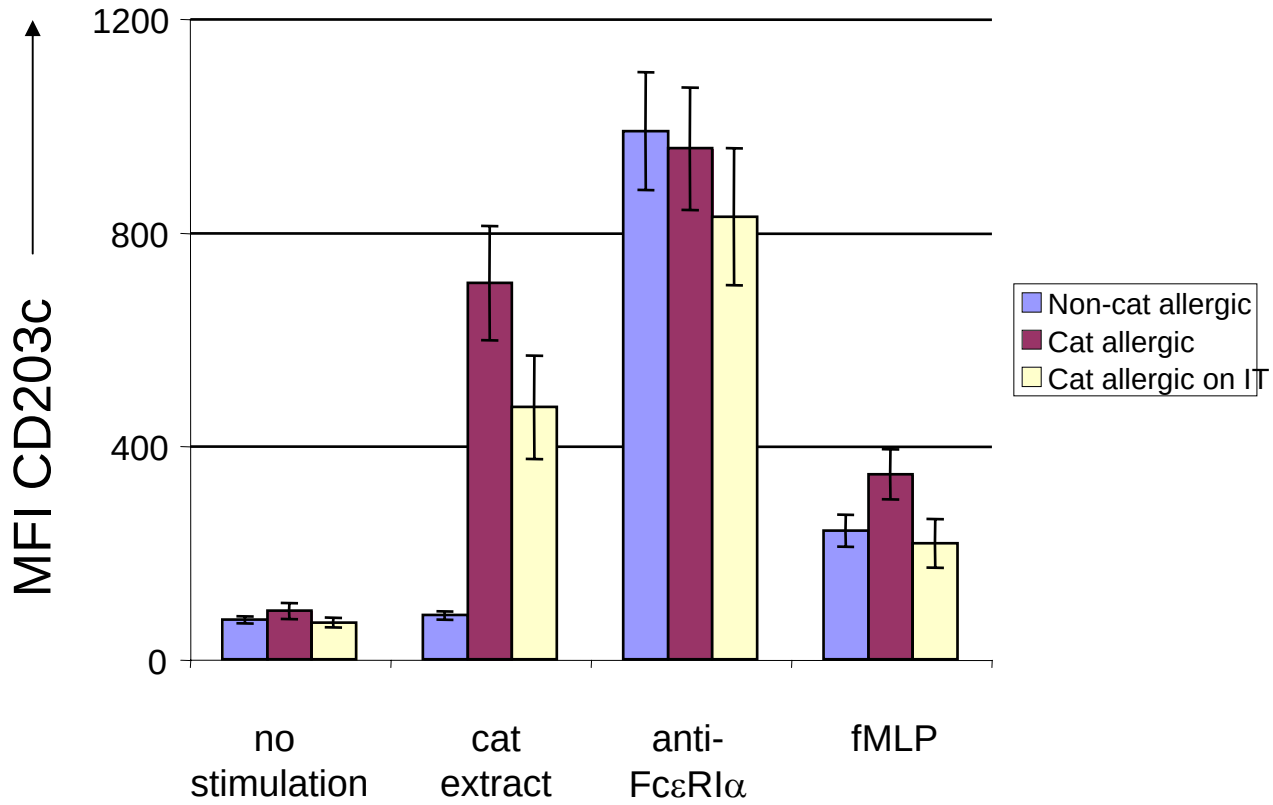








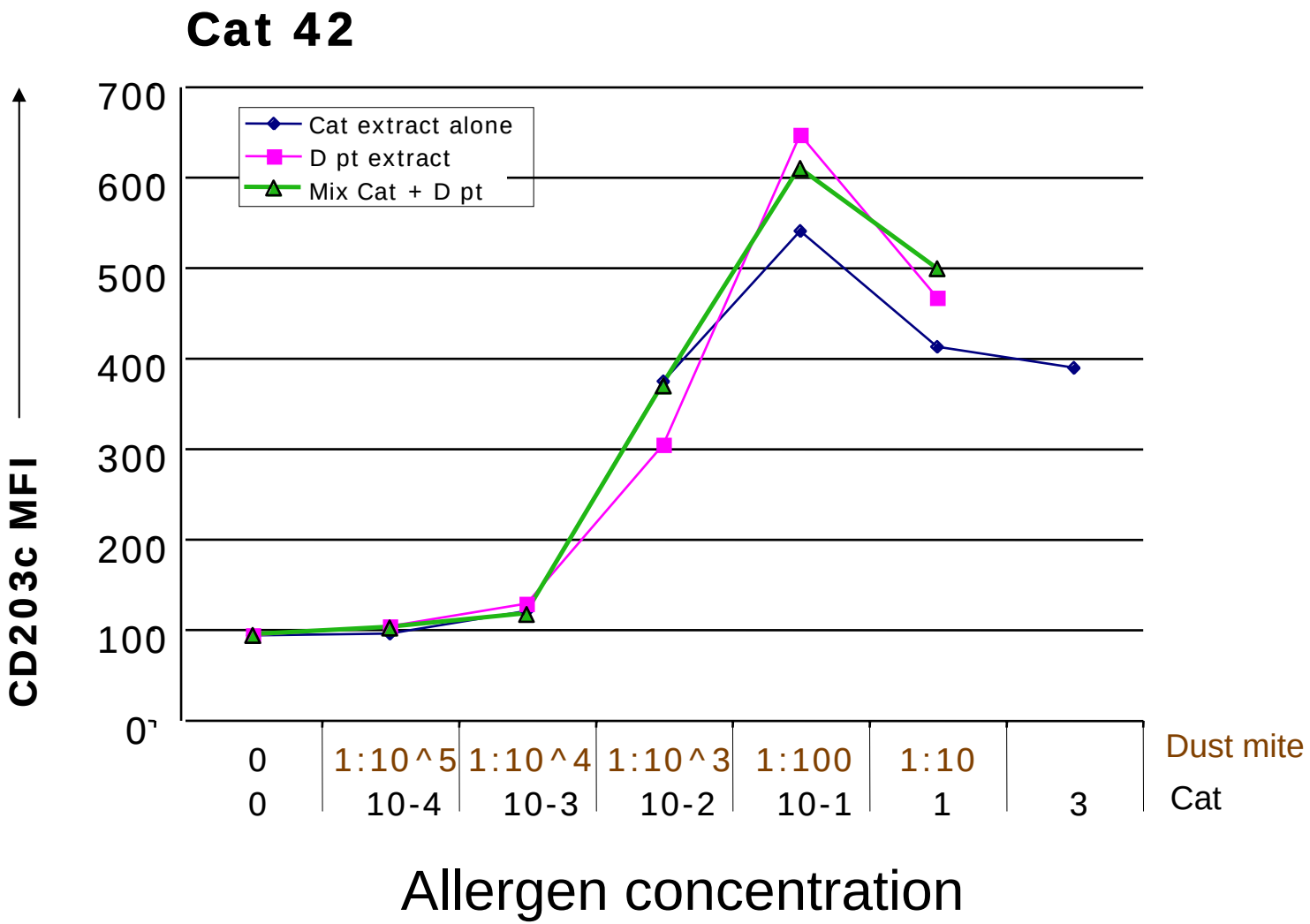
Increase in CD203c Expression is a Sensitive and Specific Marker for Basophil Activation



