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THE 62nd ANNUAL
Swineford Allergy
CONFERENCE

The Allergic Effector Unit and the Mast cells as Masterminds from Inflammation to its Resolution

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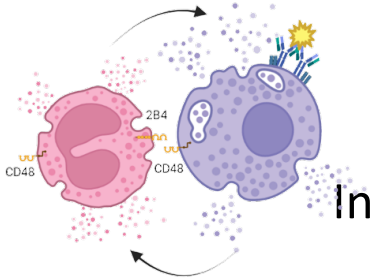

Mast Cells, Eosinophils and Allergy

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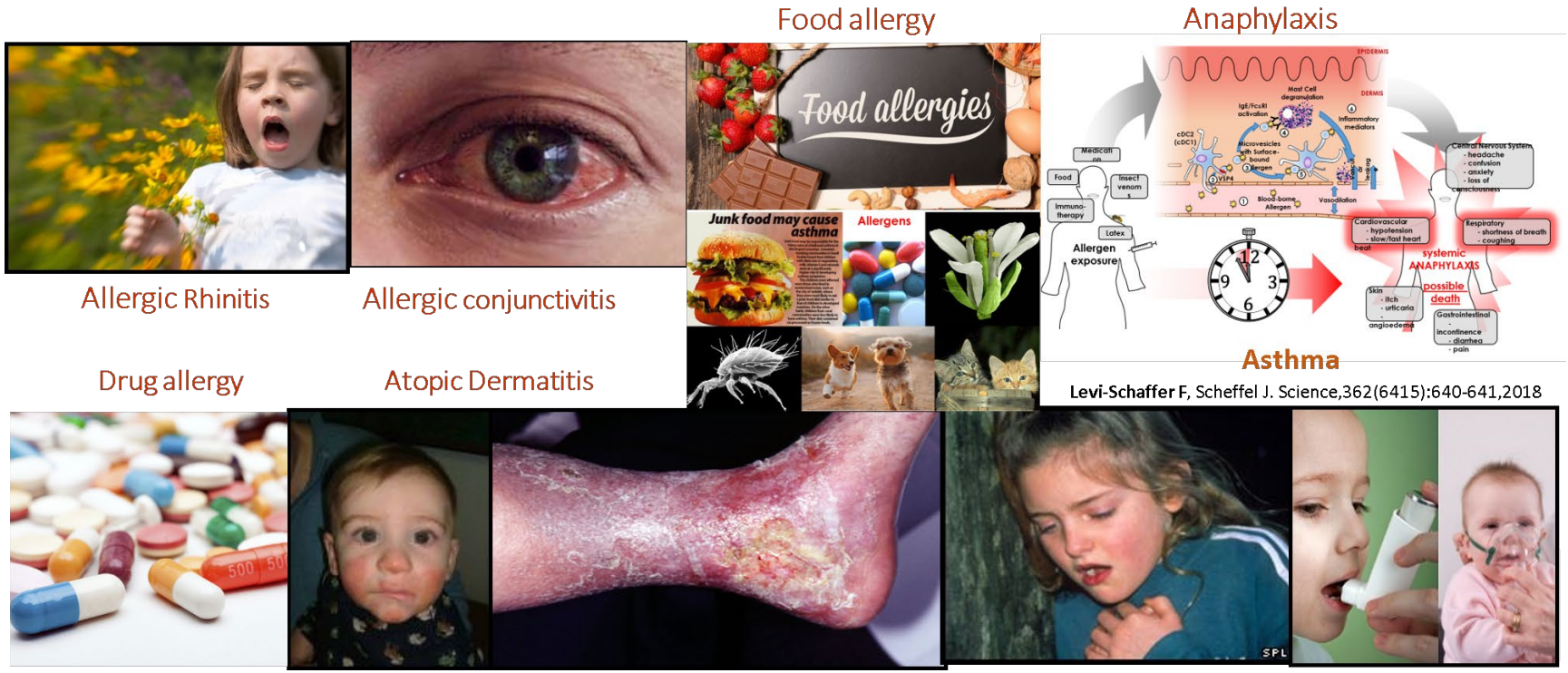
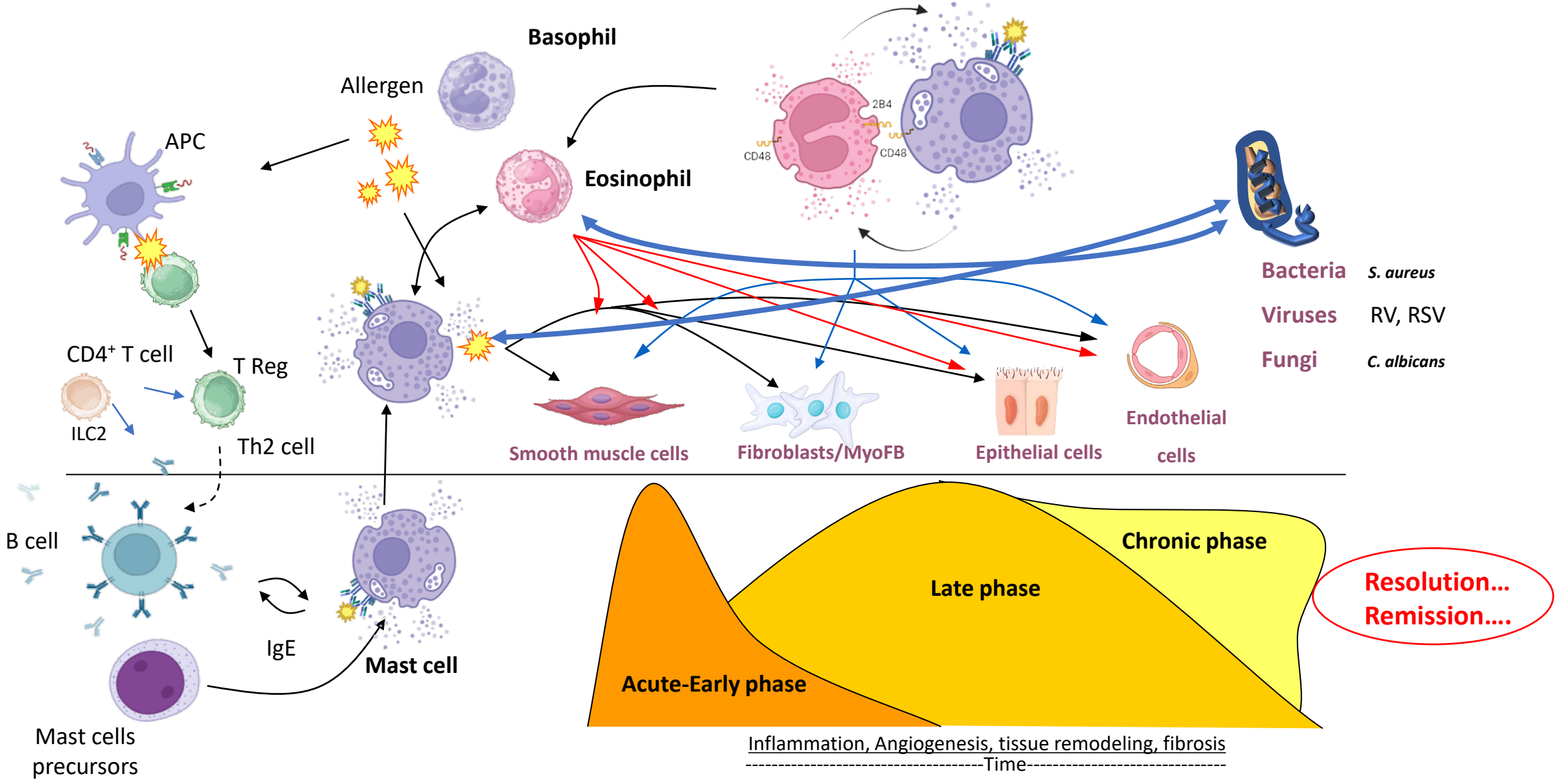
In ALLERGIC DISEASES (but also in several other diseases with different etiopathogenesis), MAST CELLS are usually associated with EOSINOPHILS.

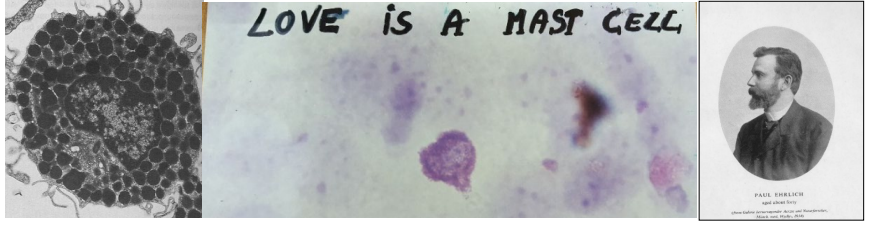
My research GOAL has long been to determine new immunopharmacological targets, principally for the treatment of allergy, focusing on mast cells, on eosinophils and on their soluble and physical cross-talk, that we have defined, i.e., the "Allergic Effector Unit" (AEU).



Our Oversimplified View of the Allergic Inflammatory Reaction

The "Allergic Effector Unit"





The Human Mast Cell

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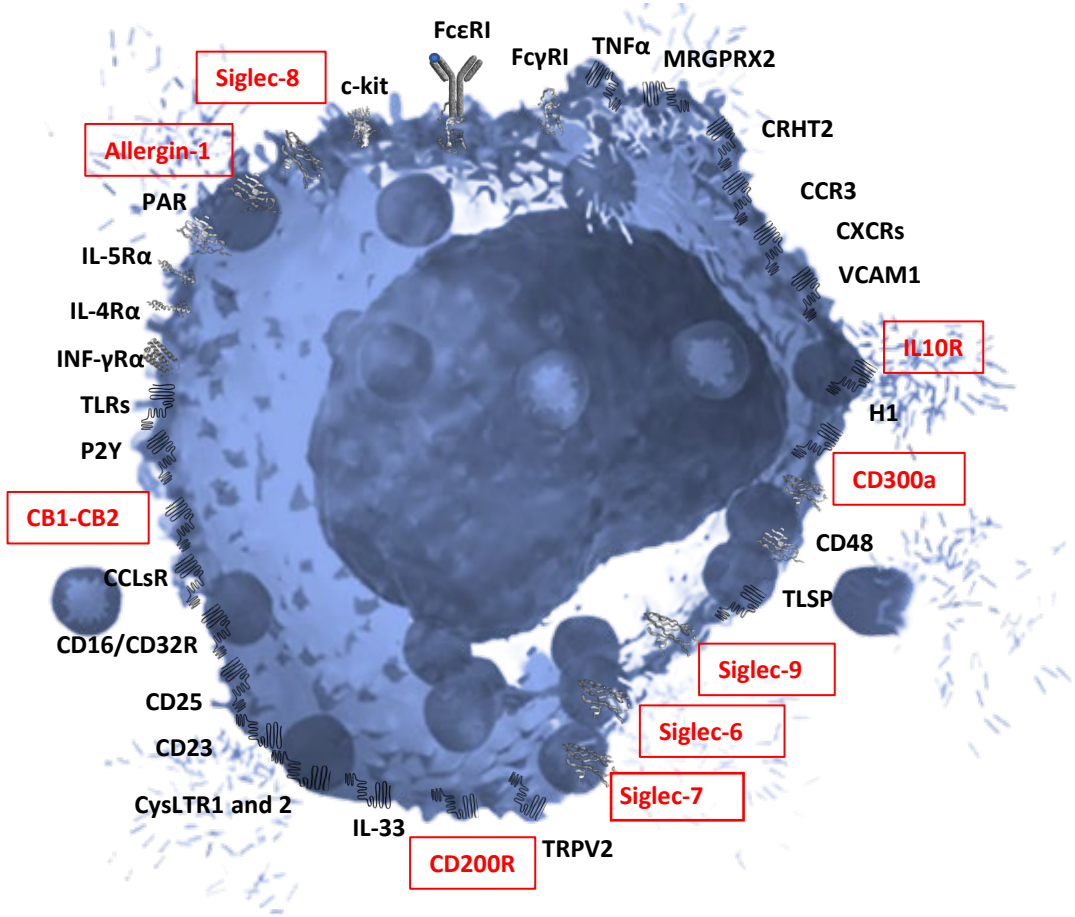


Preformed Mediators (Minutes)
- Histamine
- Proteases
- TNF-α
- Proteoglycans

Newly formed Lipid mediators (Minutes)
- LCT4
- PGD2
- LTB4
- SPMs?

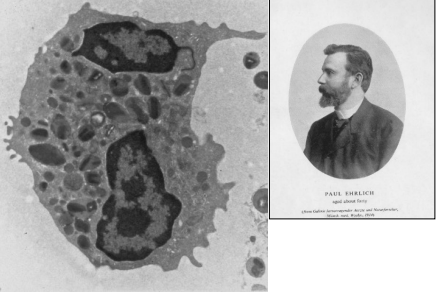
Induced Cytokines/ Chemokines (Hours)
- IL-3
- IL-4
- IL-5
- IL-6
- IL-8
- IL-9
- IL-11
- IL-13
- MIP-1α
- MIP-1β
- MCP-1
- TNF-α
- IL-25
- etc.

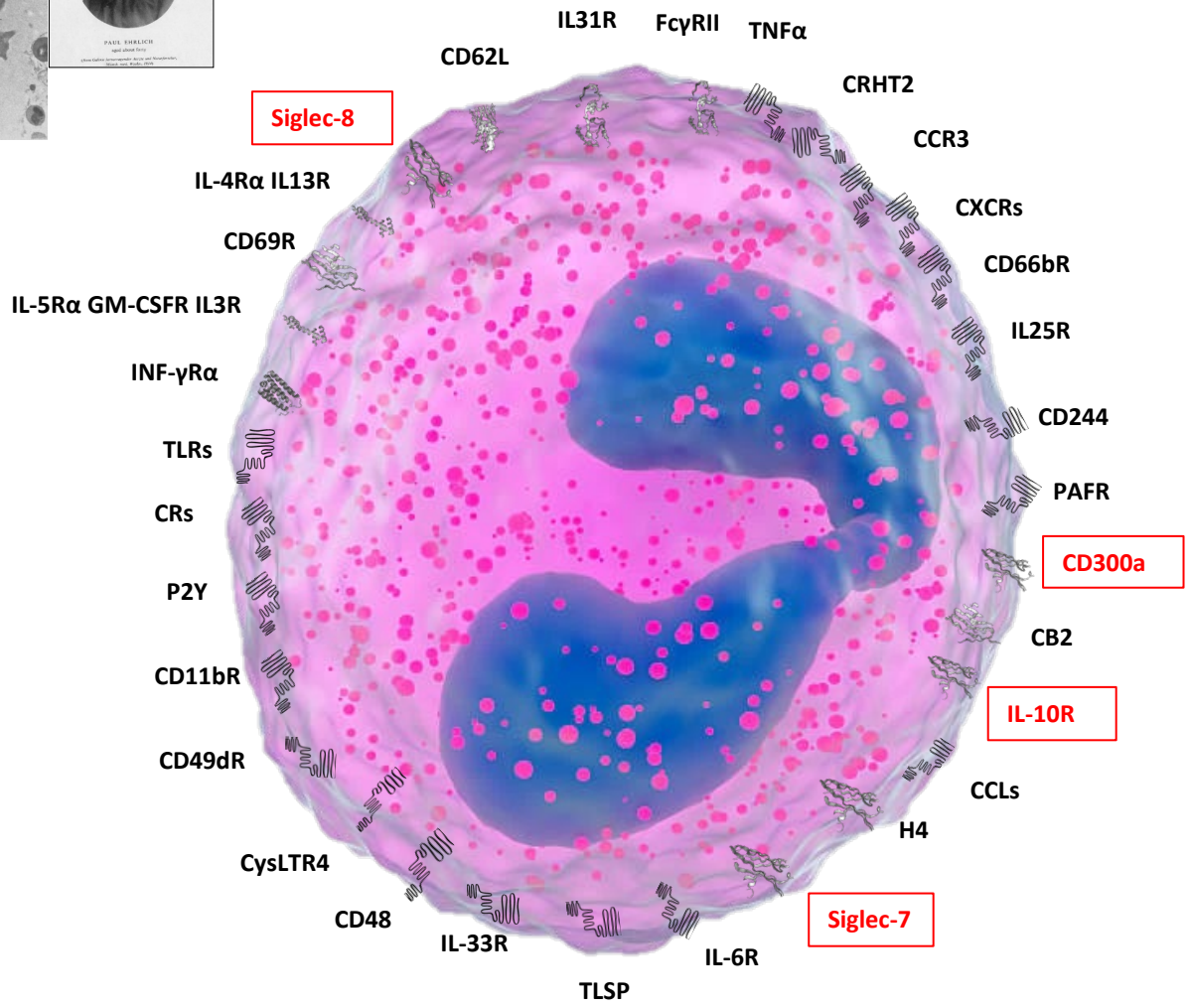
Early
Edema
Vasopermeability

Late
Inflammation
(cell recruitment)

Mast cells (MCs) are high affinity IgE-receptor bearing cells with prominent cytoplasmic granules containing histamine, specific neutral proteases, proteoglycans etc, which stain metachromatically with cationic dyes and commonly reside in tissues.

The Human Eosinophil





Preformed Mediators	MBP, ECP, EDN, EPO/EPX, CLC	Late phase/chronic phase Direct tissue damage Vasopermeability Platelets activation
Newly formed Lipid mediators	LCT4, PGE1, PGE2, LTB4, PAF	
Preformed and induced Cytokines/ Chemokines	IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-13, TNF-α, IL-25, etc.	Late phase/chronic phase Cell recruitment and continuation of inflammation ,tissue damage, fibrosis

Eosinophils (Eos) are granulocytes containing highly basic pre-formed mediators and cytokines, usually residing in the blood entering in the tissues where there is an inflammatory response. They also reside in some tissues, such as the gut, mammary glands, uterus, adipose tissue, thymus, lungs and might influence tissue homeostasis.

Same important facts on mast cells and on eosinophils

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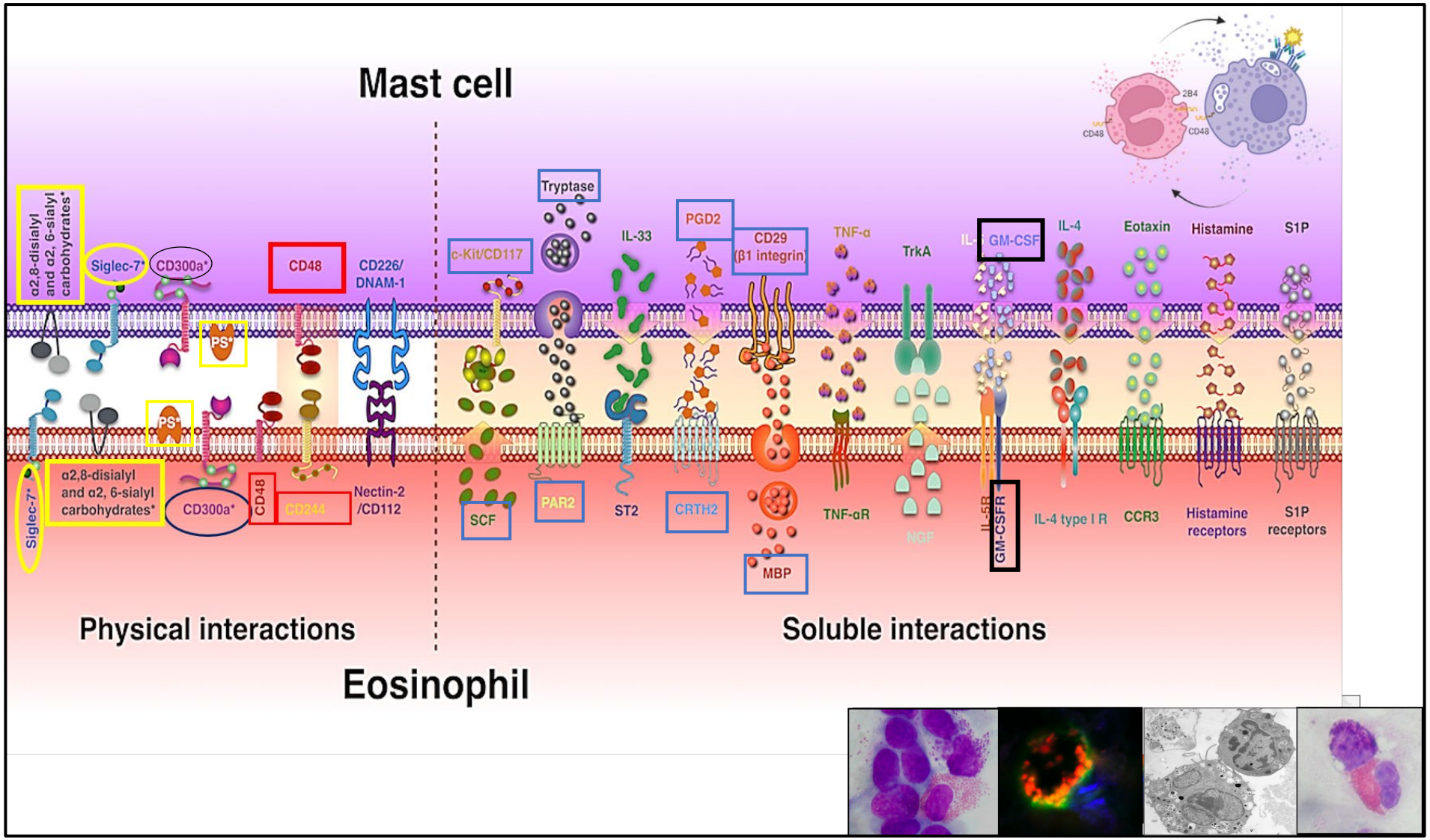
Mast cells are long lived cells that, after activation, regenerate their mediators and their potential to be re-activated Levi-Schaffer F et al. **Cell Immunol.** 1989; Levi-Schaffer F et al. **Eur J Immunol.** 1990; Levi-Schaffer F et al. **J Immunol.** 1990; Levi-Schaffer F et al. **Cell Immunol.** 1993

Eosinophils Maintain Their Capacity to Signal and Release Eosinophil Cationic Protein Upon Repetitive Stimulation with the Same Agonist Simon HU et al **J Immunol**. *2000*

Proteomic analysis of human eosinophil activation mediated by mast cells, granulocyte macrophage colony stimulating factor and tumor necrosis factor alpha

GM-CSF provided the strongest signal and the highest rate of protein synthesis (1018 spots) followed by TNF- (747 spots) and HMC-1 sonicate (611 spots). Levi-Schaffer F, et al. **Proteomics**. 2002

The Allergic Effector Unit: Mast Cells and Eosinophils as Key Players of AI



Landolina N et al., **Adv Immunol**, 2015, Gangwar RS, Landolina N et al, **Pharm Ther**, 2016
Shamri R et al., **Clin Exp Allergy**, 2018 , Puzzovio PG and Levi-Schaffer F, **J All Clin Immunol Pract**, 2021
Puzzovio PG and Levi-Schaffer F, **Front Pharmacol**, 2021, Levi-Schaffer F et al.,**JACI**, 2022, Zoabi Y et al **Biomedicines** , 2022

Therapeutic Strategies for Allergy
MC and Eos "Soluble" and Cellular Targets

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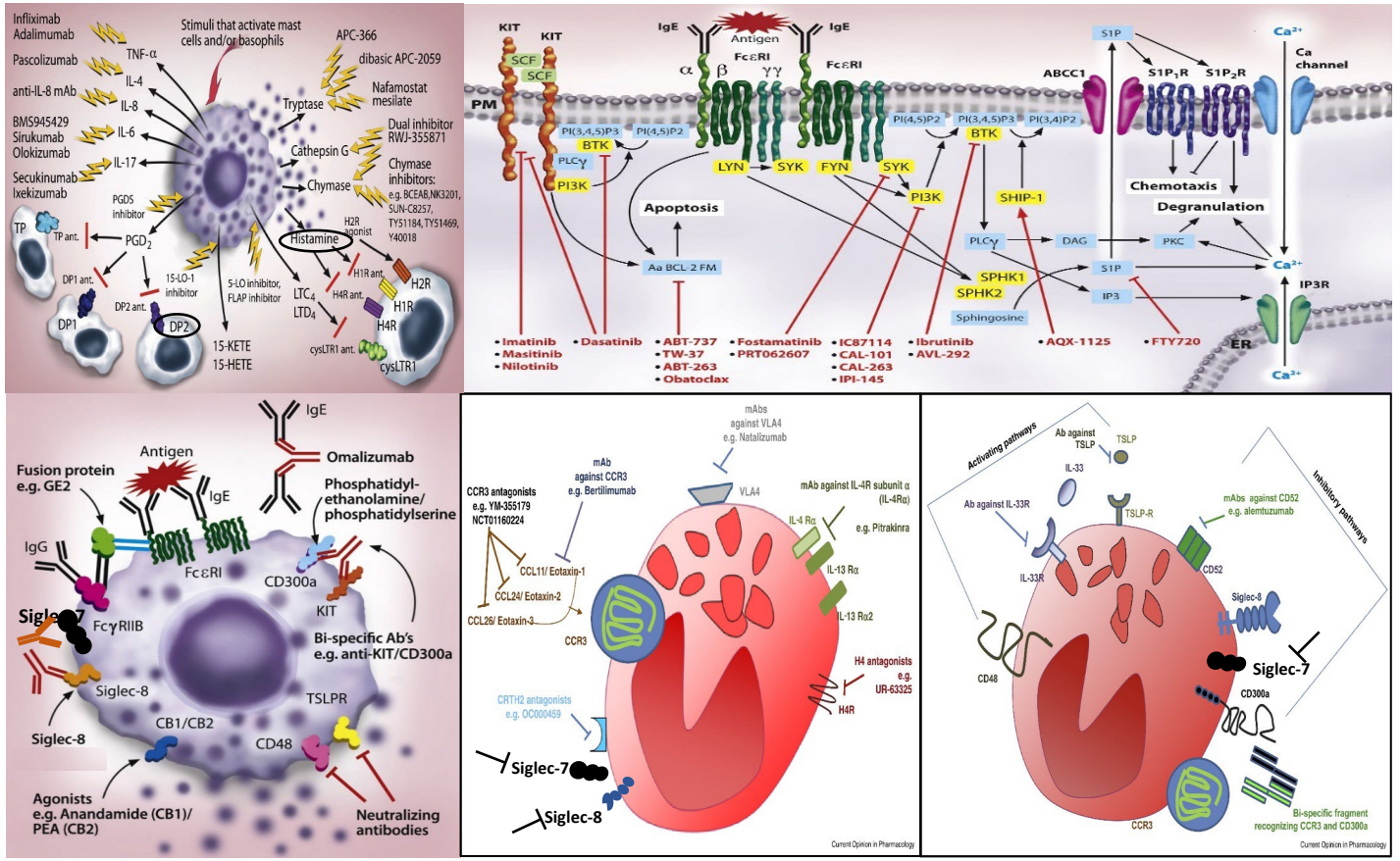
Older strategies

- Anti-cholinergic, β2 agonists, Anti-histamines (H1)
- MC stabilizing drugs: Cromones, Puzzovio PG et al., Pharmacol. Res., 2022
- Anti-inflammatory drugs:
- Glucocorticosteroids, Anti-leukotrienes



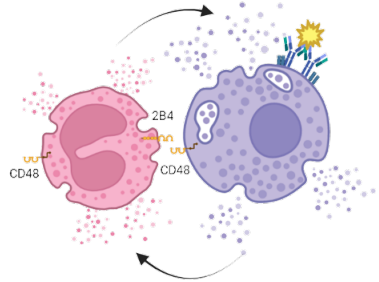
Newer strategies

- Anti-IgE mAb (omalizumab, ligelizumab)
- Anti-TSLP mAb
- Anti-IL-5/IL-5Rα (mepolizumab, reslizumab, benralizumab)
- Anti-IL-4Rα (dupilumab)
- Anti-TNFα (adalimumab, infliximab)
- Anti-IL-13 (lebrikizumab, tralokinumab)
- Anti-Siglec 8 (lirentelimab)
- KIT inhibitors (imatinib, avapritinib, masitinib, fenebrutinib, midostaurin)
- Anti-KIT mAbs(barzolvolimab)
- Jak inhibitors (abrocitinib, ruxolitinib, upadacitinib, delgocitinib)

Harvima IT et al, **J Allergy Clin Immunol,** 2014; Landolina N et al., **Curr Opin Pharm**, 2014 ; Bulfone-Paus S et al**, Trends Immunol**, 2017 ;Gangwar RS et al, **Pharmacol Ther**, 2017; Puzzovio P and Levi-Schaffer F, **Comprehensive Pharmacology,** 2020

Puzzovio PG and Levi-Schaffer F**, Frontiers in Pharmacology** 2021; Puzzovio P et al., **J Allergy and Clin Immunol in practice**,2022; Levi-Schaffer F. et al, **J Allergy Clin Immunol,** 2022; Zoabi Y et al, **Biomedicines** , 2022; Metz M et al, **Allergy**,2023; Metz et al. **Mast cell silencing:** A novel therapeutic approach for urticaria and other mast cell-mediated diseases. **Allergy** 2023

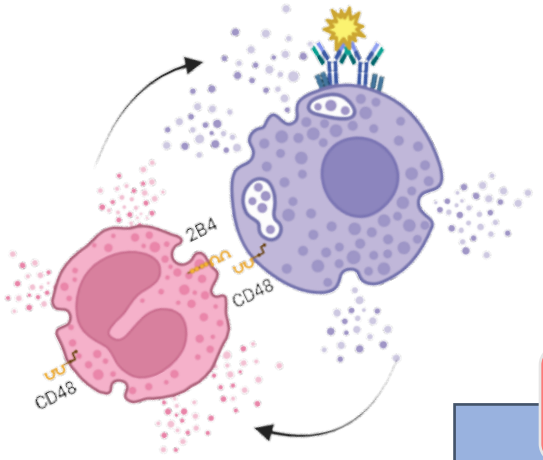
The Allergic Effector unit (AEU): always the Ugly and the Bad?



FLS: the AEU

Dynamics of AEU

Main Points Of The Lecture



- Pro-inflammatory AEU soluble interactions : GM-CSF
- Pro-inflammatory AEU physical interactions : CD48
- Pro-resolution AEU: CD300a, RvD1

What is the best target for therapy?

AEU soluble interactions: Human skin mast cells-derived GM-CSF is the main mediator responsible for eosinophil activation and survival

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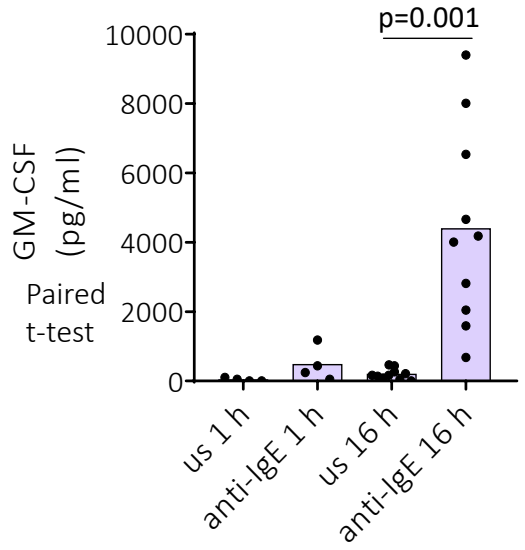
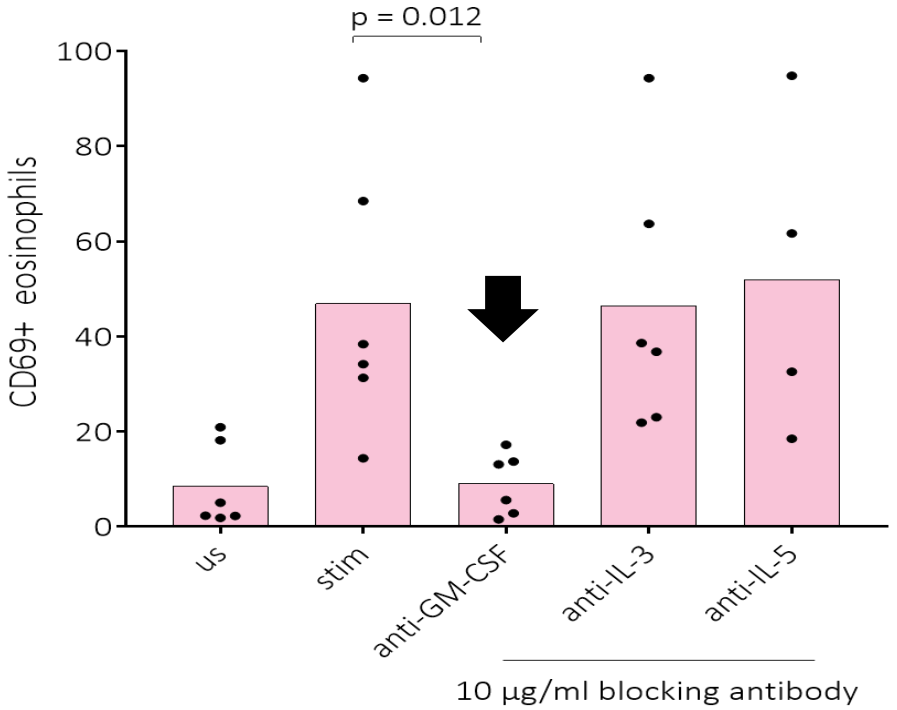
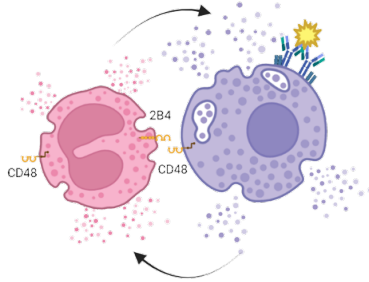
Human skin mast cells activated by an IgE-dependent mechanism release GM-CSF

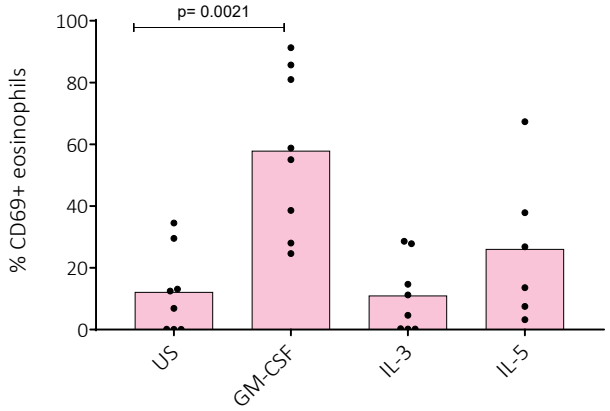


The AEU soluble interactions : Supernatants from FcεRI-activated human skin mast cells stimulate eosinophils via GM-CSF (1 h)

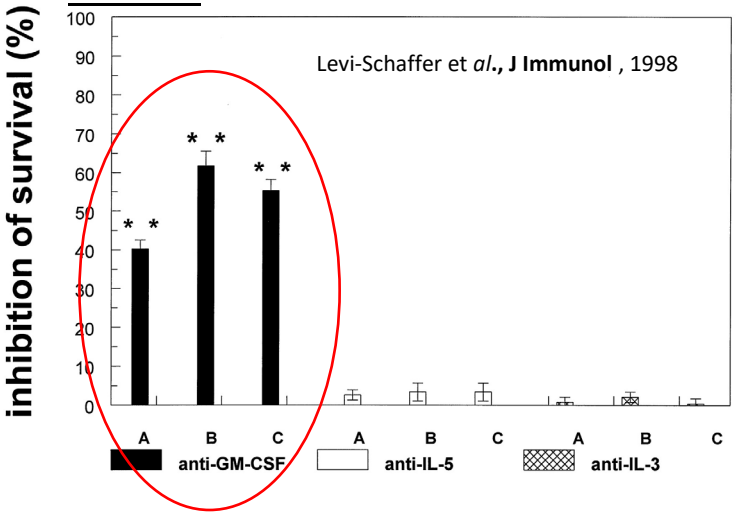




GM-CSF activates human eosinophil

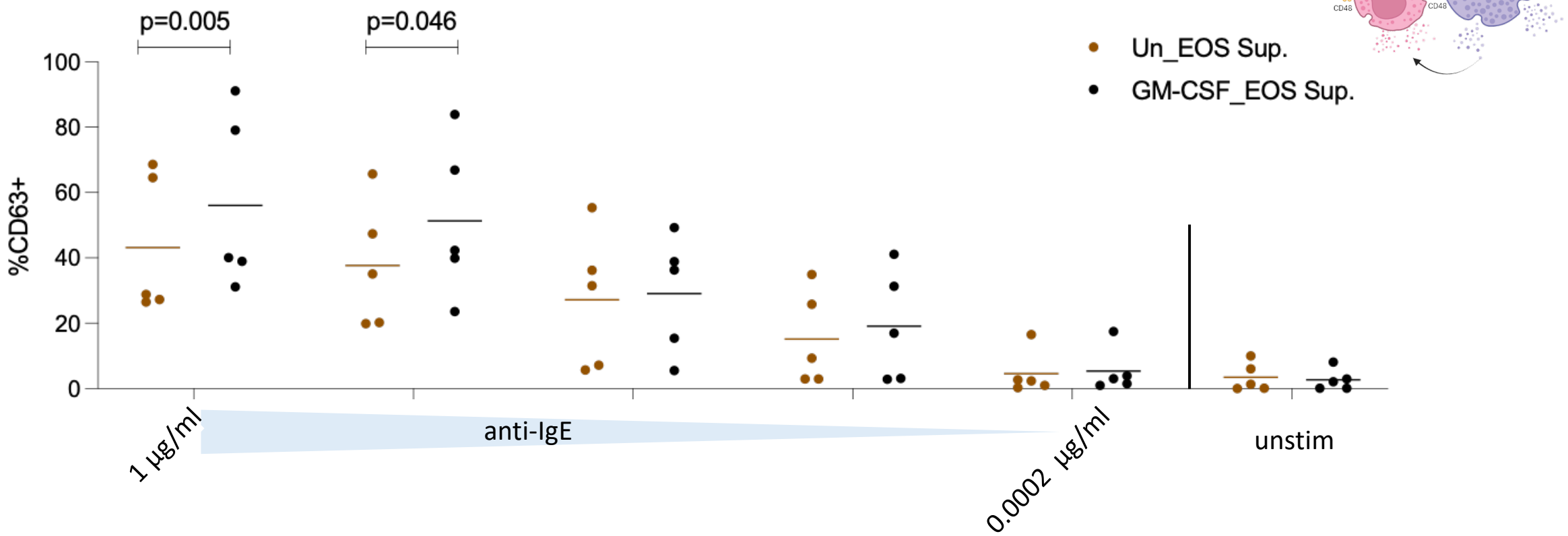


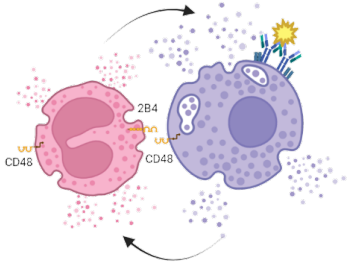
Anti-GM-CSF reduces human eosinophil survival



Luo, Levi-Schaffer, Maurer, Frischbutter manuscript in preparation

AEU soluble interactions: Supernatants from GM-CSF-treated eosinophils amplify human skin mast cell IgE- dependent activation





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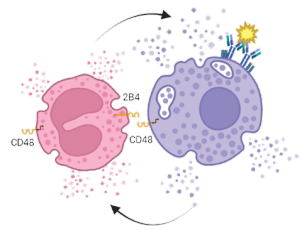

Our Main Immunomodulatory Strategies

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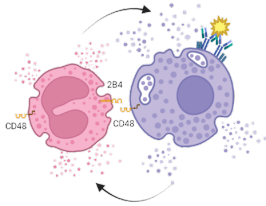
We aim to target receptors that are shared by MCs and Eos
to downregulate their functional activity

Activating receptors:
CD48 (MC and EOS);
Relevant Cytokines: GM-CSF

Our strategy consists in blocking these activating receptors/cytokines.

Inhibitory receptors:
CD300a (MC and EOS)
Siglec-7 (MC and EOS)

Our strategy consists in activating these inhibitory receptors by agonistic monoclonal antibodies.



The CD2 family: CD48 and 2B4 (CD244) role in the AEU physical interactions

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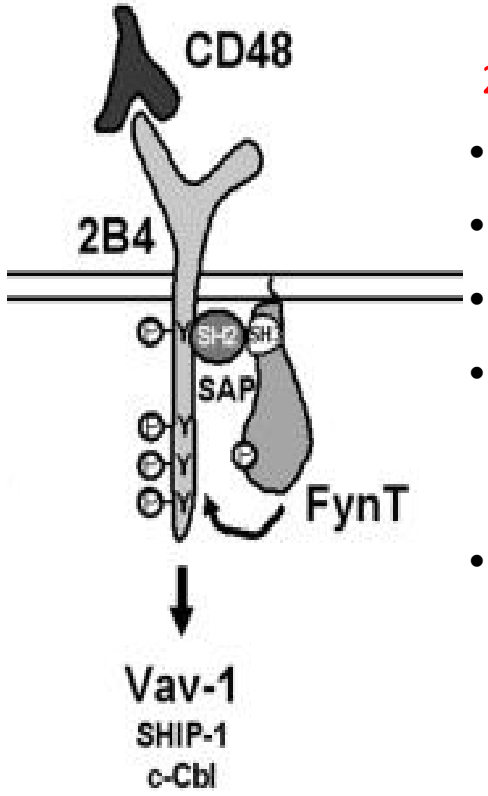
CD48

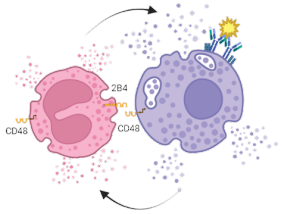
- GPI (glycosylphosphatidylinositol)
- Soluble form
- Membrane bound form on leukocytes
- Co-activating and activating receptor
- High affinity ligand for 2B4



2B4

- SLAM related
- 4 ITSM
- High affinity ligand for CD48
- NK and eosinophils activating receptor. Not expressed on human MCs
- On mouse MCs and NKs it can behave as an inhibitory receptor

The human AEU physical interactions



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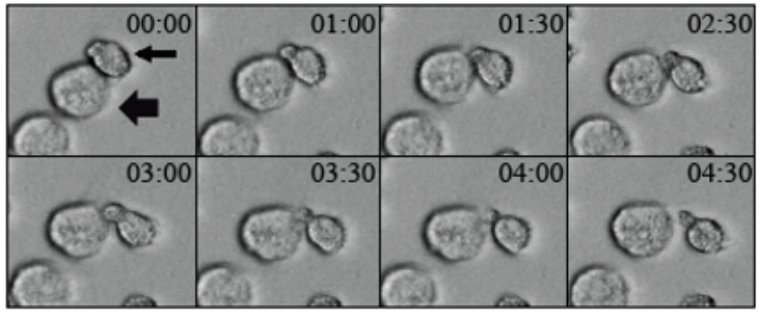
Eosinophils are quickly attracted by mast cells to form “synapse-like” couples

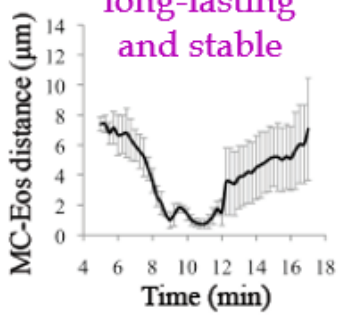
Cord blood derived mast cells (CBMC) + Peripheral blood eosinophils (pbEos)

Physical interactions of CBMC-pbEos in short-term co-cultures:

CBMCs and pbEos interact in short co-cultures (time-lapse photo-micrographs)