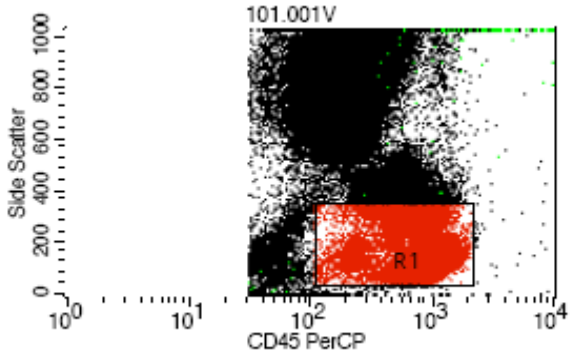
Acknowledgements

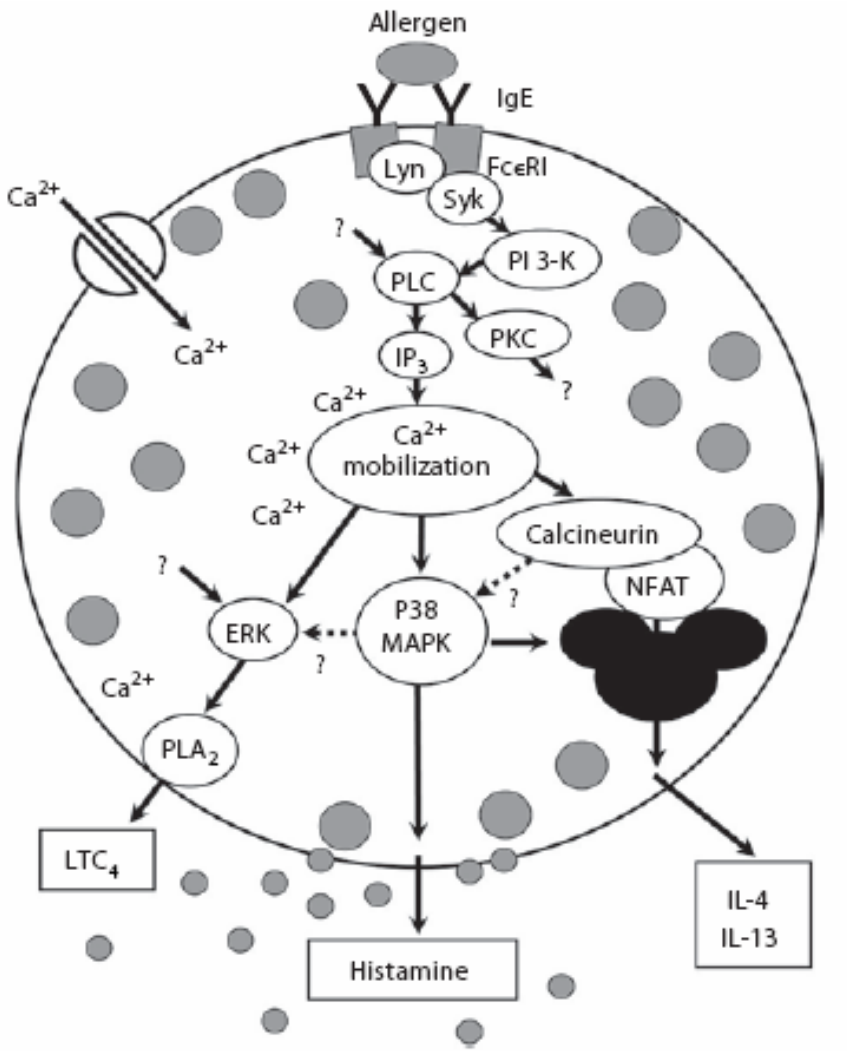
- Karen Yasnowsky-Andrews
- Carol Cady
- Melissa Boyne
- Ben Efaw
- Weiming Shen
- Rafeul Alam
- Steve Dreskin

Effect of adding multiple allergens to basophil sensitivity



Identification of Peripheral Blood Leukocytes by Flow Cytometry





From: Kleine-Tebbe J, Erdmann S, Edward EF, et al. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. Int Arch Allergy Immunol 2006;141:79-90.

Mediators Released by Mast Cells and Basophils

Mast cells

- **Histamine**
- **LTC_4**
- **PGD_2**
- **Tryptase**
- **Chymase**
- **Chemokines**

Basophils

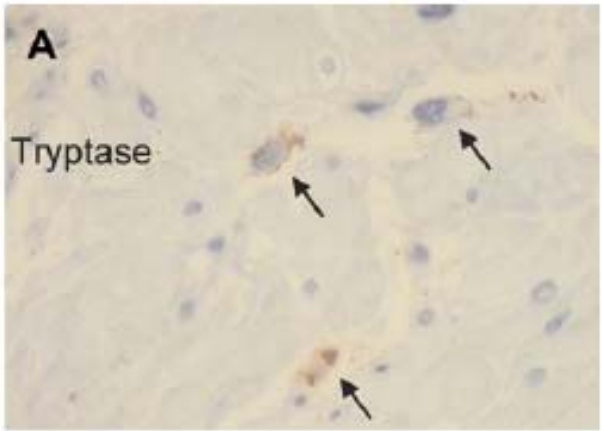
- **Histamine**
- **LTC_4**
- **Tryptase**
- **Basogranulin (a component of the granules)**
- **PAF**

CD203 upregulation technique has been used to:

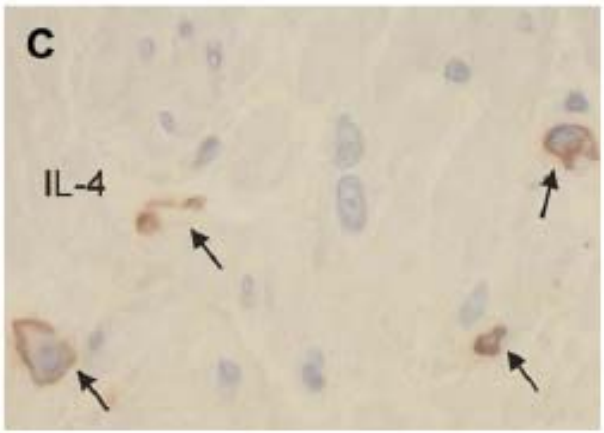
- To demonstrate allergen specific responses, e.g., to latex.
- In chronic urticaria where individuals may have an autoantibody against their FcεR1.

Utility in Allergic Diagnosis

- Latex
- Pollen
- Food
- Beta-lactam antibiotics
- Dust Mite
- Venom

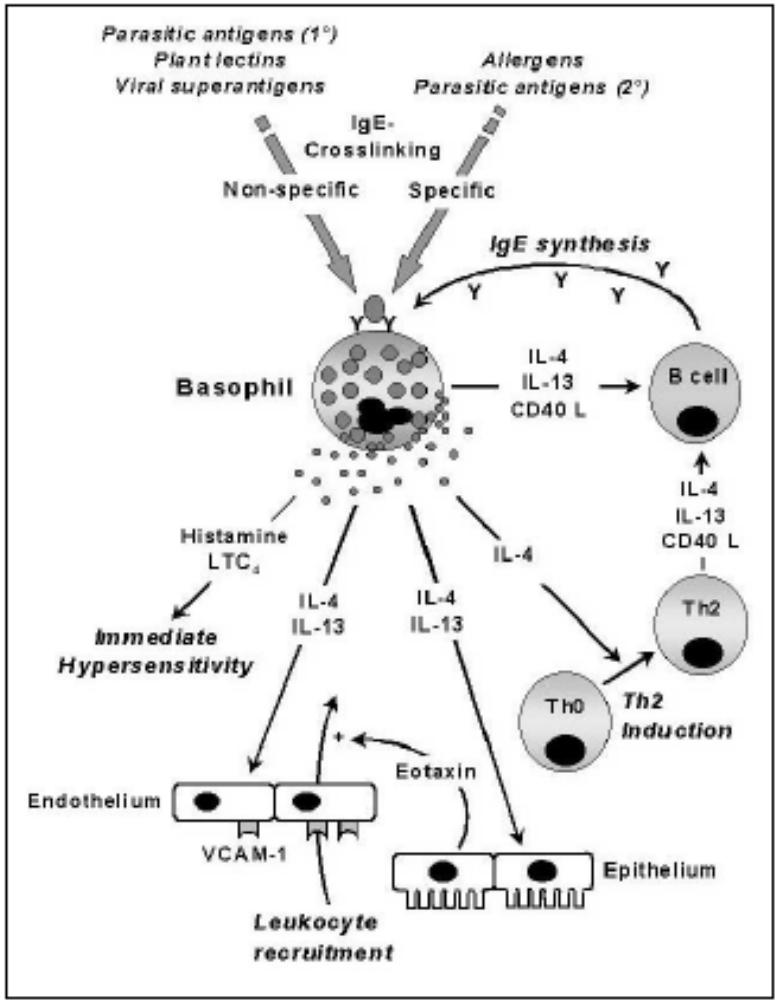


A. Section of bronchus from a patient with asthma showing degranulated tryptase+ mast cells within an ASM bundle.



B. IL-4+ mast cells within ASM bundle.

Main Biological Activities of Basophils Following IgE-dependent Activation



Differences in Mast Cells and Basophils: Cytokines

Mast cells

- **SCF**
- **IL-3**
- **IL-5**
- **IL-6**
- **IL-8**
- **IL-13**
- **IL-16**
- **IL-18**
- **TGFβ**
- **IL-25** ***(induces IL-4 and IL-13 gene expression)***
- **GM-CSF**
- **TNFα**
- **VEGF**