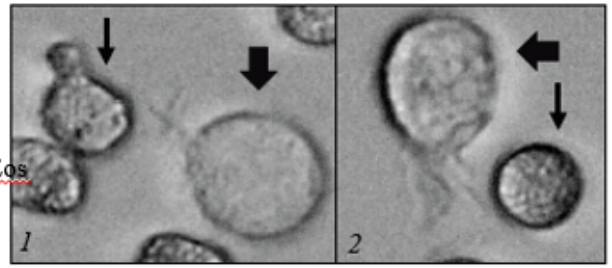


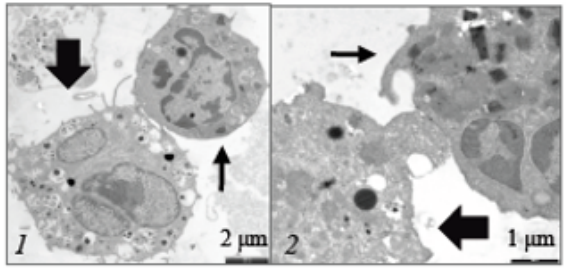
MC-Eos interactions are long-lasting and stable



Binding dynamics (quantifying MC–Eos membrane distances)

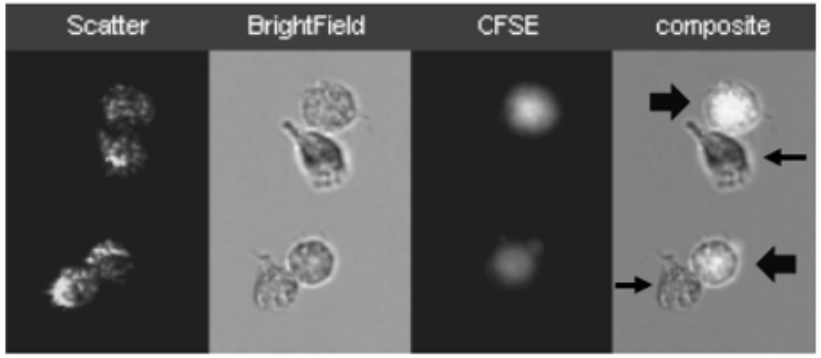
CBMCs send pseudopodia to pbEos

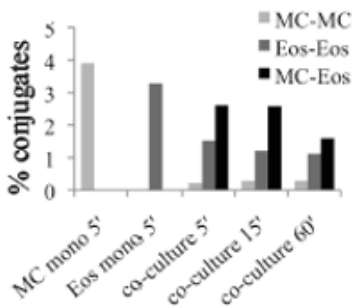




TEM in MC-Eos cc (1h) MC with lipid bodies, Eos with vacuoles

Multispectral imaging flow cytometry of Eos interactions with CFSE-labeled MCs

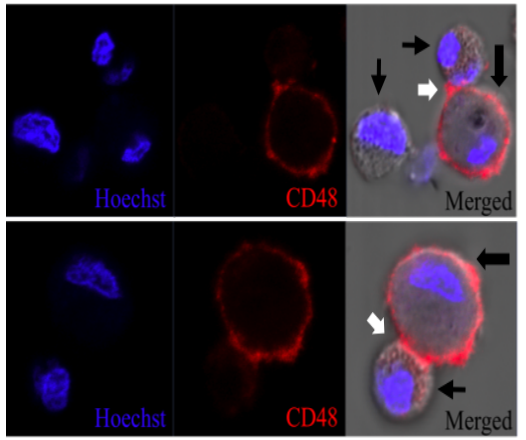


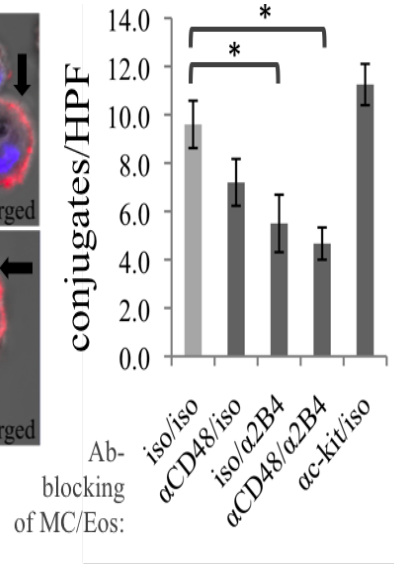


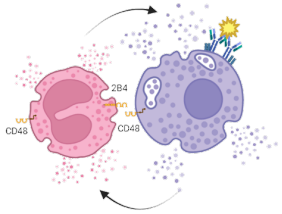
Binding rates in MC-Eos cc (5’- 1h)

Elishmereni M et al, Allergy 2011

Physical contact involves MC-CD48 and EOS-2B4







The human AEU physical interactions

CD48-2B4 interaction between the two cells is one of the underlying mechanisms for the physical contact induced Eos viability that is also carried out by soluble interactions

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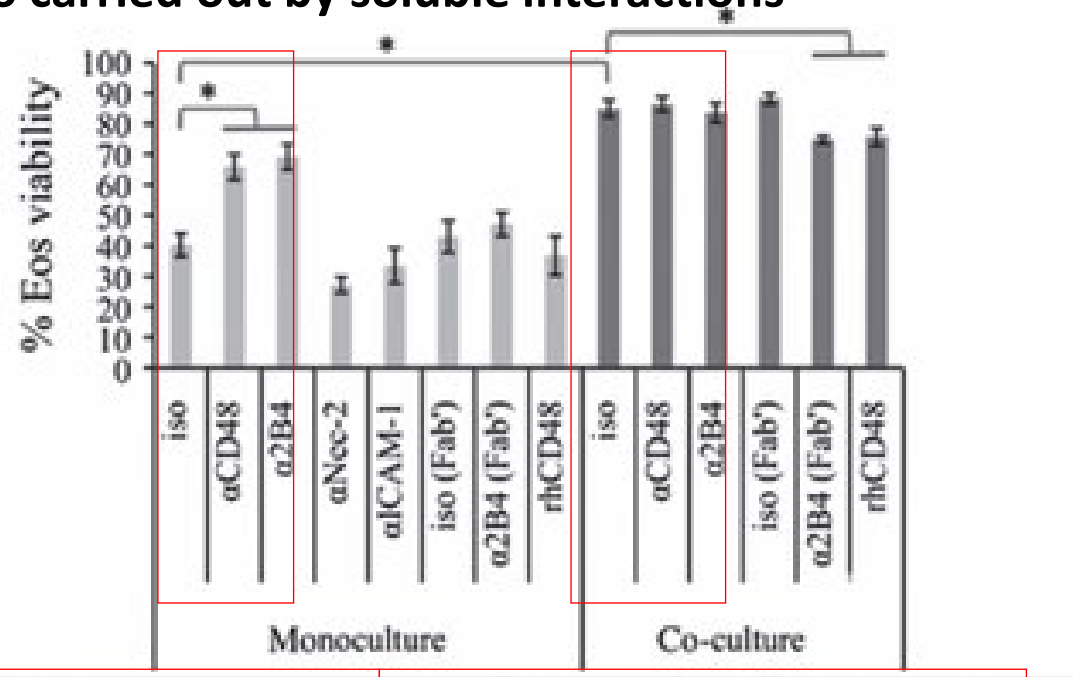
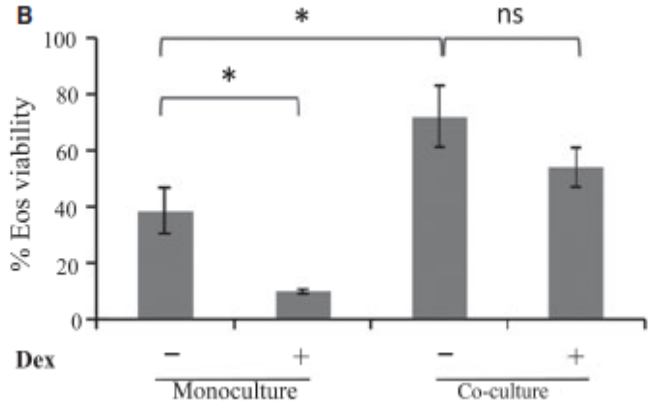
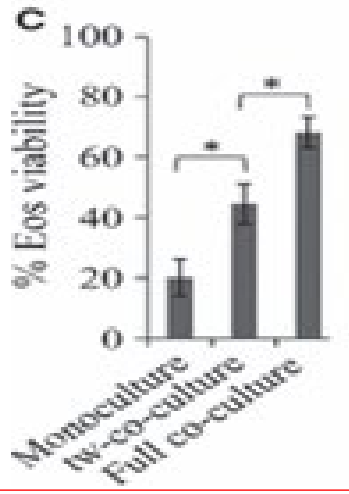
- MCs increase Eos survival.
  The effect requires both **soluble** and **physical** communication.
- **GM-CSF** is critical for the soluble effect, but is overridden by the physical contact.
- It involves **2B4-CD48** interactions.
- .Eos increased slightly but significantly MC survival.

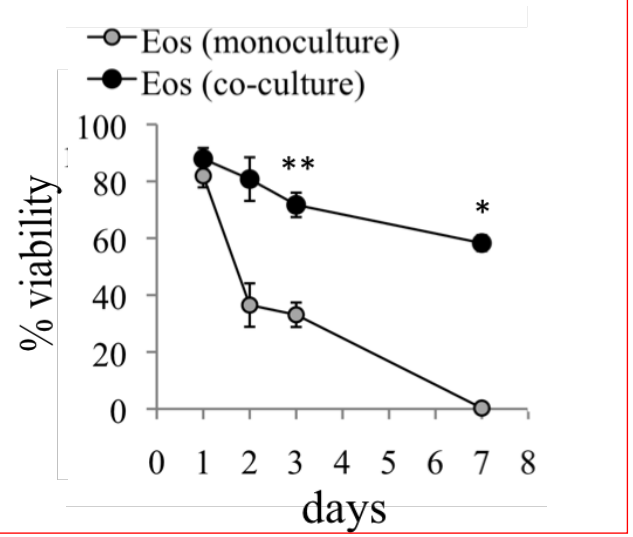


Dexamethasone does not inhibit MCs induced Eos survival

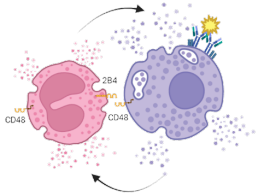


24 hr



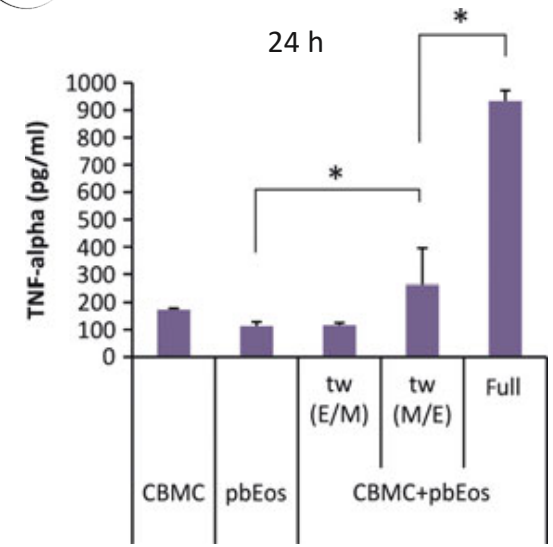


Elishmereni M et al, **Allergy**, 2011

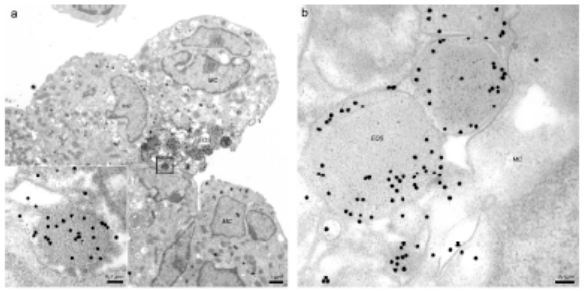


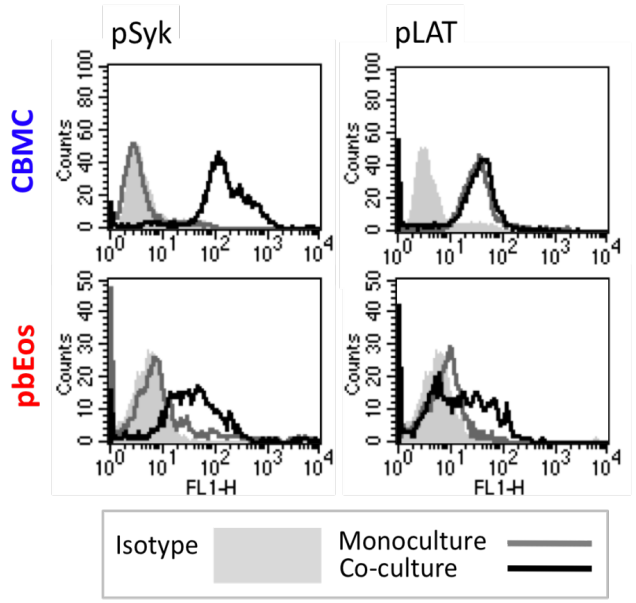
The Human AEU Physical interactions

Cell activation: MCs and Eos

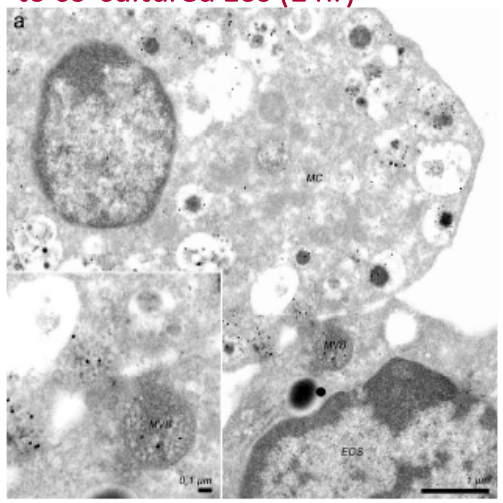


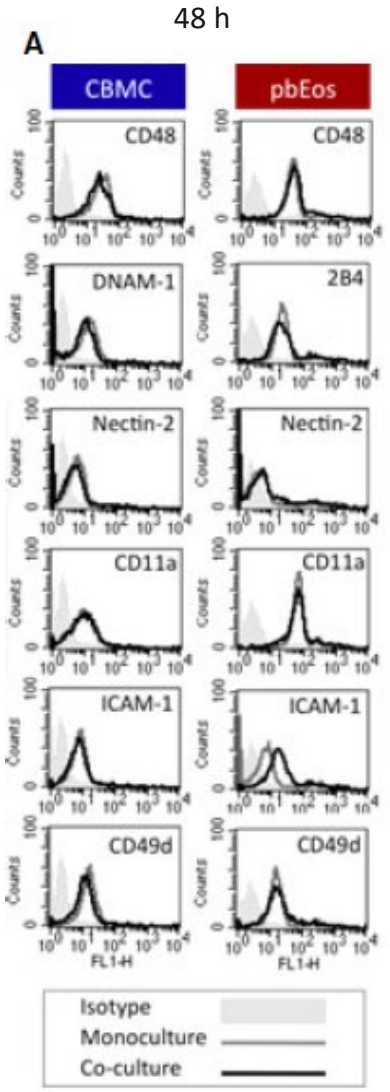
EPO is transferred from Eos to cocultured MCs (1 hr)

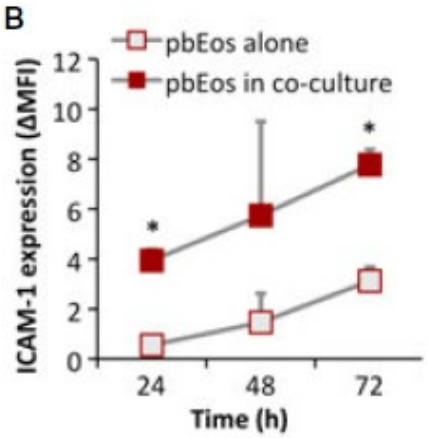


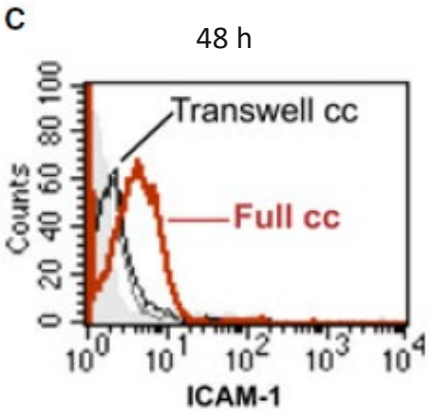


Tryptase is translocated from MC to co-cultured Eos (1 hr)

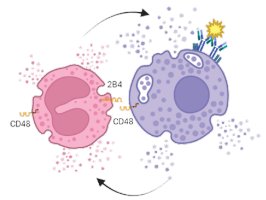








Elishmereni M and Levi-Schaffer F, **Int J Biochem Cell Biol**, 2010;
Minai-Fleminger Y et al., **Cell Tissue Res**, 2010;
Elishmereni M et al., **Allergy**, 2011;
Elishmereni M et al., **Allergy**, 2013;
Elishmereni M et al, **JID**, 2014.



The Human AEU

Cell activation: MCs and Eos

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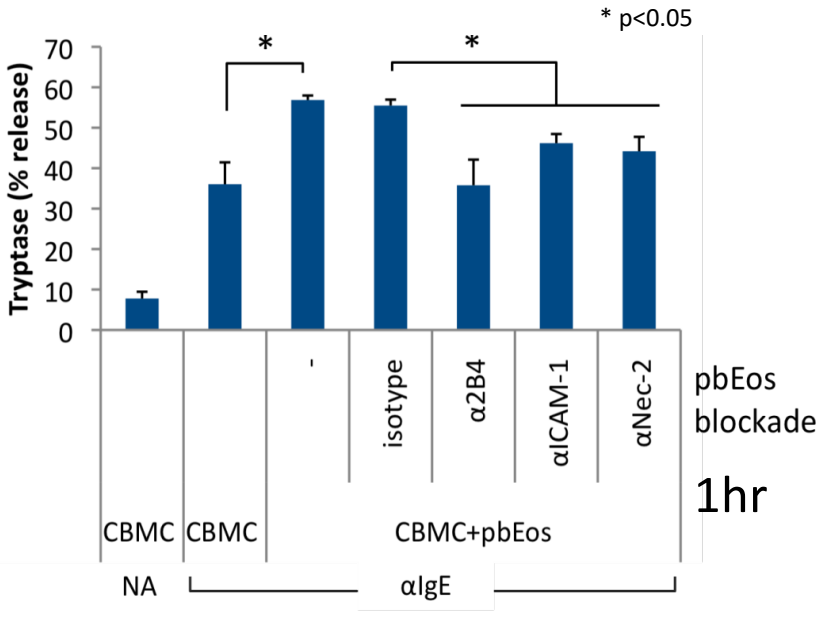
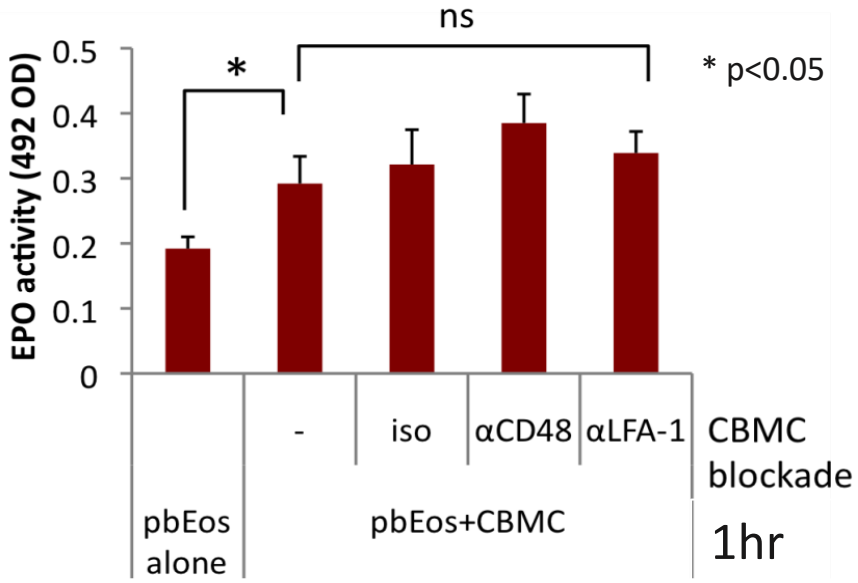
MCs activation is induced by Eos via CD48/2B4

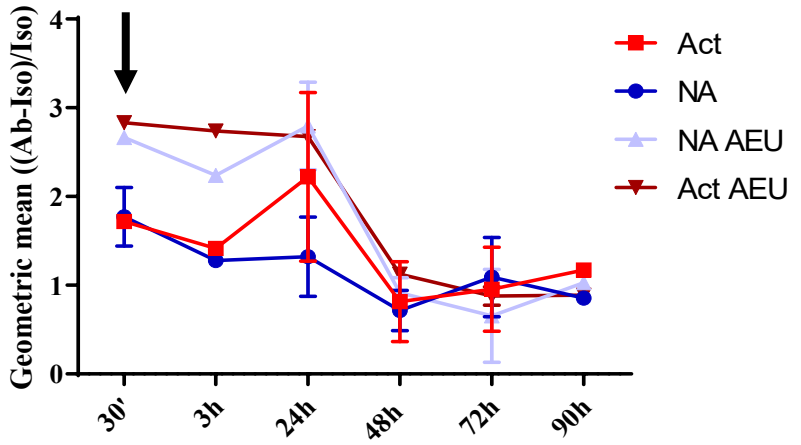
Eos activation is induced by MCs but CD48/2B4 is not involved.
It happens also when cells are not in physical contact

Ab-neutralization of CD48 / 2B4 Inhibits MC activation in co-culture

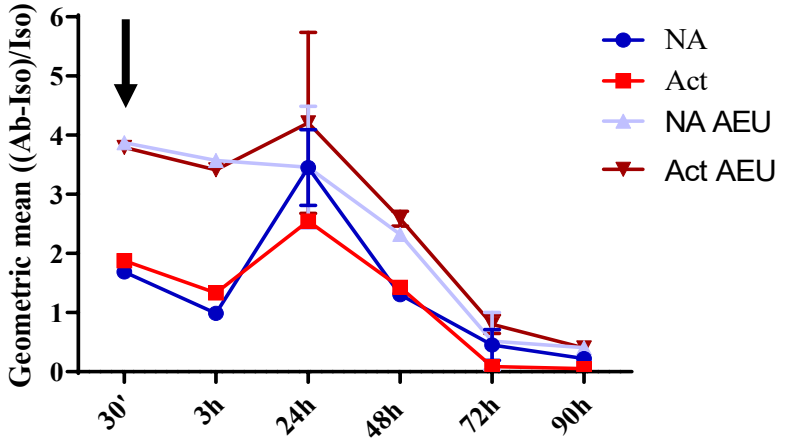




CD48 MC



CD48 Eos

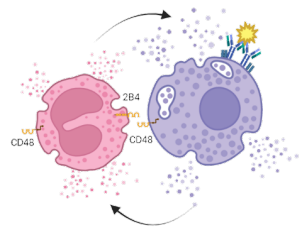


+DHA

*MCs soluble/released specific mediators such as PGD2 and tryptase and other non-MCs specific ones such as **GM-CSF** and TNF-α are probably more prominent than the physical contact in inducing Eos EPO/EPX release.*

Minai-Fleminger et al, **Cell and Tissue Research** 2010; Elishmereni M et al, **Allergy** 2013

George T and Levi-Schaffer F **unpublished**



Influence of IgE-dependent mast cell activation in the human AEU: is resolution of the pro-inflammatory AEU possible?

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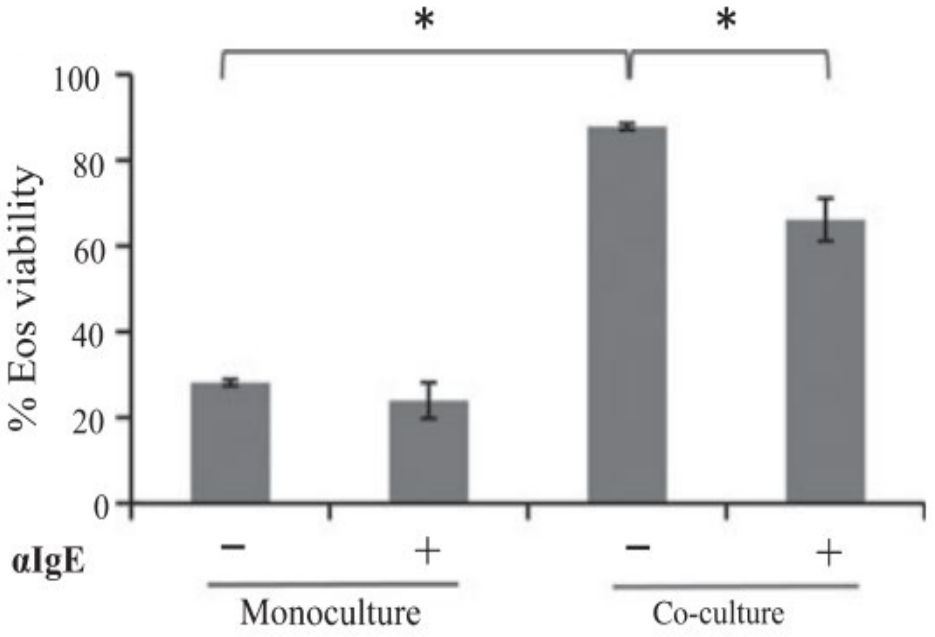
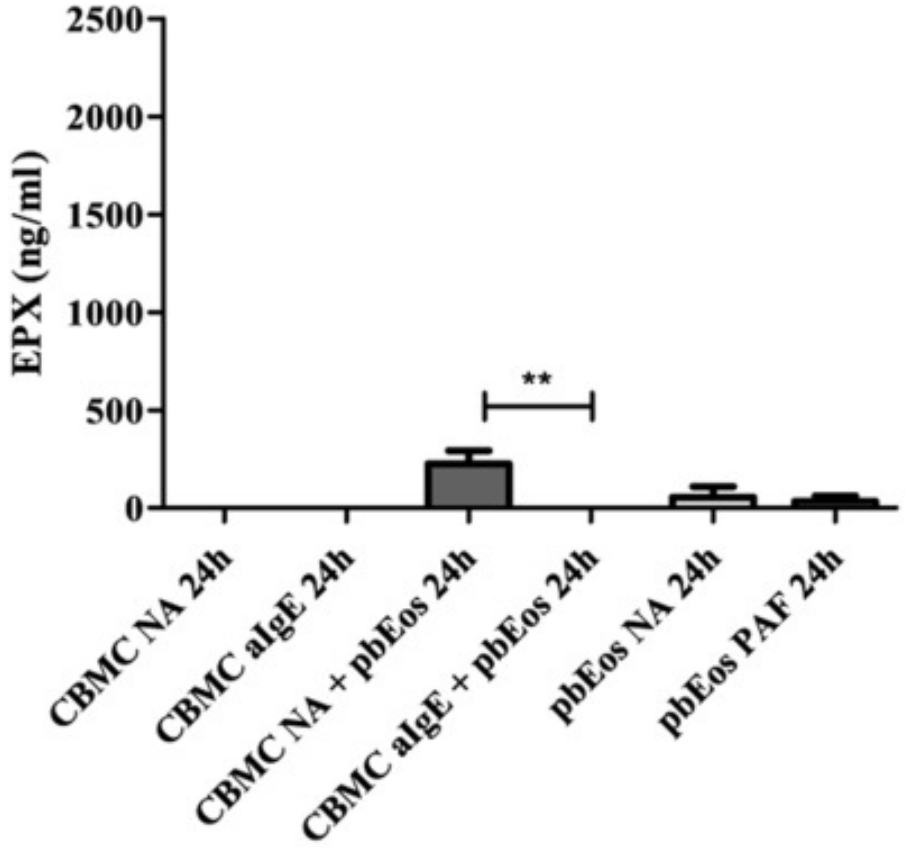


72 hr



24 hr

Elishmereni M et al., **Allergy**, 2011; Puzzovio PG, Hadas P, George T et al, **Pharmacol Res**, 2023

CD48 as Target for Anti-Allergy/Anti-Inflammation Intervention by "Inhibiting Activation"

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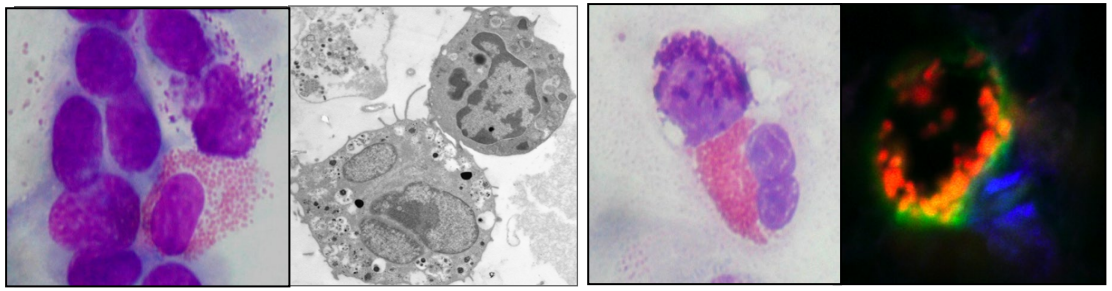
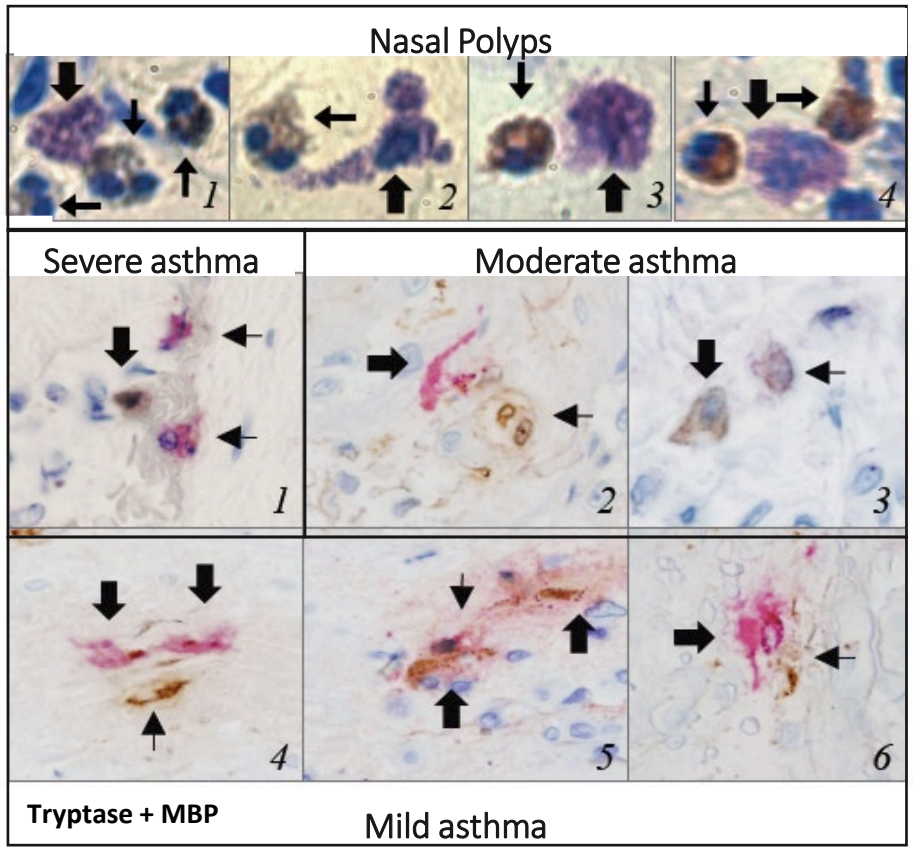
- CD48 is one of the 291 mouse asthma signature-genes *(*Zimmerman N et al., **J Clin Invest,** 2003).
- Allergic lung inflammation is inhibited in mice treated with anti-CD48 blocking Abs. 2B4 is an activating receptor on Eos **(**Munitz A et al., **J Immunol,** 2005 and **Am J Respir Crit Care Med,** 2007).
- MC-CD48 is important in the pro-inflammatory AEU as ligand of Eos-2B4 (Elishmereni M et al., **Allergy,** 2011 and J **Invest Dermatol,** 2014).
- The severity of AD in 2B4KO mice is reduced (Minai-Fleminger Y et al., **Clin Exp allergy,** 2014; Elishmereni M et al., **J Invest Dermatol,** 2014).
- **Both MCs and Eos express CD48, a main player of their interaction with *S. aureus* (Rocha-de-Souza C. M. et al., Infect Immun, 2008; Minai-Fleminger Y et al., Clin Exp allergy, 2014; Gangwar RS and Levi-Schaffer F, Allergy, 2016).**
- Eos-associated CD48 is modulated by cell activation and gives rise to soluble CD48 (sCD48). sCD48 is a decoy receptor (in vitro and in vivo) (Gangwar RS and Levi-Schaffer F, **Allergy,** 2016).
- Human asthma: mCD48 and sCD48 are potential new biomarkers for the disease (Gangwar RS et al., **Allergy,** 2017).
- Is CD48 a biomarker for airway inflammation and non-allergic asthma? (Breuer O et al., **J Immunol Res,** 2018).
- CD48 expression on nasal polyps eosinophils is a biomarker for non-allergic asthmatics (Zoabi Y. et al, **Int Arch allergy Immunol**,2021)
- CD48 expression on nasal polyps eosinophils is a biomarker for non-allergic asthmatics (Zoabi Y. et al, **Int Arch allergy Immunol**,2021)
- COVID-19 patients present with upregulation of CD48 expression on leukocytes (Pahima H et al., **Ann Allergy Asthma Immunol,** 2022)

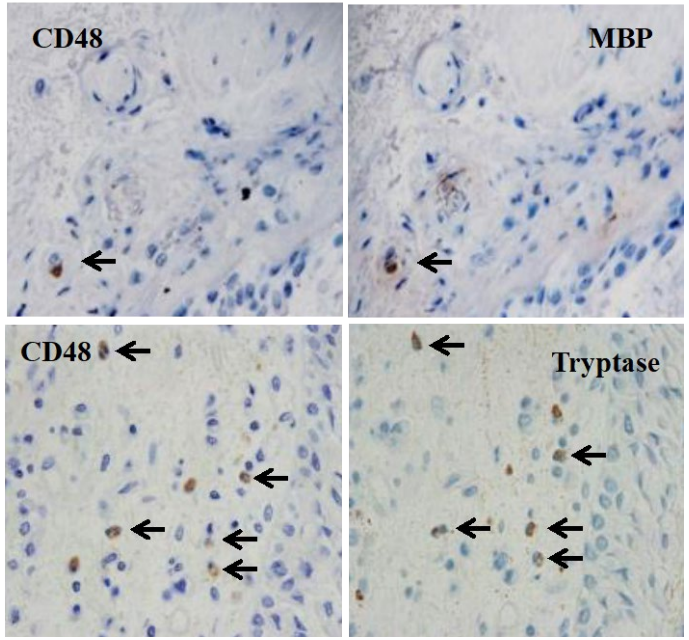
The Human AEU and CD48 in asthma, nasal polyposis, and atopic dermatitis



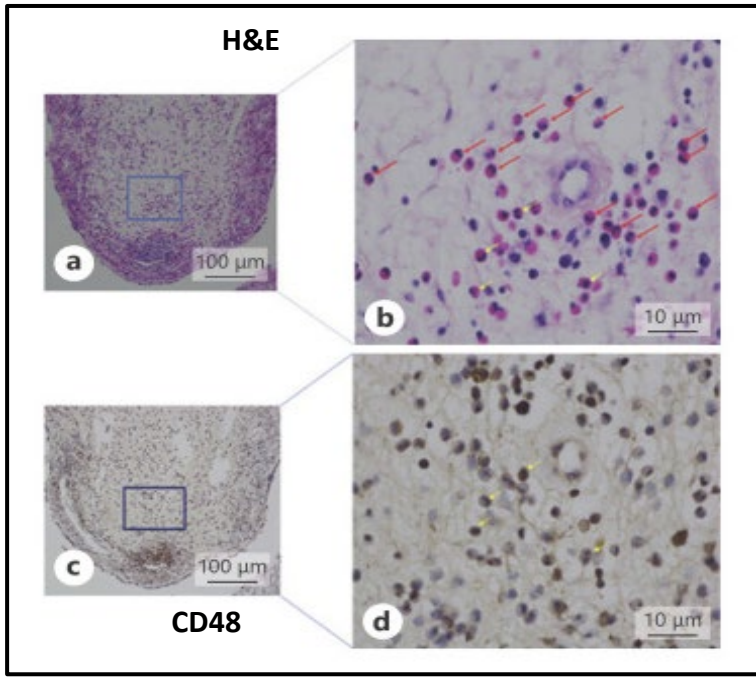
Congo-red + Toluidine Blue



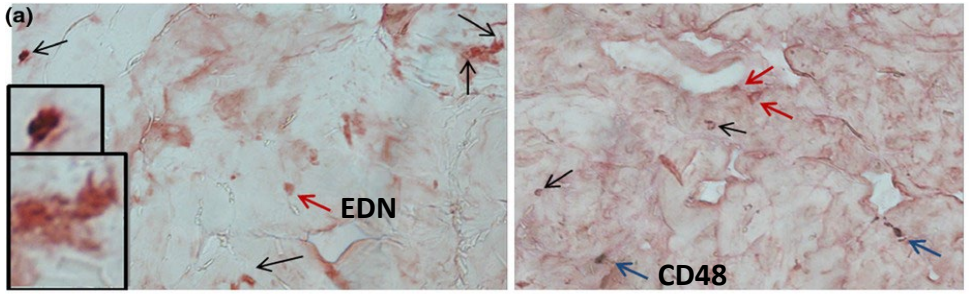
Asthma



Nasal Polyps

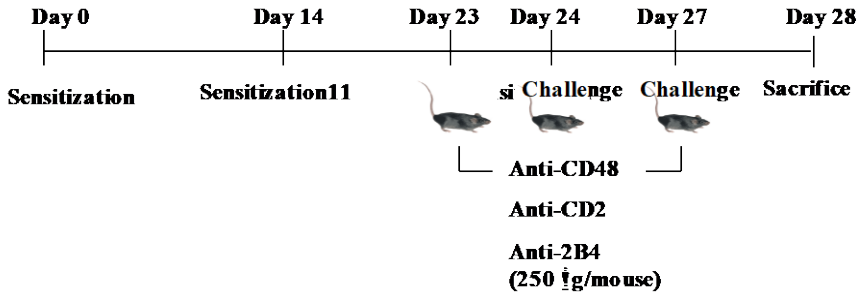


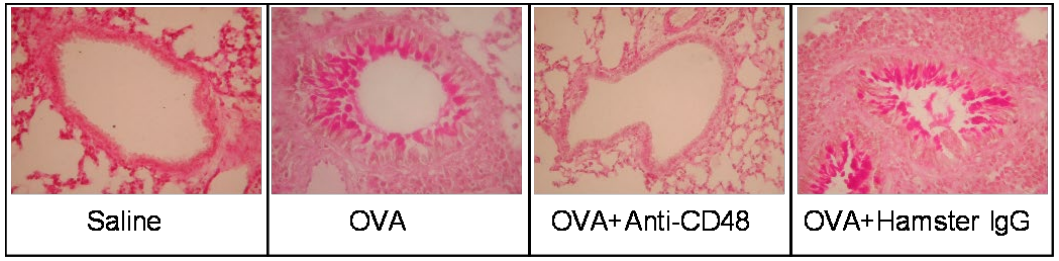
Atopic Dermatitis

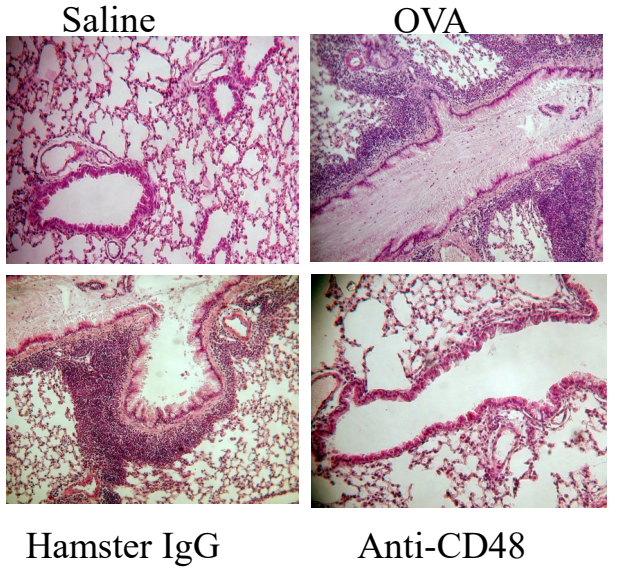


Elishmereni M and Levi-Schaffer F, **Int J Biochem Cell Biol**, 2010; Minai-Fleminger Y et al., **Cell Tissue Res**, 2010; Elishmereni M et al., **Allergy**, 2011; Elishmereni M et al, **Allergy** 2013; Minai-Fleminger Y et al**, Clin Experimental Allergy** 2014; Gangwar R et al, **Allergy** 2017; Zoabi Y et al., **Int Arch Allergy Immunol** 2021

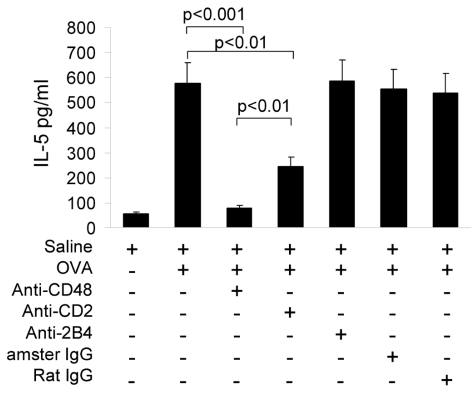
Neutralization of CD48 but not of 2B4 Inhibits Mouse Asthma