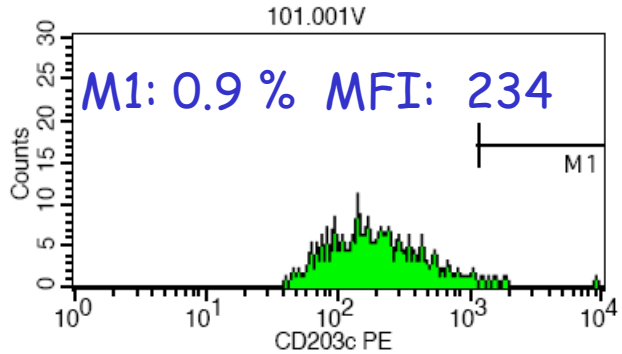
Basophils

Restricted to Th2 cytokines:

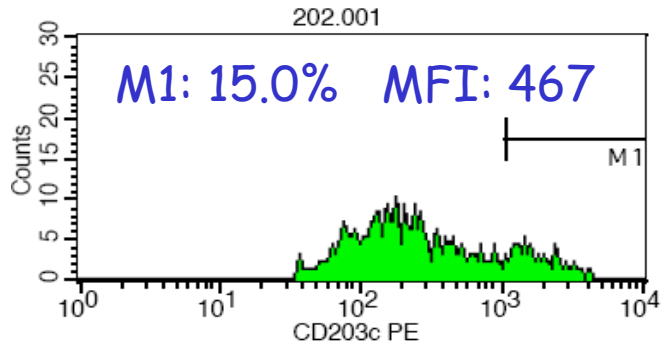
- **IL-4**
- **IL-13**
- **VEGF**
- **CCL3**
- **CXCL8**