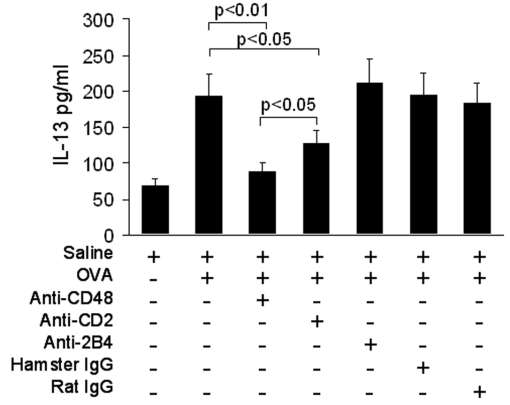


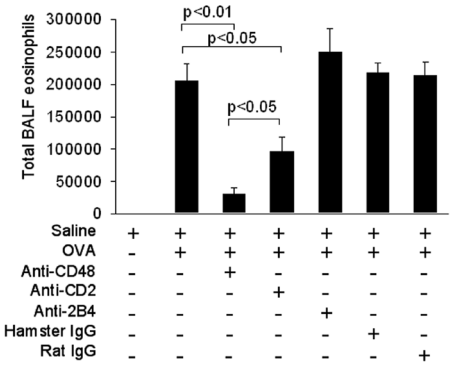




Decrease of eosinophilia, cytokine and chemokine production

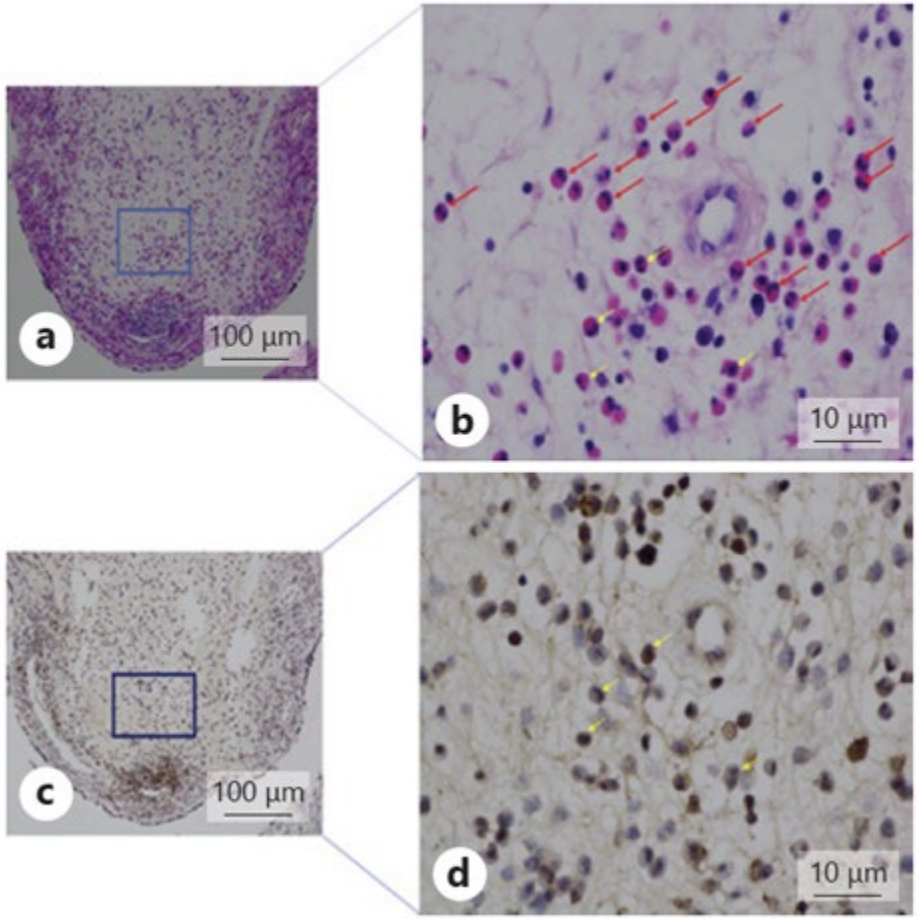


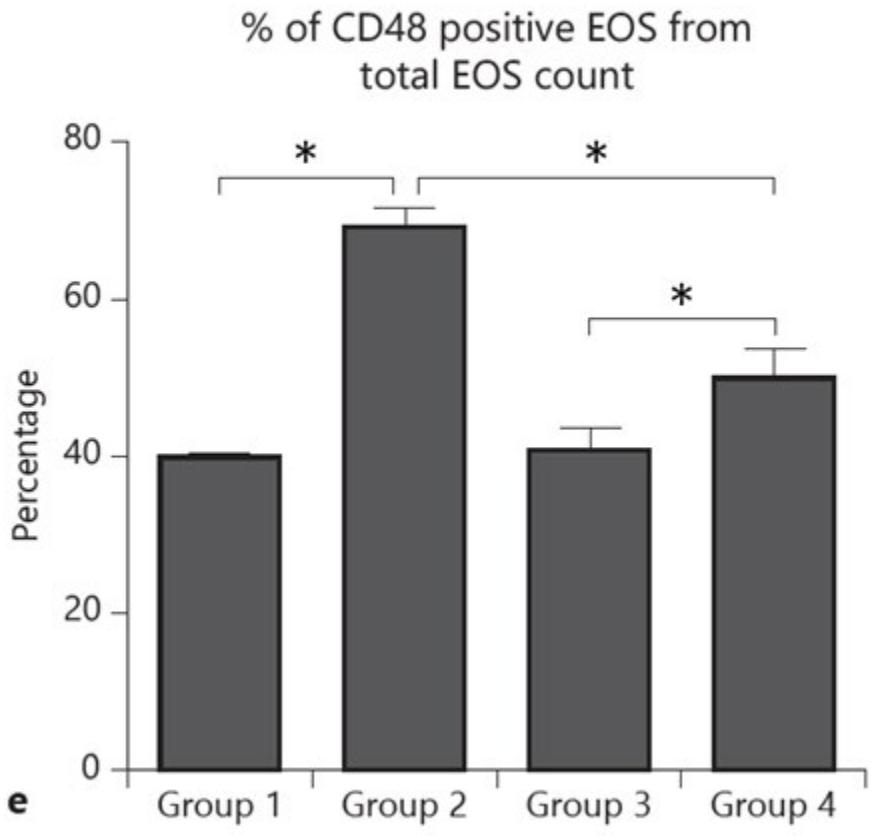




Munitz et al., **Am J Respir Crit Care Med,** 2007

Nasal Polyps: CD48 Expression is increased on Eosinophils in Chronic Rhinosinusitis /Asthmatic Patients

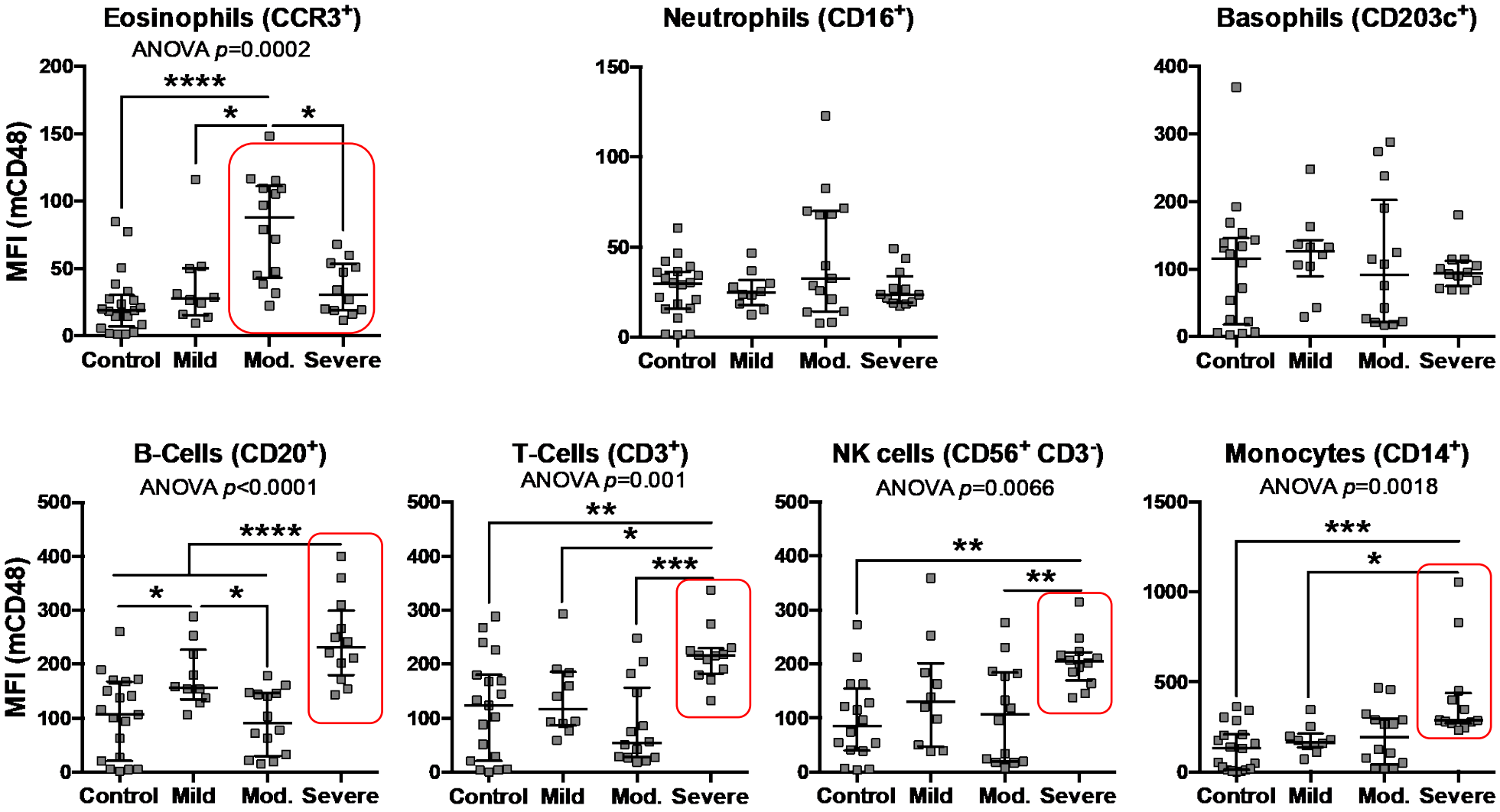




Immunohistochemical analysis of **CD48 positive eosinophils in NPs**. Eosinophils in *allergy−asthma−* NP (group were identified by H&E staining; or with anti-CD48 mAb. Red arrows show CD48 negative eosinophils. Yellow arrows show CD48 positive eosinophils; Comparison of the percentage of CD48 expressing eosinophils among the different groups . Group 1:allergy−asthma− ;Group 2:**allergy− asthma+;**Group 3:allergy+ asthma−;Group 4:allergy+asthma+

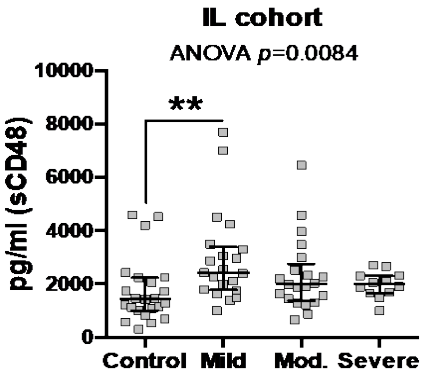
Zoabi Y et al., **Int Arch Allergy Immunol,** 2021

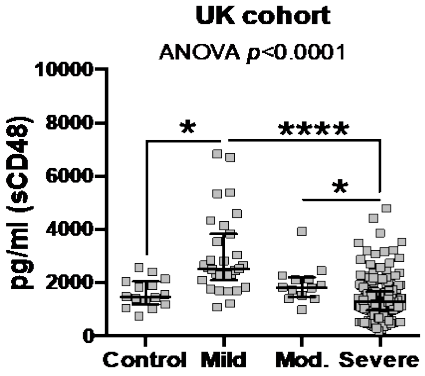
mCD48 is Differentially Expressed on Blood Leukocytes of Asthma Patients with Varying Severity

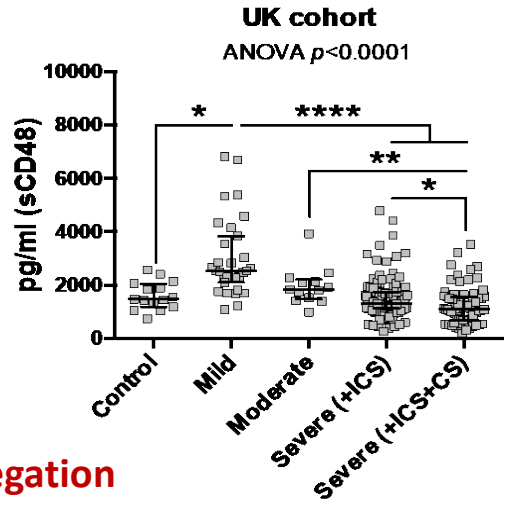


Gangwar RS et al. **Allergy** 2017

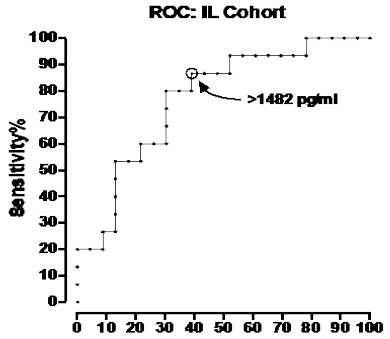
sCD48 is Elevated in Serum of Mild Asthma and Decreased in Moderate and Severe Asthma



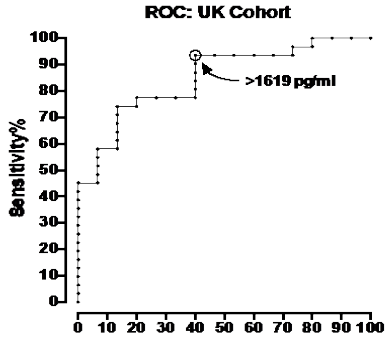




Prediction of a Cut-off Value of sCD48 for Segregation between Asthma (Mild, Steroid Naive) and Health



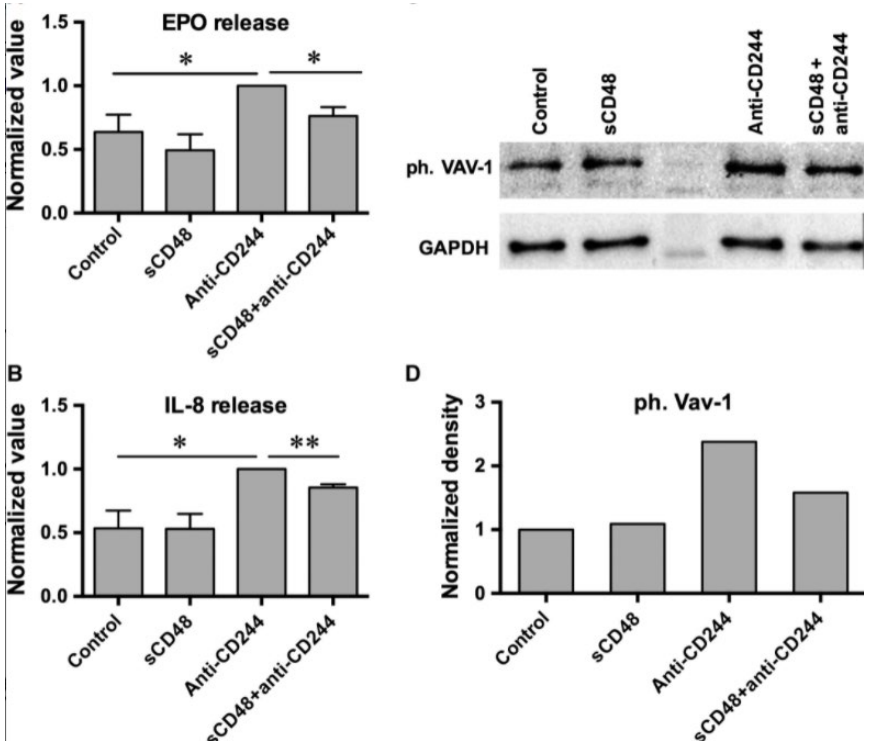
	Results
Area under the ROC curve	
Area	0.7710
Std. Error	0.07732
95% confidence interval	0.6194 to 0.9226
P value	0.0052



	Results
Area under the ROC curve	
Area	0.8495
Std. Error	0.05729
95% confidence interval	0.7371 to 0.9618
P value	0.0001

No correlation was found between sCD48 and atopy, IgE levels, Eos numbers and percentages, gender, age, smoking, BMI

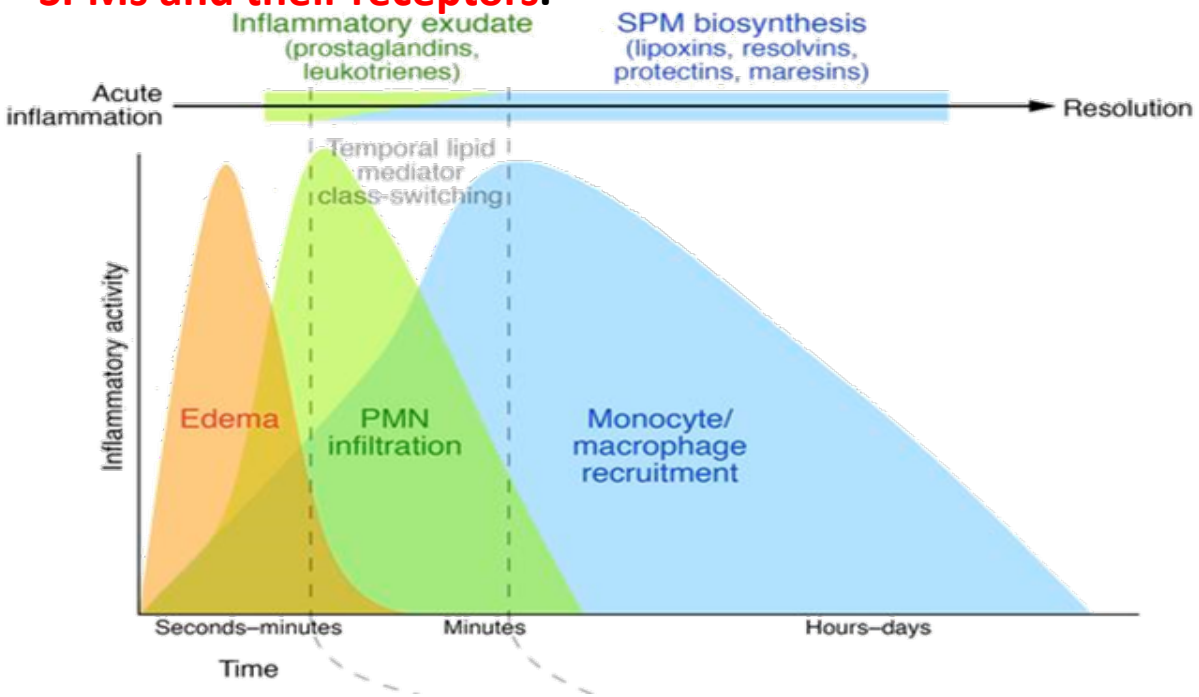
sCD48 decreases CD244-induced eosinophil activation: decoy receptor

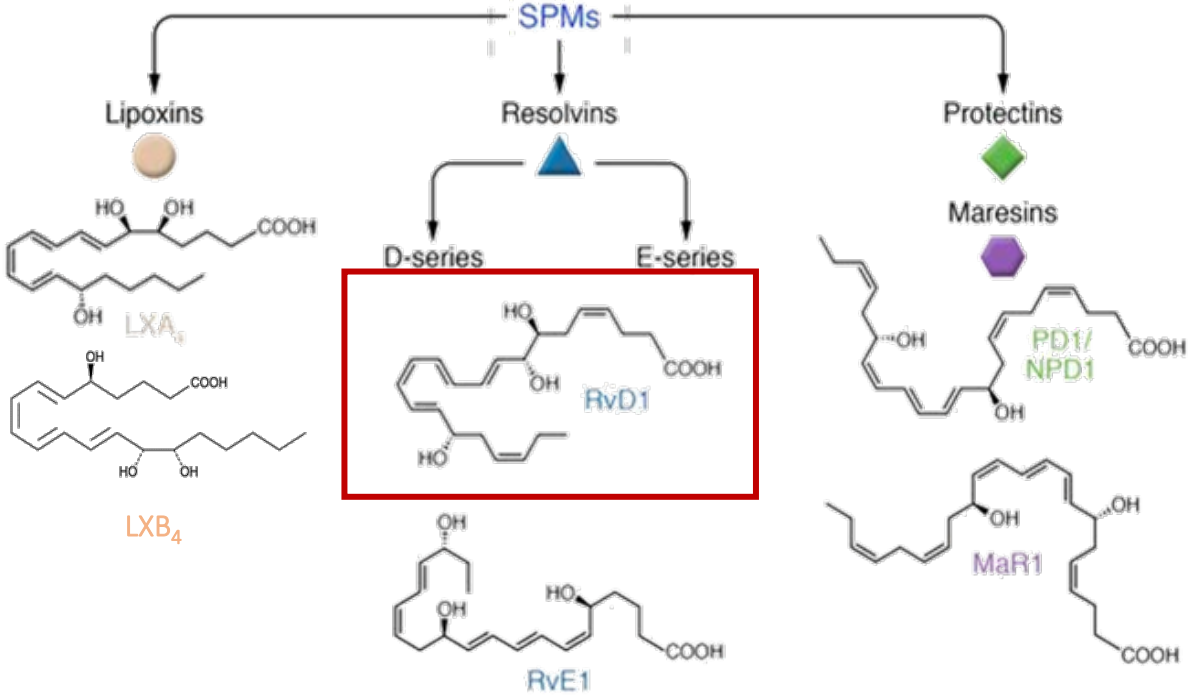


Gangwar RS et al. **Allergy** 2017

Resolution of inflammation-SPMs: last but not least!!!

AI is usually a chronic disease characterized by absence of resolution, but sometimes by remission phases. Resolution of inflammation is an active process regulated by the release of specialized pro-resolving lipid mediators (SPMs) from leukocytes. We have hypothesized that in AI mast cells can produce pro-resolution mediators and orchestrate not only the initiation but also the resolution of AI. Resolution in AI can also be modulated via the activation of inhibitory receptors (IRs) such as CD300a, a "threshold" IR, expressed and functional on the membrane of mast cells and eosinophils, by their natural ligand/s. Moreover, resolution can be orchestrated by a cross-talk between IRs and SPMs and their receptors.

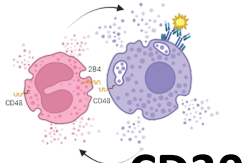




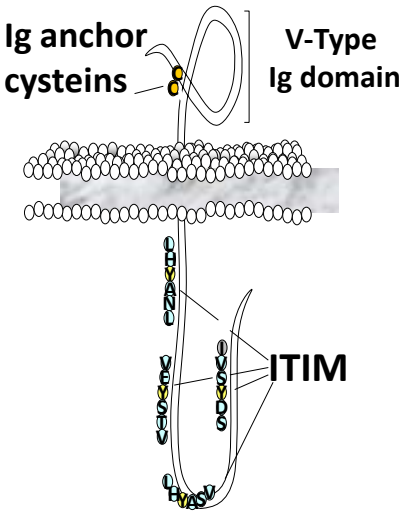
SPMs are a physiological mechanisms for AI resolution. Importantly in asthma their levels are reduced in adult patients (Planagumà et al.**, AJRCCM,** 2008**) and in pediatric severe asthma patients (**Hasan et al.**, Pediatr Crit Care Med,** 2012**).**

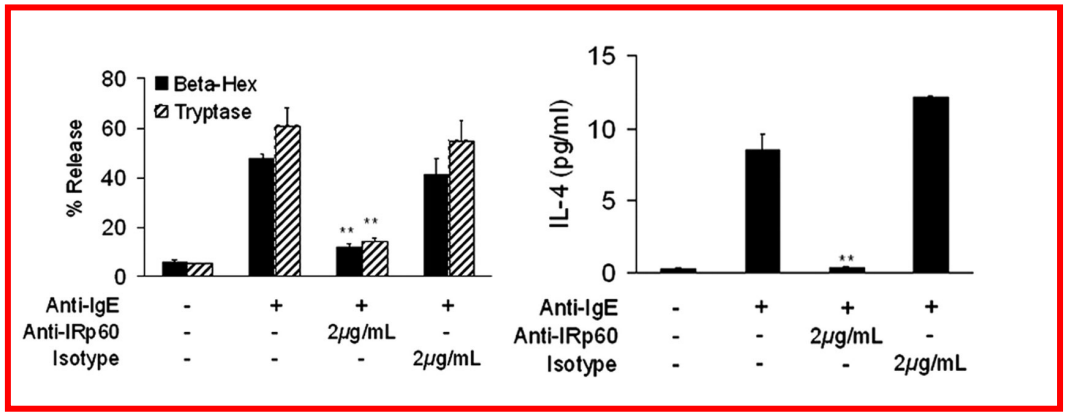
Modified from: Serhan C and Levy BD, **J Clin Invest,** 2018

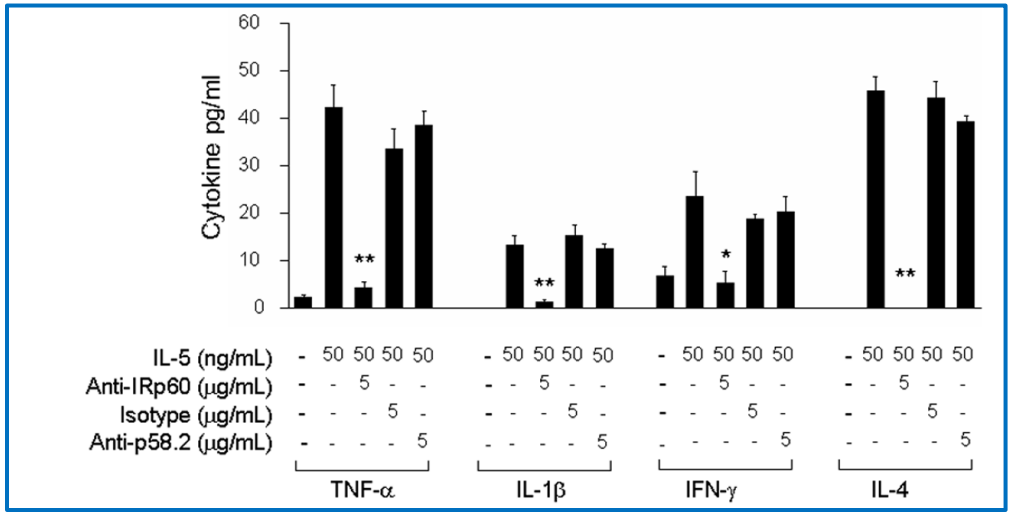
Expression of CD300a on CBMCs and on pbEos and in the AEU



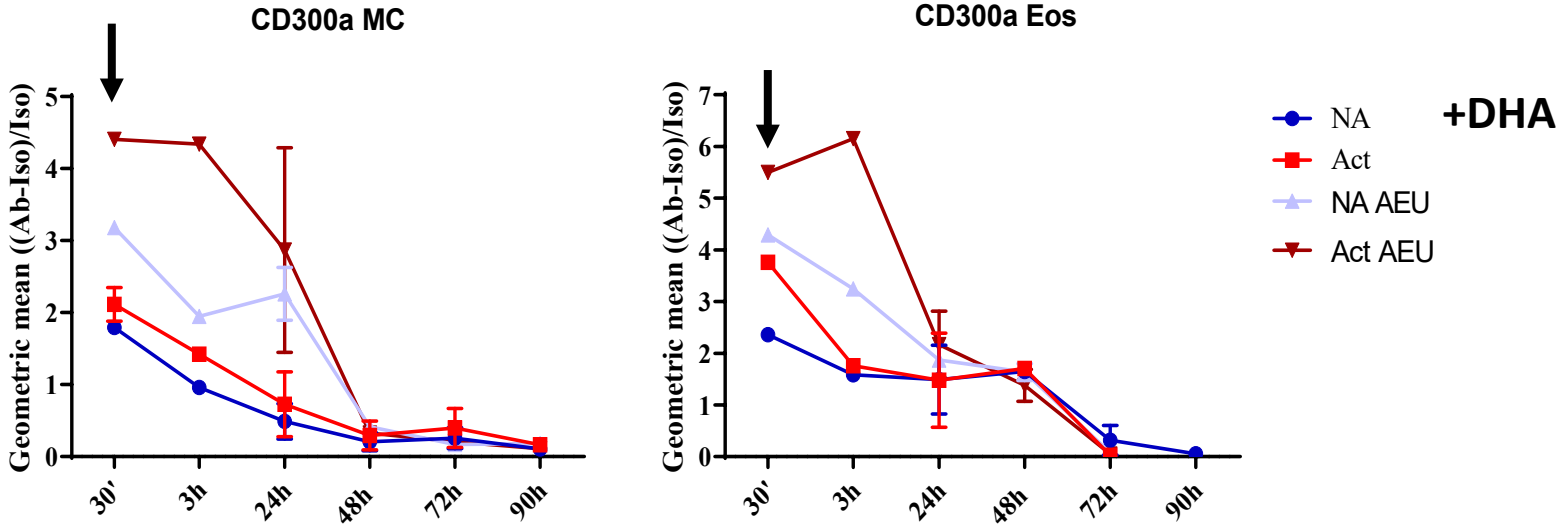
CD300a







- It belongs to the Ig superfamily. And to the CD300 family with 8 members some IRs and some ARs
- It has a mouse homologue, LMIR-1.
- 3 classical and one non classical ITIMs
- Expressed on: NK cells, neutrophils, T and B lymphocytes, **mast cells**, **eosinophils**, **basophils.** Expressed on some malignant cells.
- CD300a recognizes phosphatidylserine (PS) and phosphatidylethanolamine (PE) on apoptotic, activated ,transformed or virus infected cells .
- It has a paired AR, CD300c



Bachelet I et al., **J Immuno,** 2005; Munitz A et al., **Blood** 2006; George T and Levi-Schaffer F **unpublished**

CD300a: past and recent works

2022:New generation of engineered bispecific mAbs

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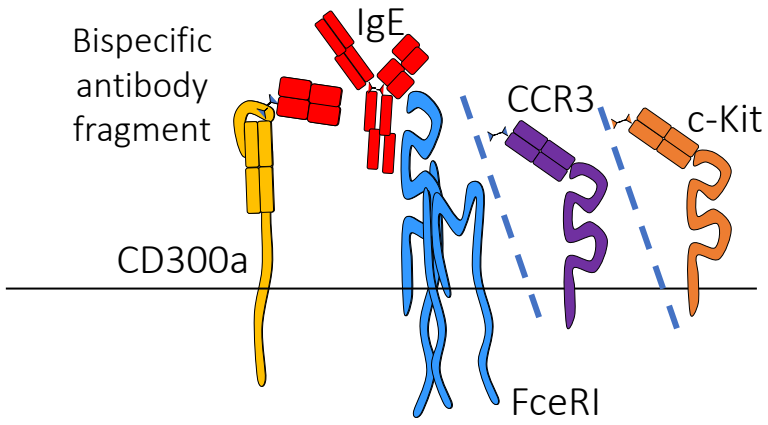


Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

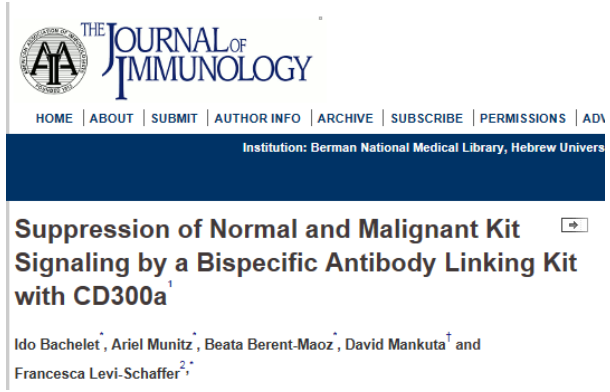
Brief Communication

The inhibitory receptor CD300a is up-regulated by hypoxia and GM-CSF in human peripheral blood eosinophils

A. H. Nissim Ben Efraim, L. Karra, M. Ben-Zimra, F. Levi-Schaffer

First published: 25 January 2013 | https://doi.org/10.1111/all.12092 | Citations: 20

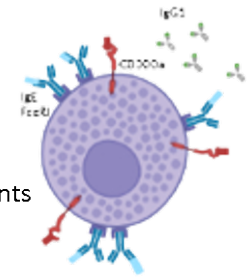
Edited by: Hans-Uwe Simon



Suppression of Normal and Malignant Kit Signaling by a Bispecific Antibody Linking Kit with CD300a[1]

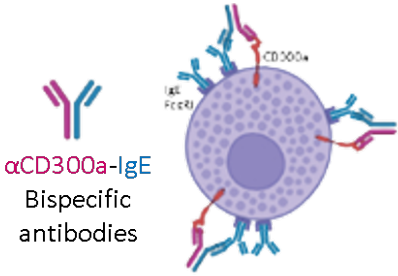
Ido Bachelet[*], Ariel Munitz[*], Beata Berent-Maoz[*], David Mankuta[†] and Francesca Levi-Schaffer[2,*]

αCD300a-IgE F(ab) fragments or IgG1



NO INHIBITION

αCD300a-IgE Bispecific antibodies



INHIBITION

Abrogation of allergic reactions by a bispecific antibody fragment linking IgE to CD300a

Ido Bachelet, MSc,* Ariel Munitz, MSc,* and Francesca Levi-Schaffer, PhD

Jerusalem, Israel

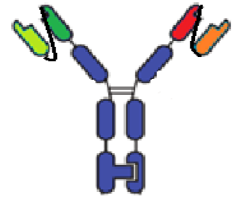
J ALLERGY CLIN IMMUNOL
VOLUME 117, NUMBER 6

ELSEVIER

Journal of Allergy and Clinical Immunology

Volume 118, Issue 5, November 2006, Pages 1082–1089

anti-CD300a anti-IgE



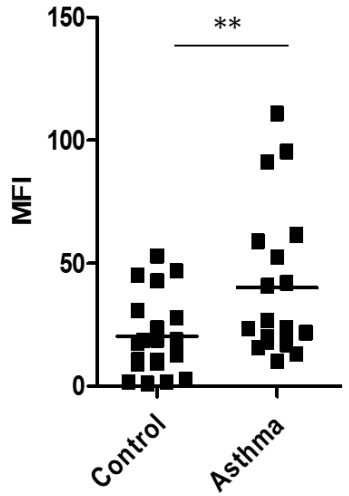
Recombinant bi-specific Ab produced by the "crossmab" technique

Mechanisms of asthma and allergic inflammation

Reversal of airway inflammation and remodeling in asthma by a bispecific antibody fragment linking CCR3 to CD300a

Ariel Munitz, MSc*, Ido Bachelet, MSc*, Francesca Levi-Schaffer, PhD

CD300a is increased on peripheral blood eosinophils of asthmatic patients (unpublished data)



P<0.05

CLINICAL & EXPERIMENTAL ALLERGY TRUSTED EVIDENCE IN ALLERGY

RESEARCH LETTER

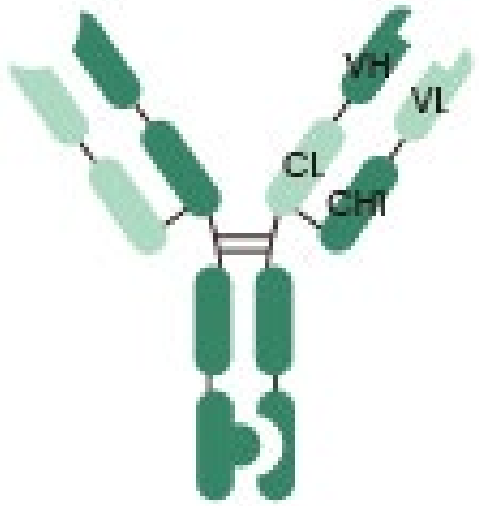
Dexamethasone and CD300a activation display additive inhibitory effect on human and murine mast cell functions

Pratibha Gaur, Fidan Rahimli Alekberli, Laila Karra, David Mankuta, Micha Ben-Zimra, Francesca Levi-Schaffer

First published: 02 April 2021 | https://doi.org/10.1111/cea.13872

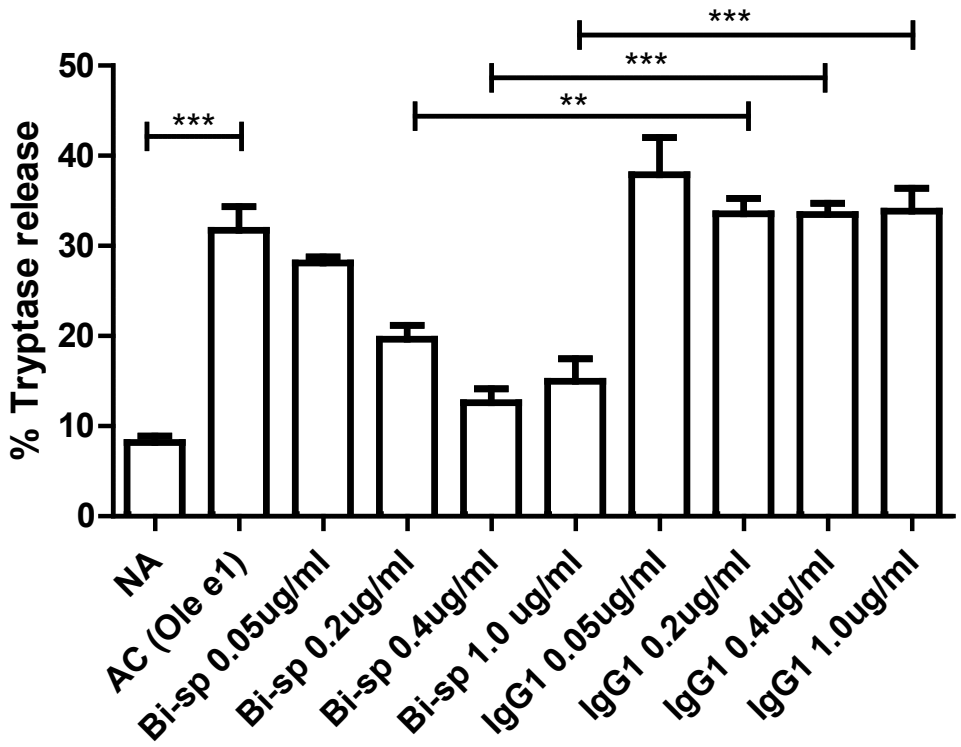
Where are we going with CD300a? Engineered αCD300a-αIgE bispecific Ab

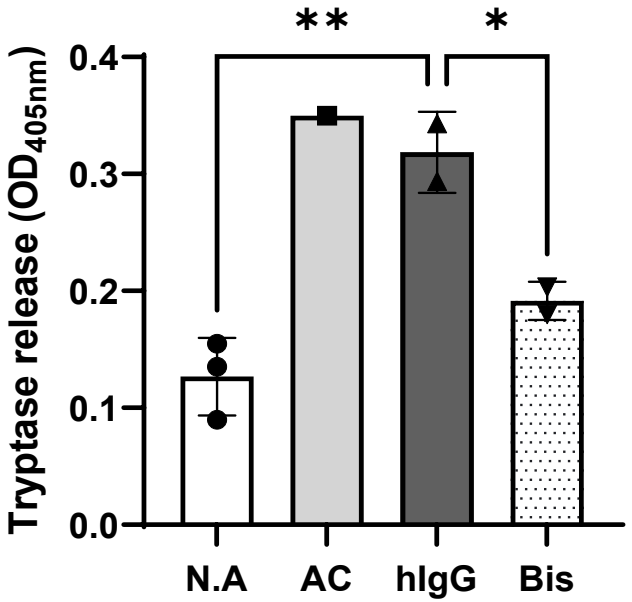
αCD300a αIgE



Roche ® Cross-Mab technique

CBMCs inhibition of "allergic activation" in vitro and in vivo (mouse)





Ben Zimra M, Zaffran I and Levi-Schaffer F, **unpublished**

CD300a expression is upregulated in human AD lesional skin, and AD-induced CD300a $^{-/-}$ mice display higher inflammatory features

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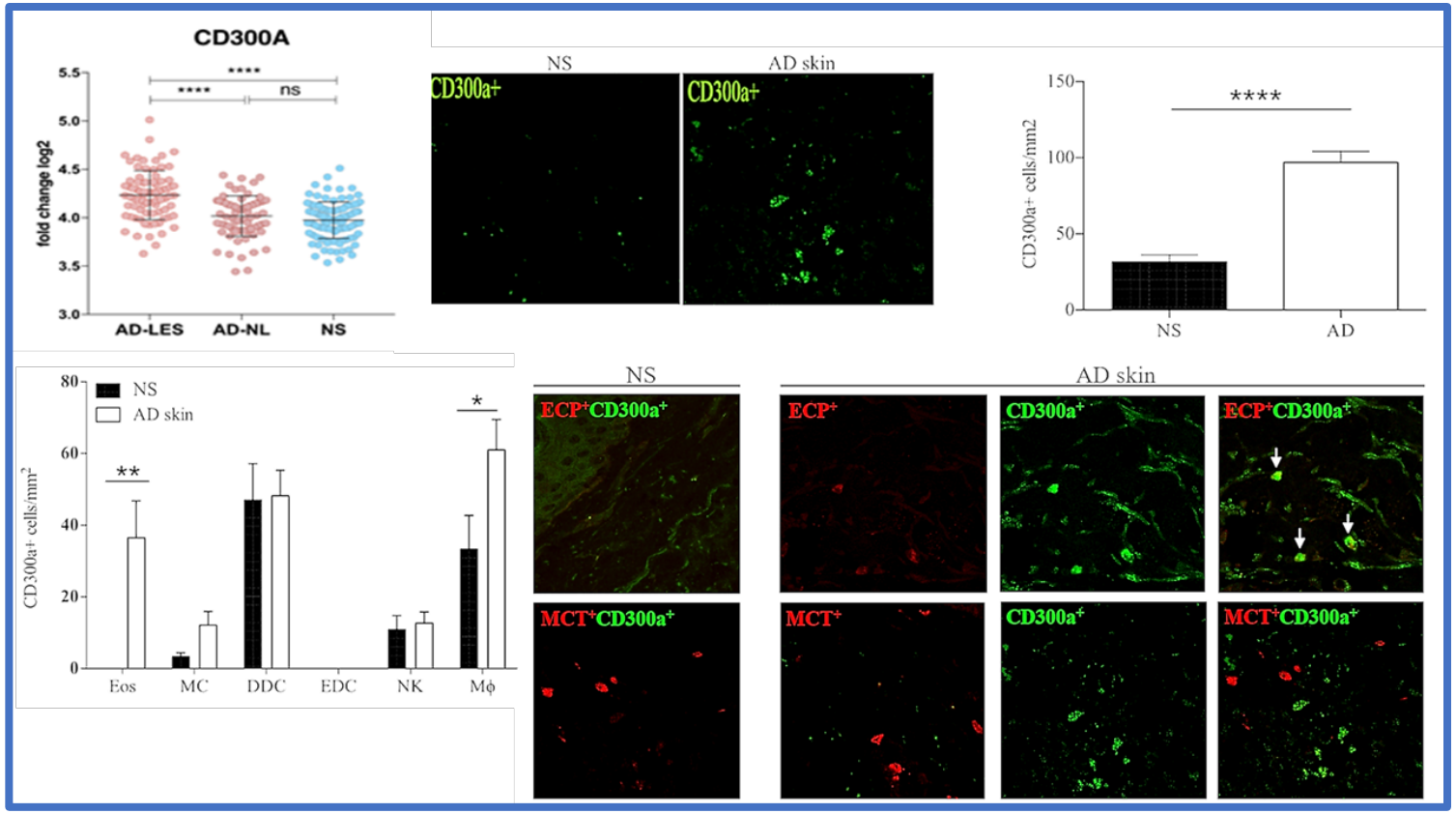
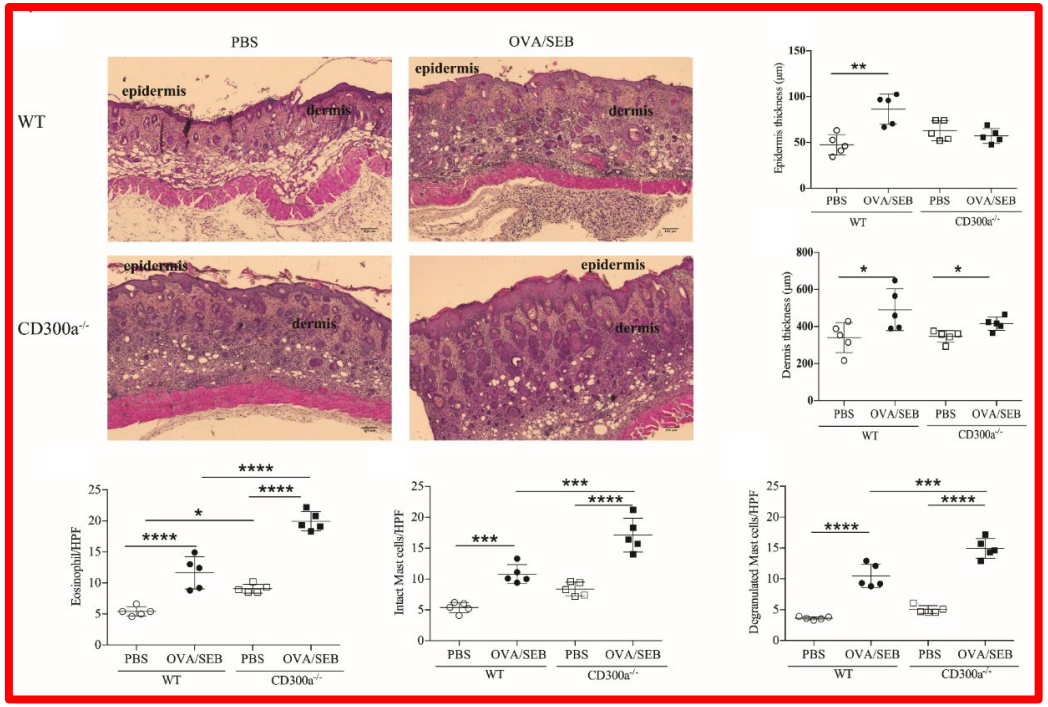
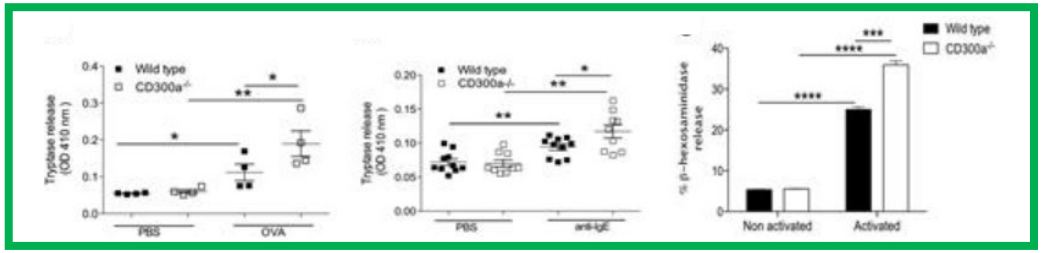
Human results