Results

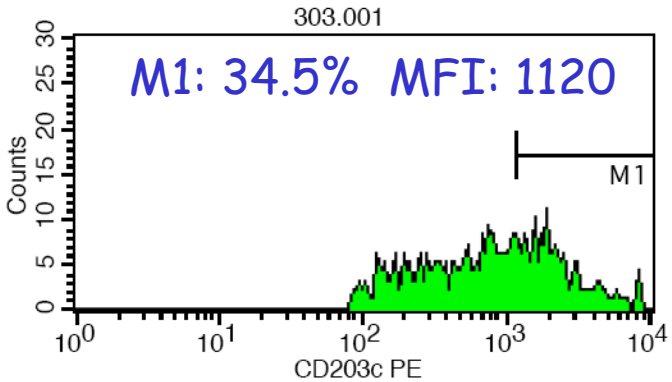
Buffer



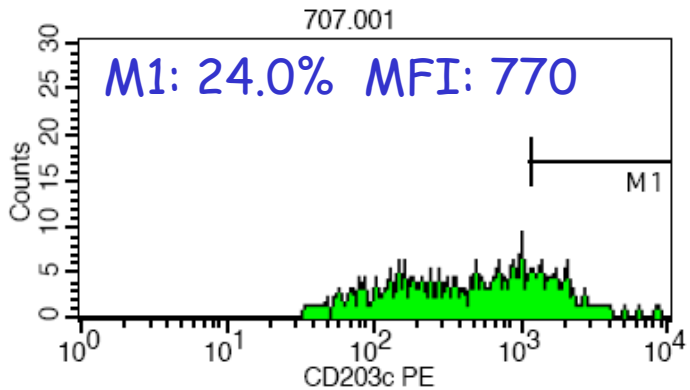
fMLP



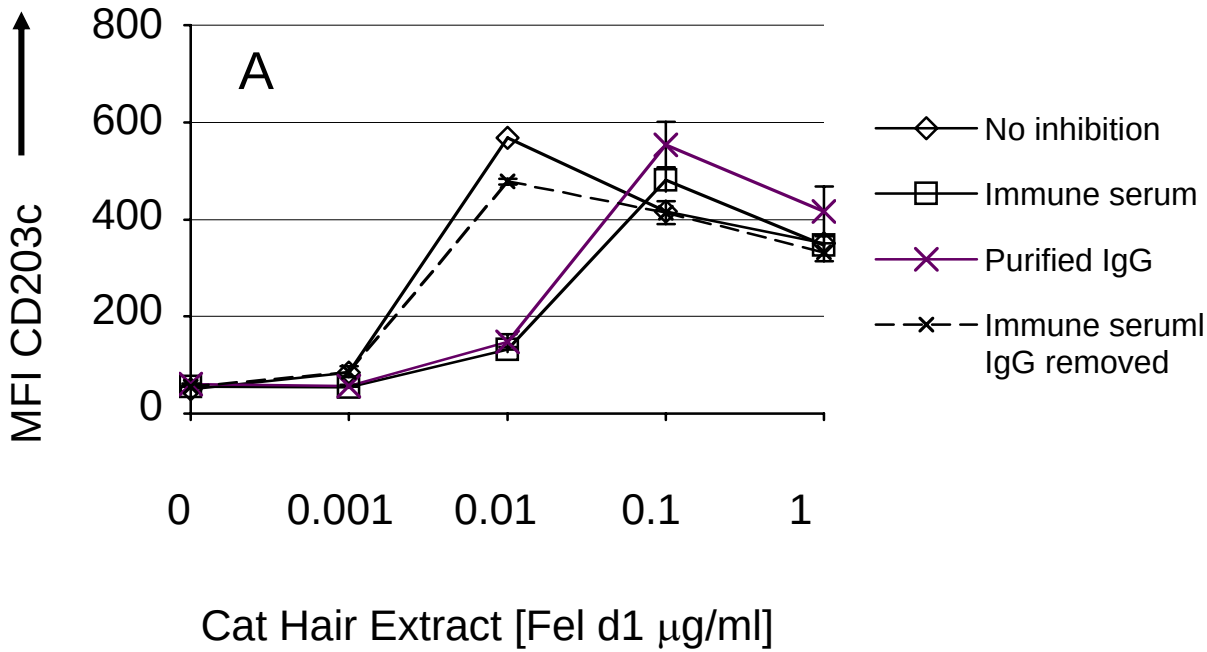
Anti-FceRI



Positive CU Serum

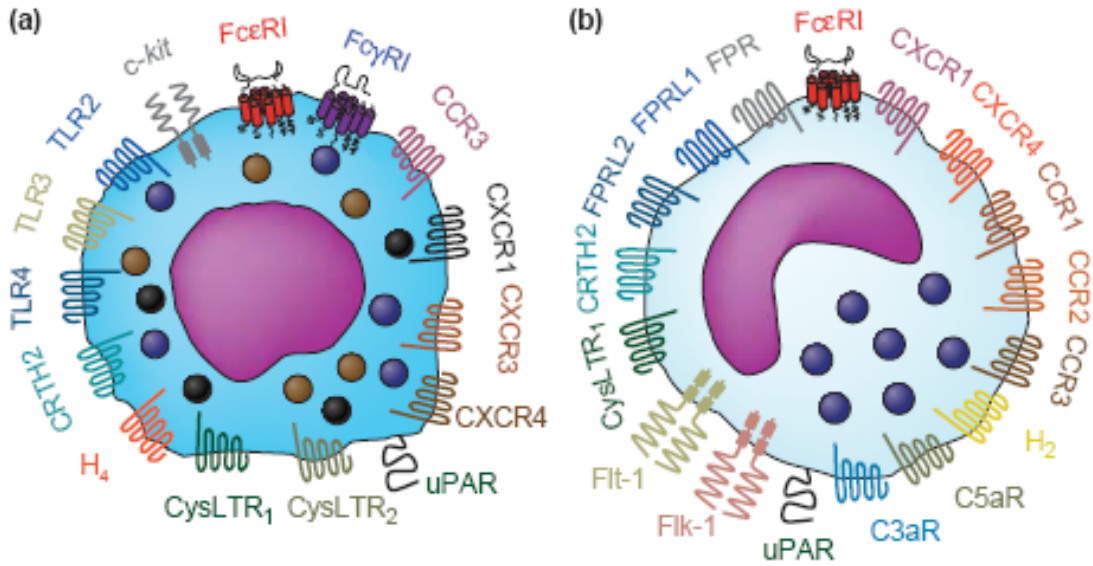


IgG antibodies produced during immunotherapy alter basophil responsiveness.