Murine results

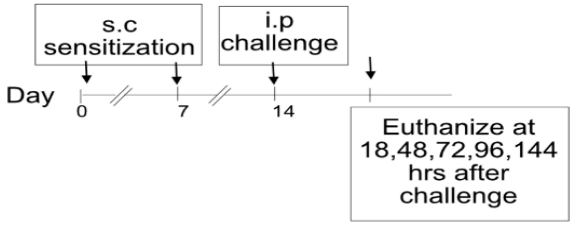




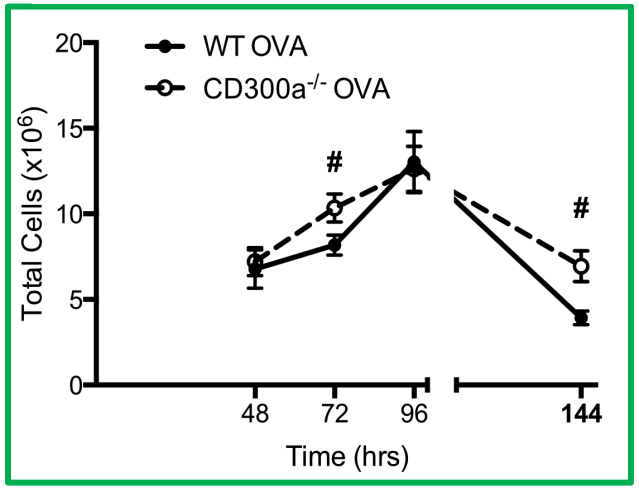
*p< 0.05, **p<0.005, ***p<0.001, ****p<0.0001

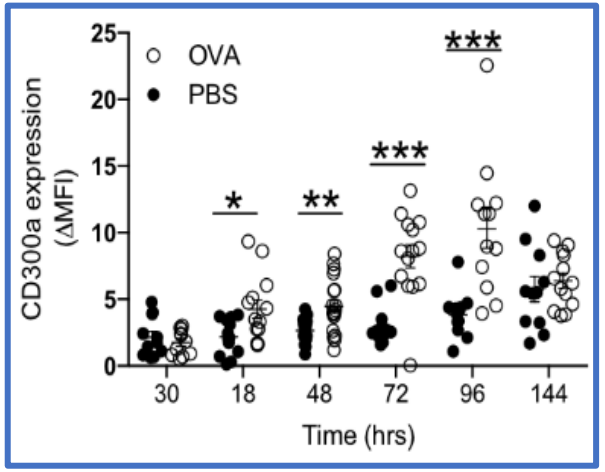
Karra L, Gangwar RS, Puzzovio PG et al., **Allergy**, 2019

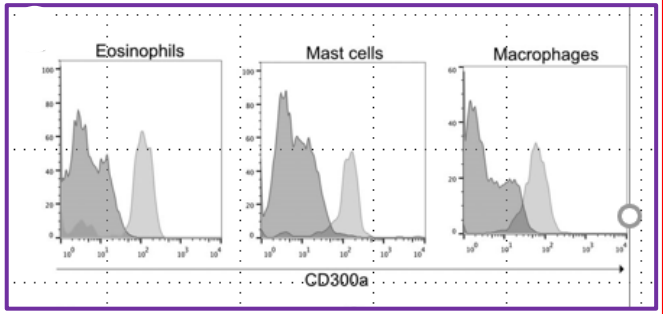
CD300a expression is timely upregulated in OVA/Alum AP model in mice and CD300a $^{-/-}$ mice display increased allergic inflammation and impaired resolution

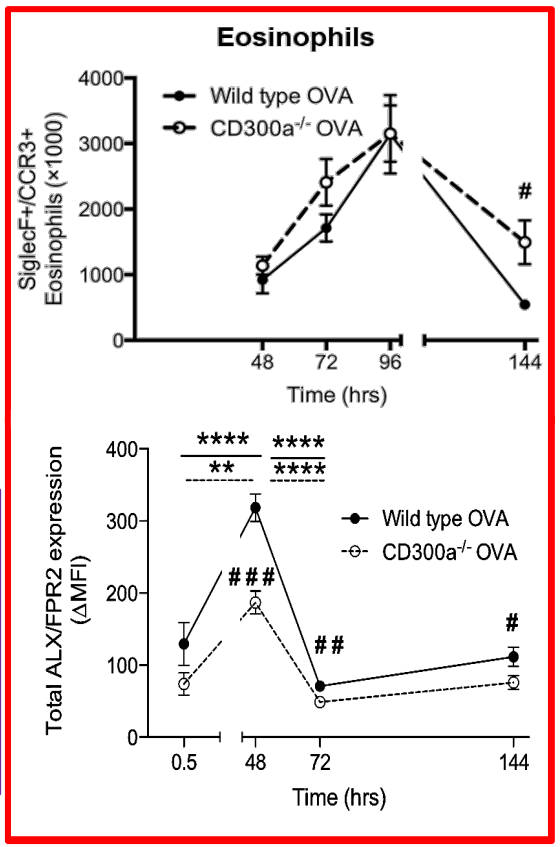


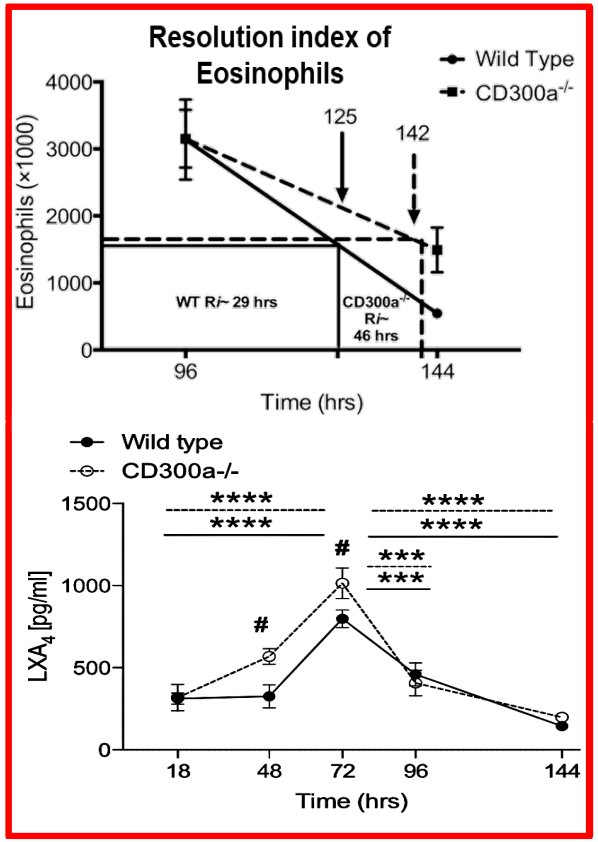
CD300a, ALX/FPR2 and LXA_4 are spatiotemporally modulated in this model.
Resolution index (R_i) is defined as the time interval for the maximum number of cells to decrease by 50%

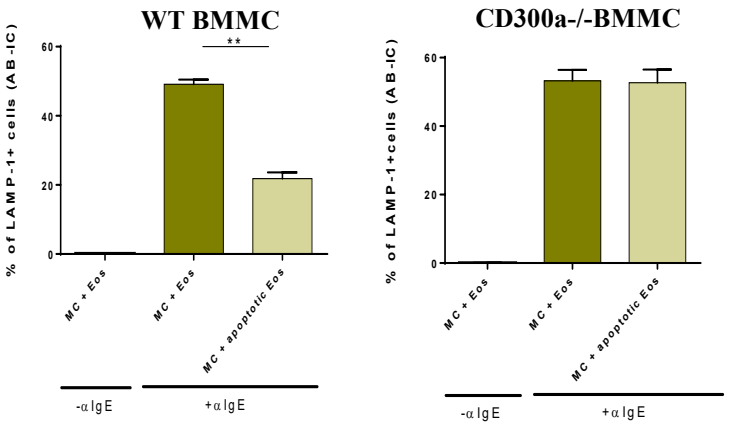








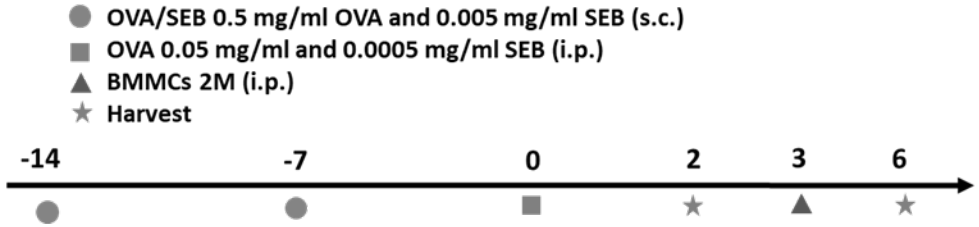


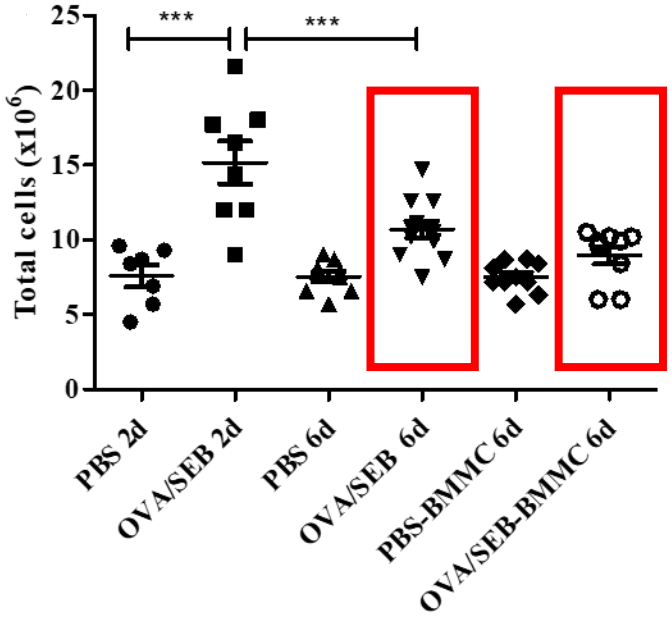


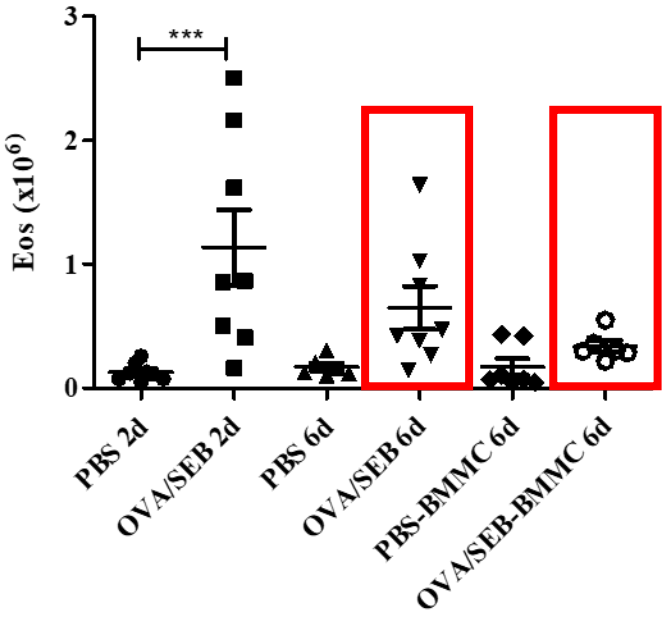
Karra L, Gangwar RS, Shamri R et al, **J Immunol**, 2018

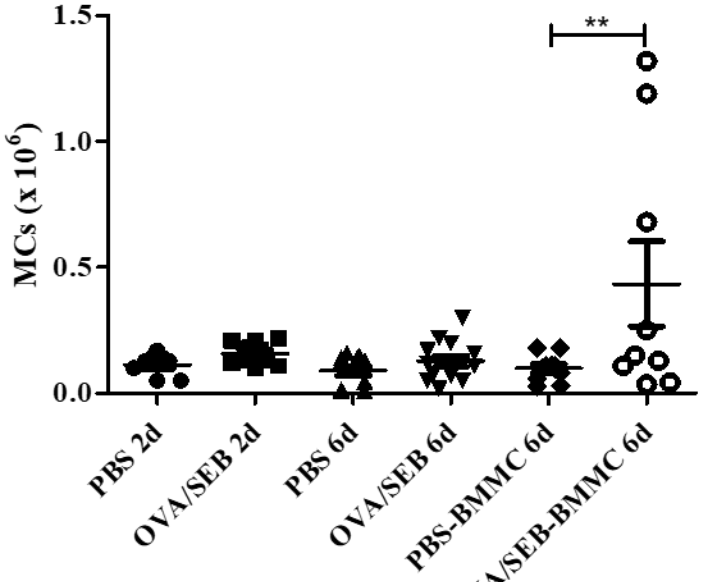
*p< 0.05, **p<0.005, ***p<0.001, ****p<0.0001

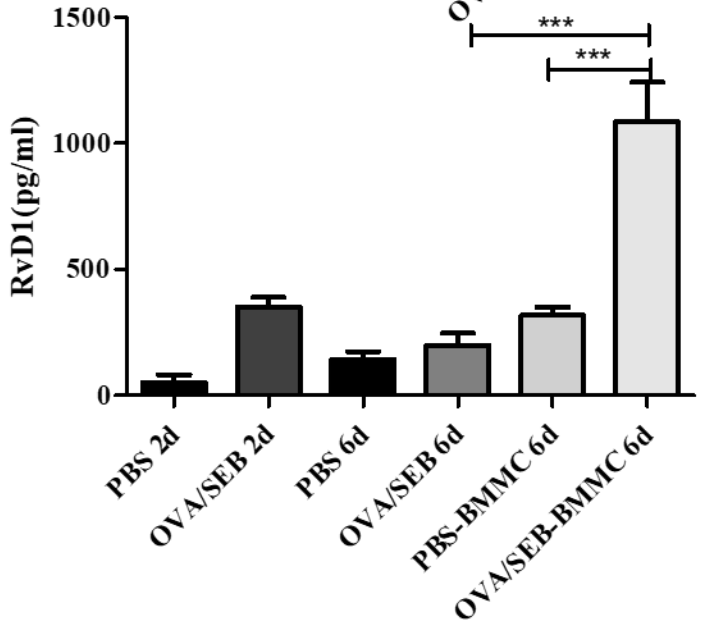
“MC Overshooted” Mice Show a Trend of Reduced Inflammation in an OVA/SEB AP model











Puzzovio PG, Hadas P, George T et al., **Pharmacol Res,** 2023

Lipoxin B4 promotes the resolution of allergic inflammation in the upper and lower airways of mice

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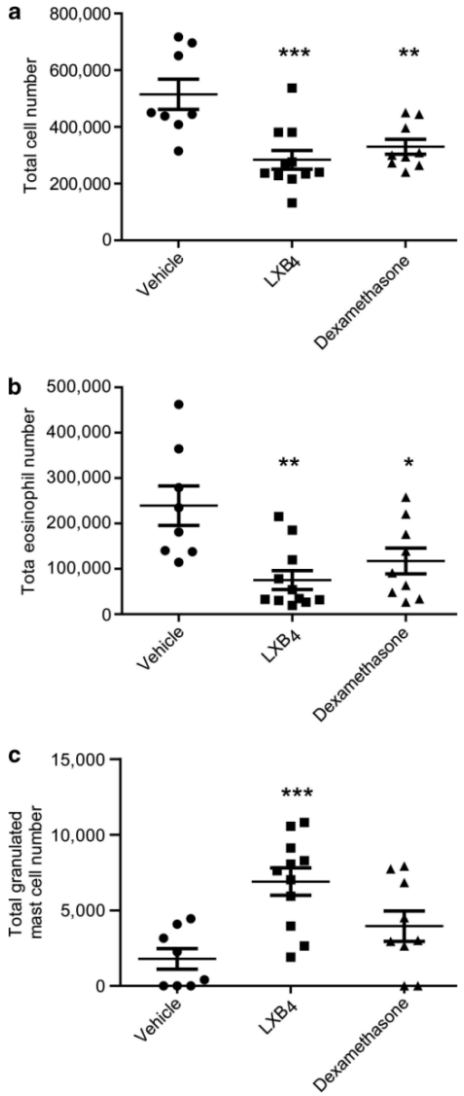
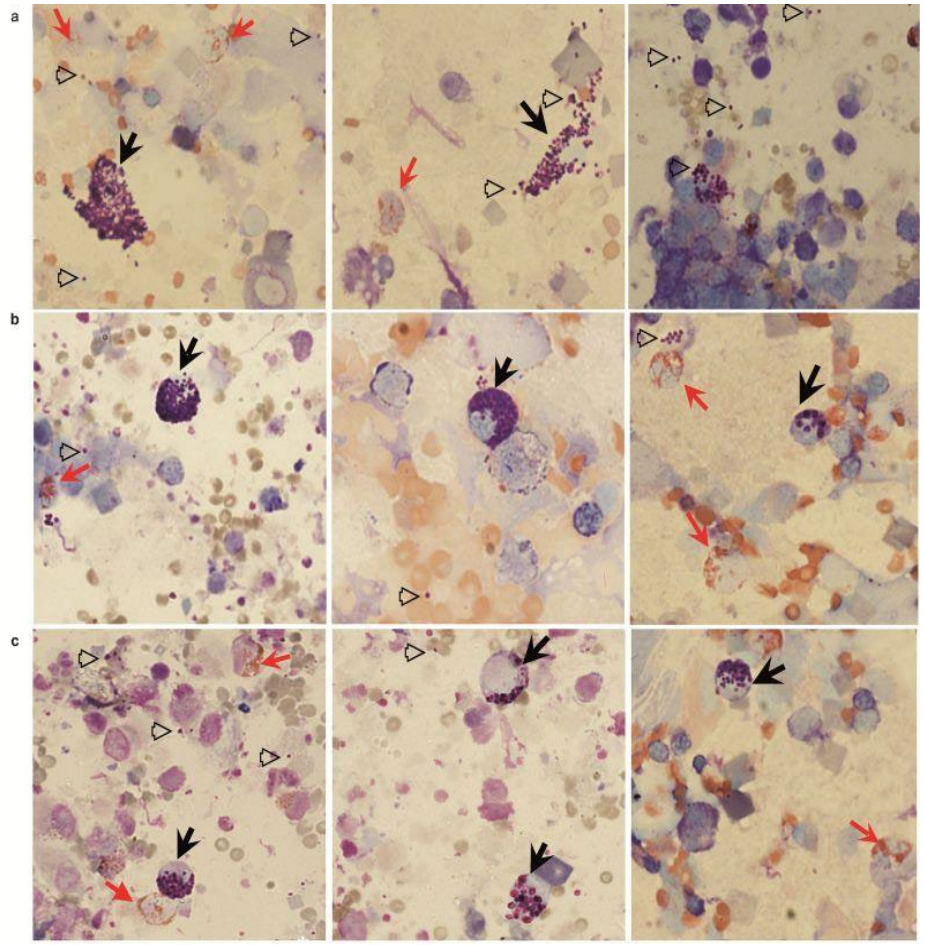
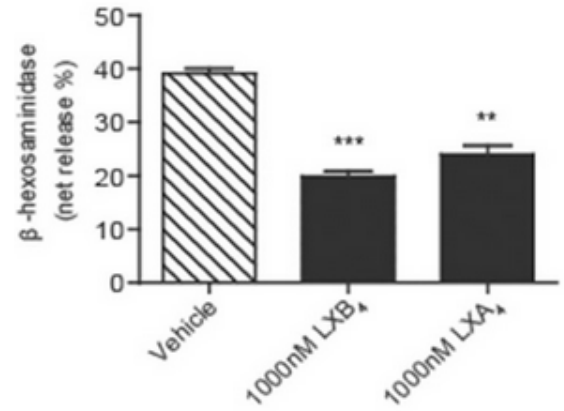
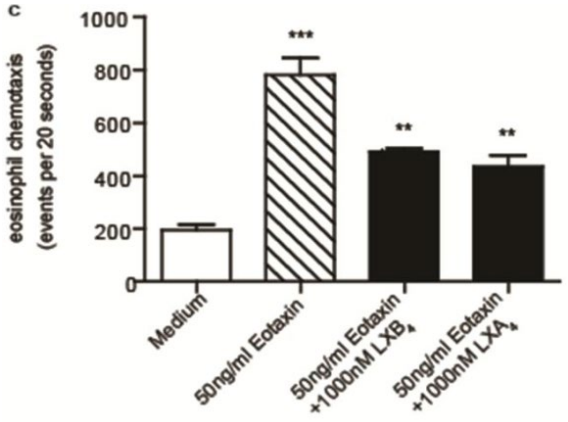
Here, we provide evidences that LXB4 mediates anti-inflammatory and pro-resolving actions for allergic airway responses in murine models of upper and lower airway mucosal inflammation









Karra L et al, **Mucosal Immunol,** 2015

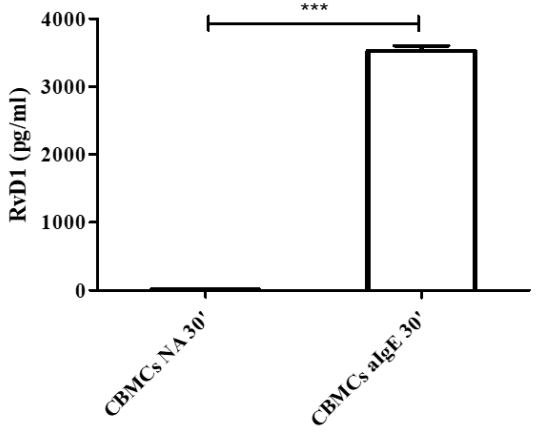
Human MCs release RvD1 after IgE-mediated activation

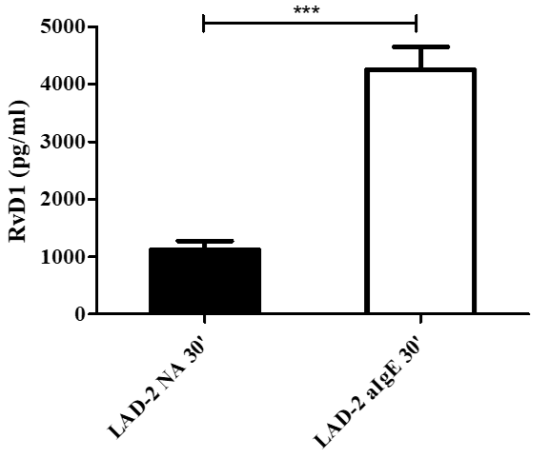
CBMCs: cord blood-derived MCs

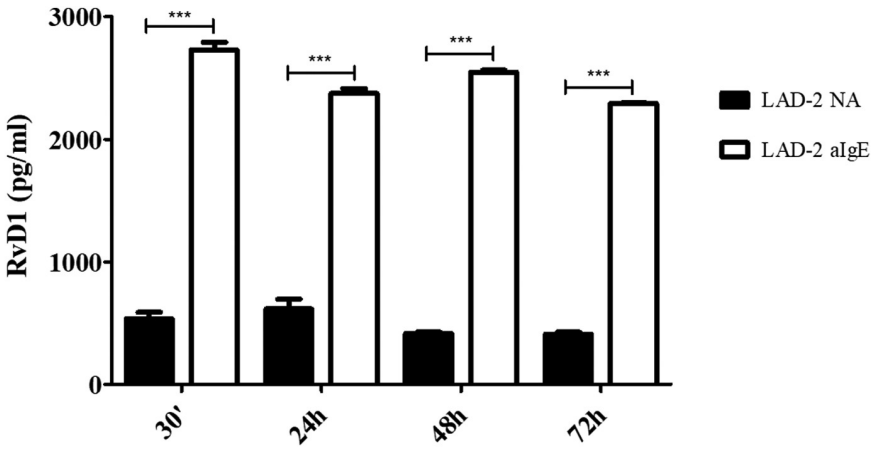
FSMCs: foreskin-derived MCs

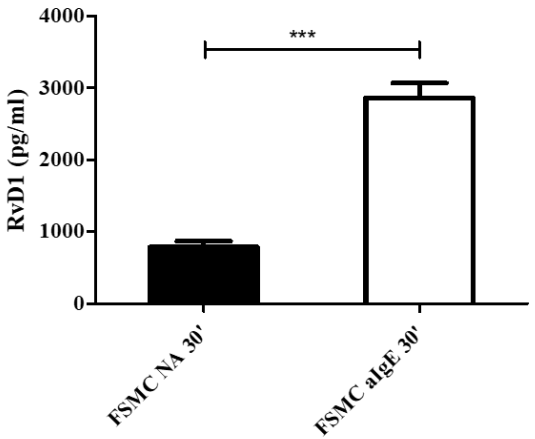
NPMCs: nasal polyps-derived MCs

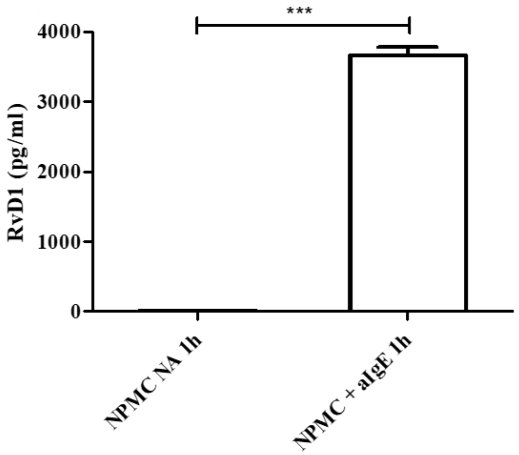
LAD-2: Laboratory of Allergic Diseases-2 cell line

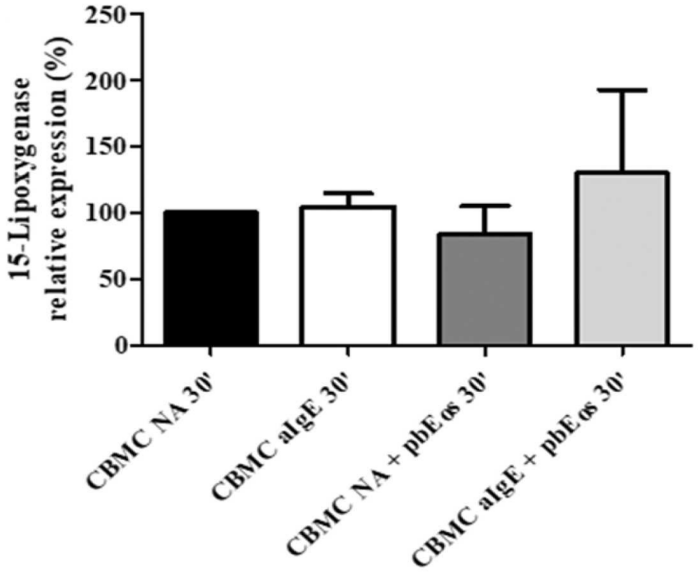






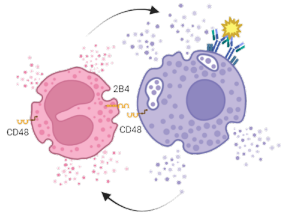






Puzzovio PG, Hadas P, George T et al., **Pharmacol Res**, 2023

The human AEU continues to produce RvD1 over time



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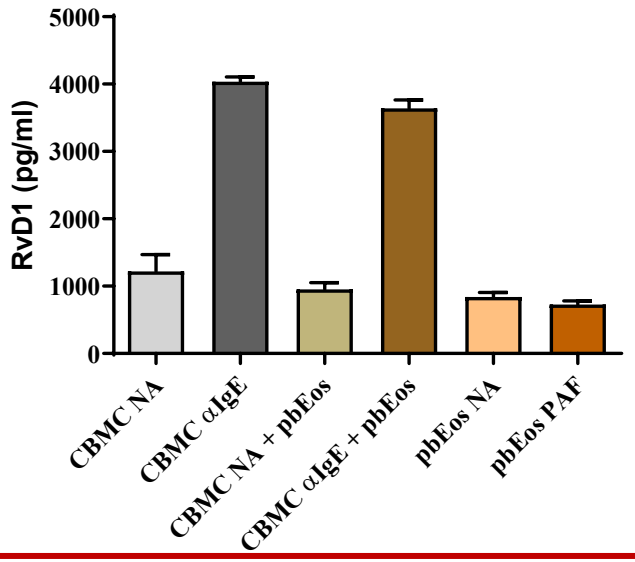
30 min



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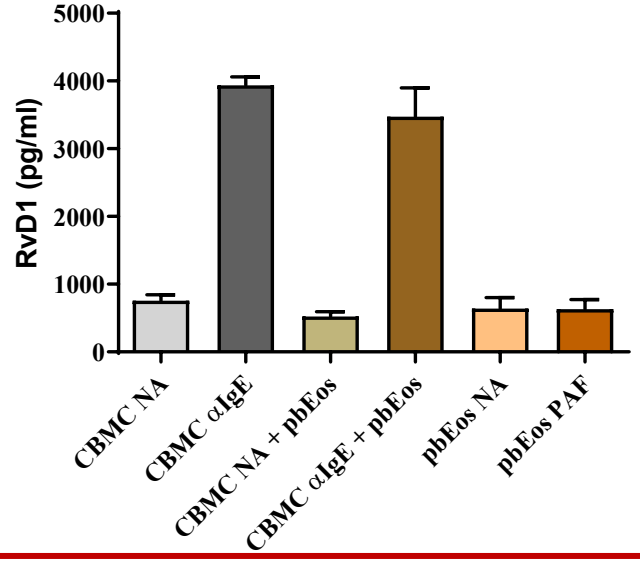

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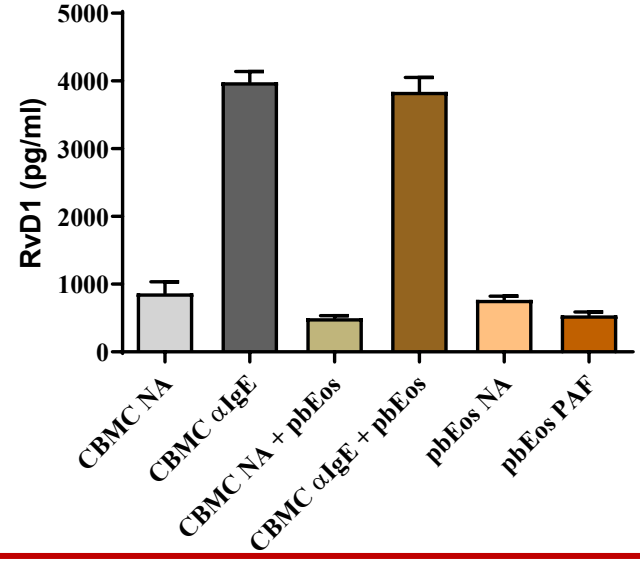
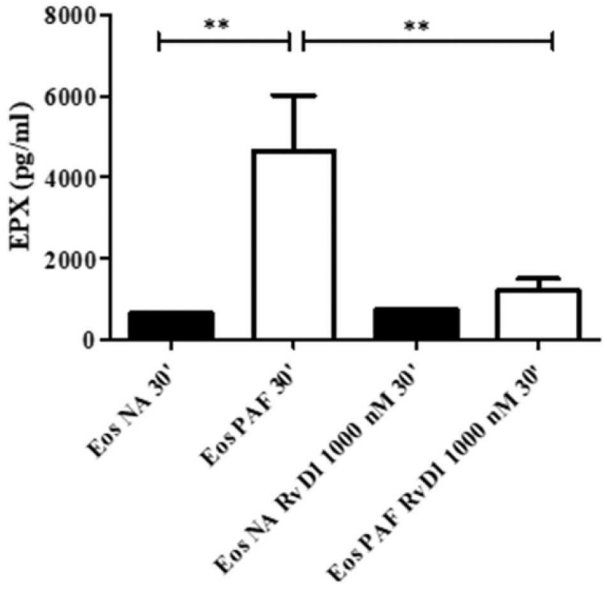
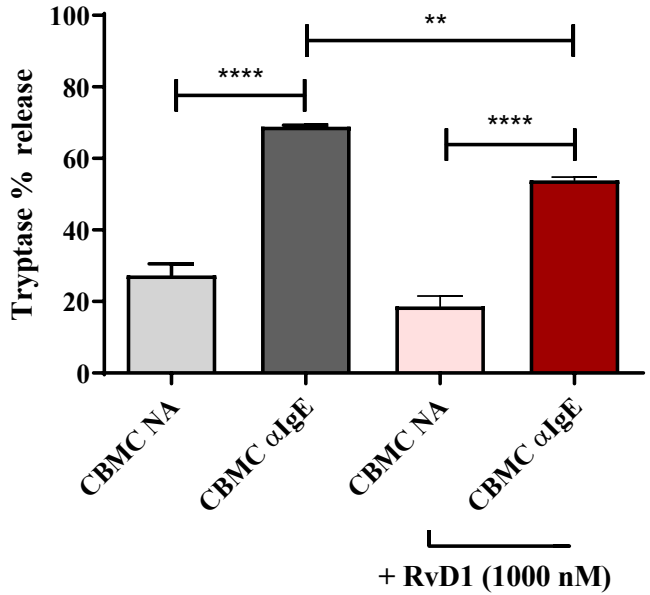




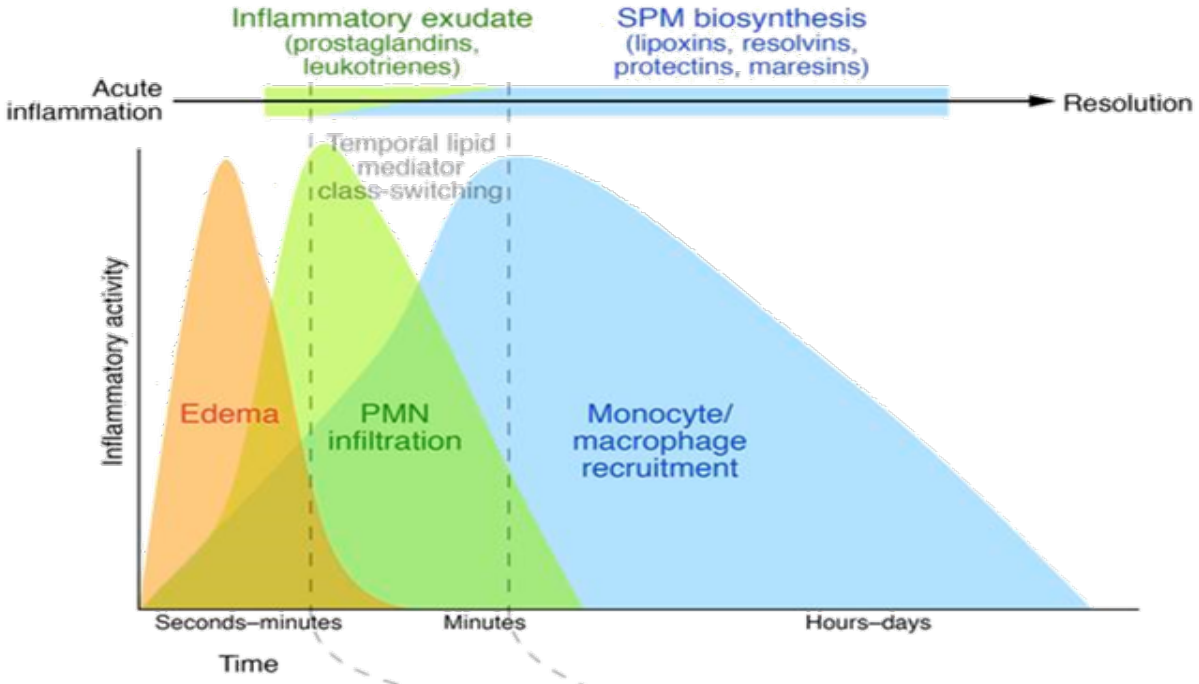


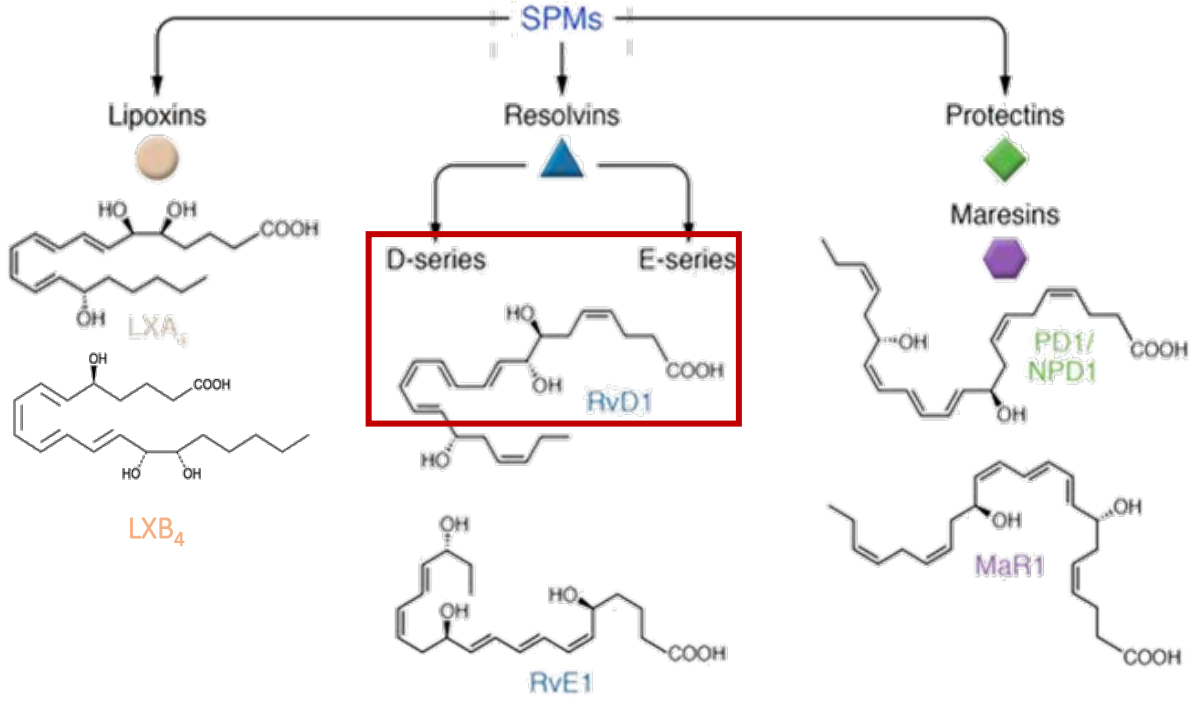
The story of mast cells in resolution is an evolving one…many other partners for mast cells are present in allergic inflammation….for next time!

Puzzovio PG, Hadas P, George T et al, **Pharmacol Res**, 2023; George T and Levi-Schaffer, **unpublished**

Resolution of inflammation-SPMs: last but not least!!!

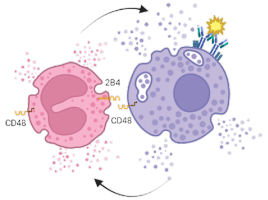
AI is usually a chronic disease characterized by absence of resolution, but sometimes by remission phases. Resolution of inflammation is an active process regulated by the release of specialized pro-resolving lipid mediators (SPMs) from leukocytes. We have hypothesized that in AI mast cells can produce pro-resolution mediators and orchestrate not only the initiation but also the resolution of AI. Resolution in AI can also be modulated via the activation of inhibitory receptors (IRs) such as CD300a, a "threshold" IR, expressed and functional on the membrane of mast cells and eosinophils, by their natural ligand/s. Moreover, resolution can be orchestrated by a cross-talk between IRs and SPMs and their receptors.





SPMs are a physiological mechanisms for AI resolution. Importantly in asthma their levels are reduced in adult patients (Planagumà et al.**, AJRCCM,** 2008**) and in pediatric severe asthma patients (**Hasan et al.**, Pediatr Crit Care Med,** 2012**).**

Modified from: Serhan C and Levy BD, **J Clin Invest,** 2018



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Summary

In the frame of allergy, we have demonstrated:

- The important pro-inflammatory role of the **AEU** and of the dominant roles of **GM-CSF** and of the activating receptor **CD48**.
- The immunomodulatory role of the inhibitory receptor **CD300a** as anti-inflammatory player and its interplay in resolution of allergic inflammation.
- The role of IgE-dependent activated MCs might play by producing **Resolvin D1** in **resolution of allergic inflammation**.

Conclusions

Allergic inflammation (and inflammation in other diseases with a different etiopathology in which MCs and Eos have prominent roles) can be **down-regulated** by immunopharmacological modulation of the **AEU** and of MCs/Eos by blocking **GM-CSF**, the activating receptor **CD48,** or by activating the inhibitory receptor **CD300a** (and **Siglec-7**), and/or by administering exogenous **SPMs in a time-dependent fashion**. Moreover, the expression/production of these receptors/mediators might provide new diagnostic tools for allergic diseases and for tailoring personalized therapy. Importantly new drugs should ideally try to specifically and timely target mast cells and eosinophils to limit the inflammation flares and induce the resolving/reparative stages, ultimately leading to a new homeostasis.

Thank you!

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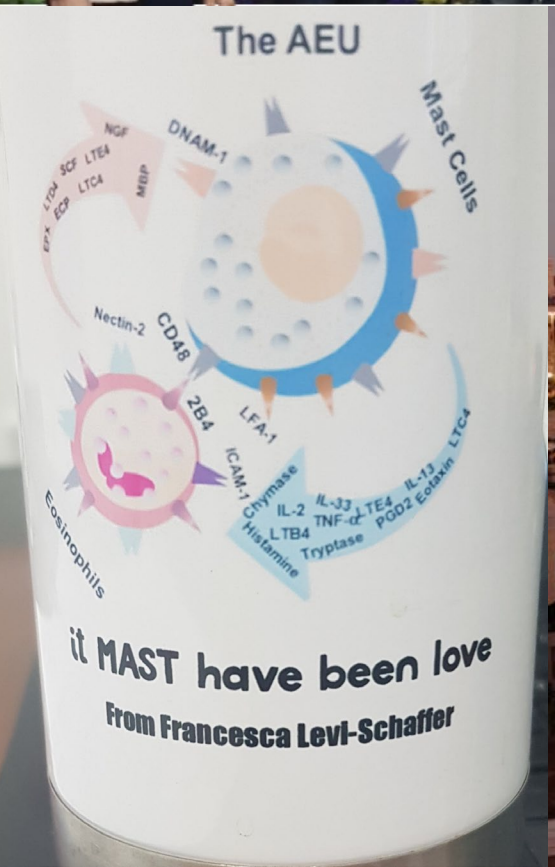

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Ilan Zaffran, MSc
Marco Zurlo, MD

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