* How assay can be used fo ex vivo diagnostics

Selective Display of Membrane Receptors on Mast Cells and Basophils

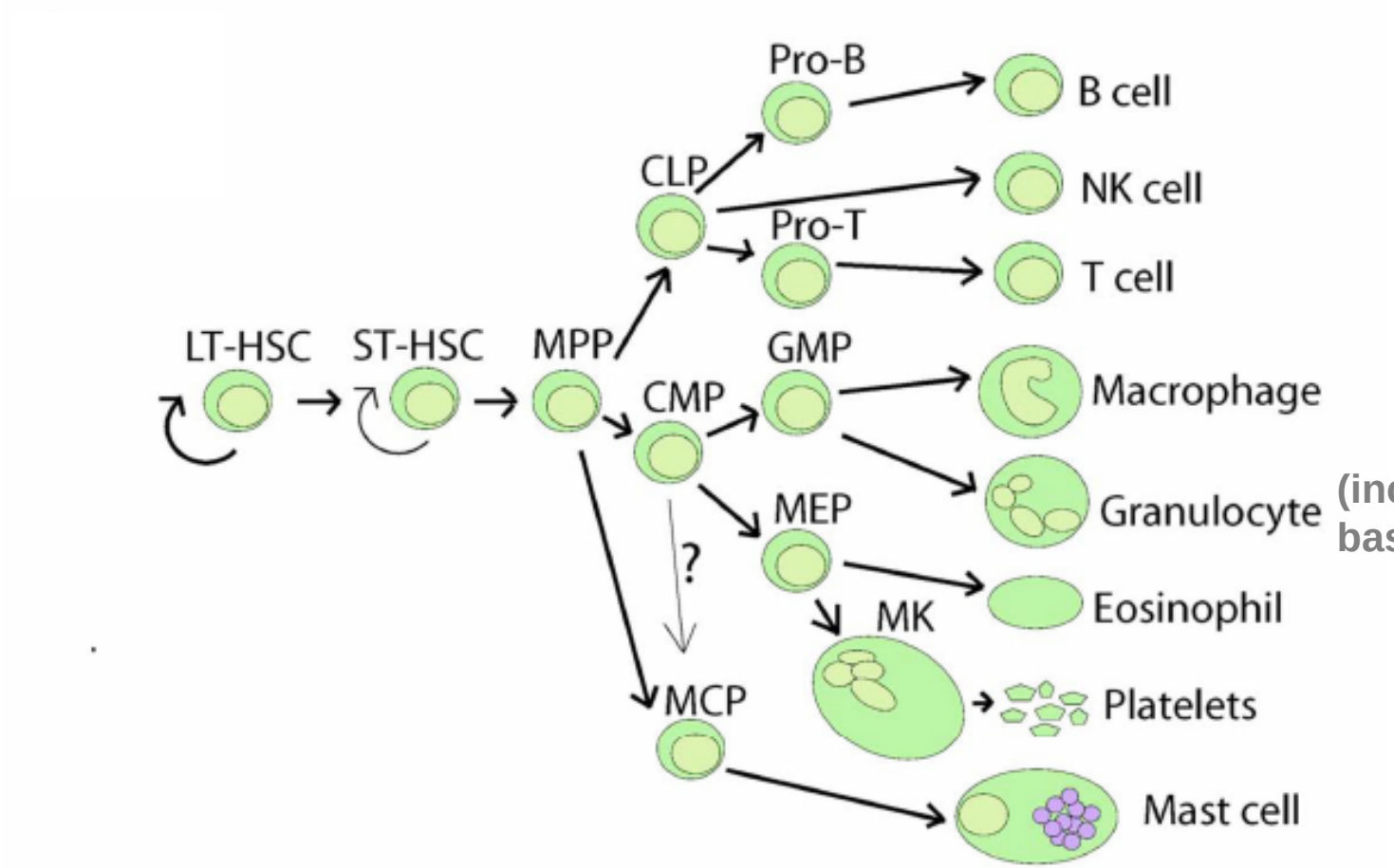


Mast cells

CCR3, CXCR1, CXCR3 and 4
TLR2, 3, 4, 5, 6, 7 and 9
***c-kit* receptor**
$CysLTR_1$ and R_2
FcγR1

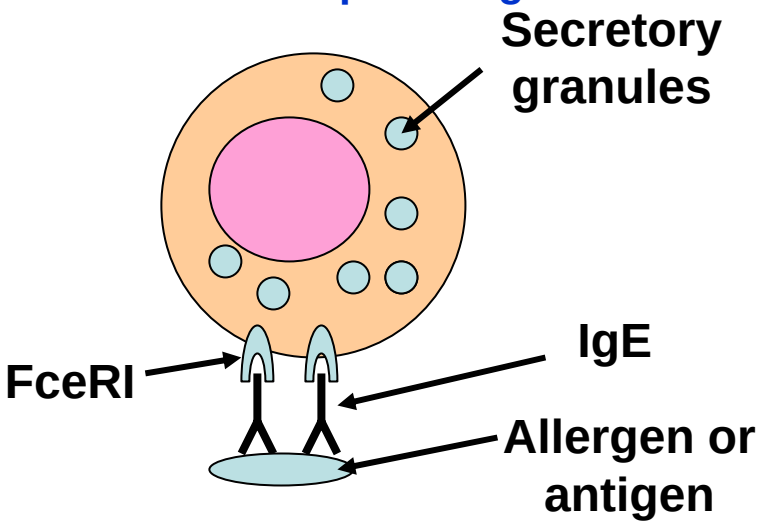
Basophils

CCR1, 2, 3, CXCR1, CXCR4, TRTH
Formyl peptide receptors
C3a and C5a
VEGF

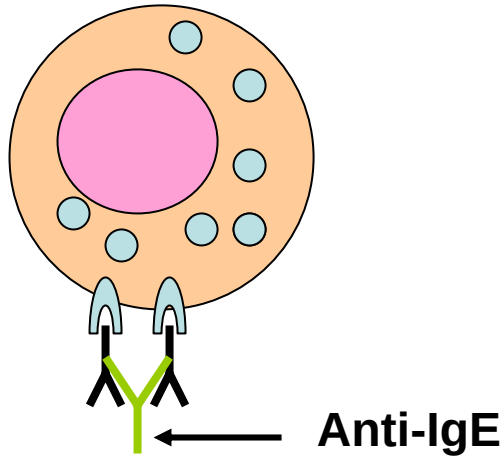

Pro-B
B cell
CLP
NK cell
Pro-T
T cell
LT-HSC
ST-HSC
MPP
GMP
CMP
Macrophage
Granulocyte
(including basophils)
MEP
?
Eosinophil
MK
MCP
Platelets
Mast cell

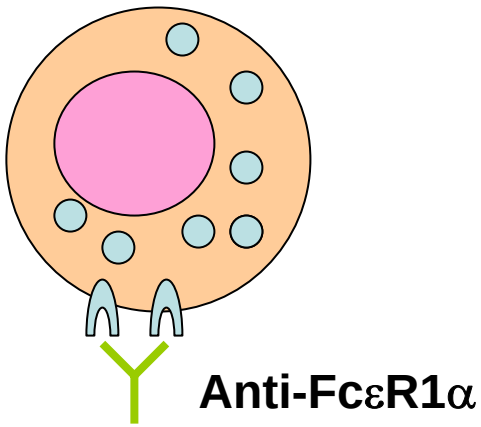
Mechanisms of Mast Cell and Basophil Degranulation

1. A multivalent antigen/allergen cross links 2 specific IgE molecule

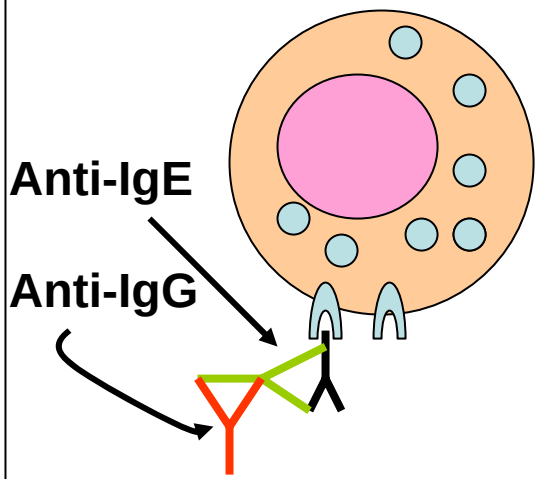


2. Anti-IgE binds to two binding sites on the Fce





3. Antibodies directed against the FceRI



4. Immune complexes composed of IgG anti-IgE and IgG (in vitro only)

Measurement of CD63 on Basophils

1. 100 μL heparinized whole blood from an appropriate basophil donor.
2. Incubate with positive and negative controls and allergen for 10 minutes.
3. Stop reaction by placing tube on ice.
4. Stain cells with:
    1. PE- anti-human CD203c
    2. PerCP-anti-human CD45
    3. FITC-anti-human IgE