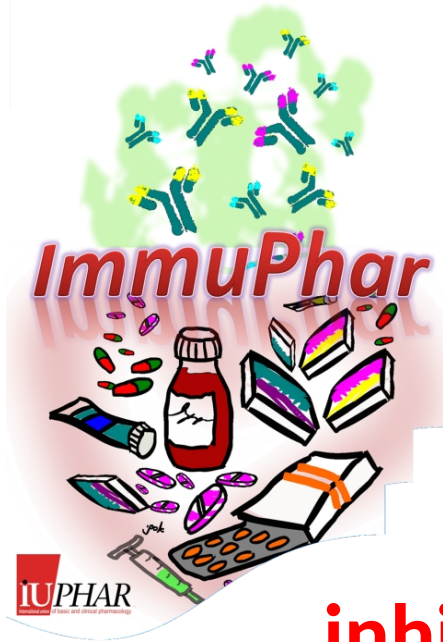


Edinburgh 1-2 October 2018

Immunopharmacology : Challenges, opportunities and research tools



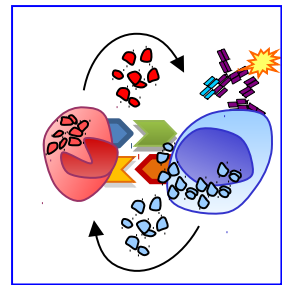
Inhibit activation or activate inhibition of Mast Cells and Eosinophils: which weapon is better to fight allergic diseases?

Francesca Levi-Schaffer

School of Pharmacy and Institute of Drug Research

The Hebrew University Medical School

Jerusalem, Israel

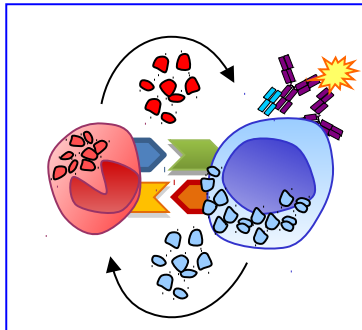


Mast Cells, Eosinophils and Diseases

In ALLERGY (but also in several other diseases with different etiopathogenesis) MAST CELLS are associated with EOSINOPHILS

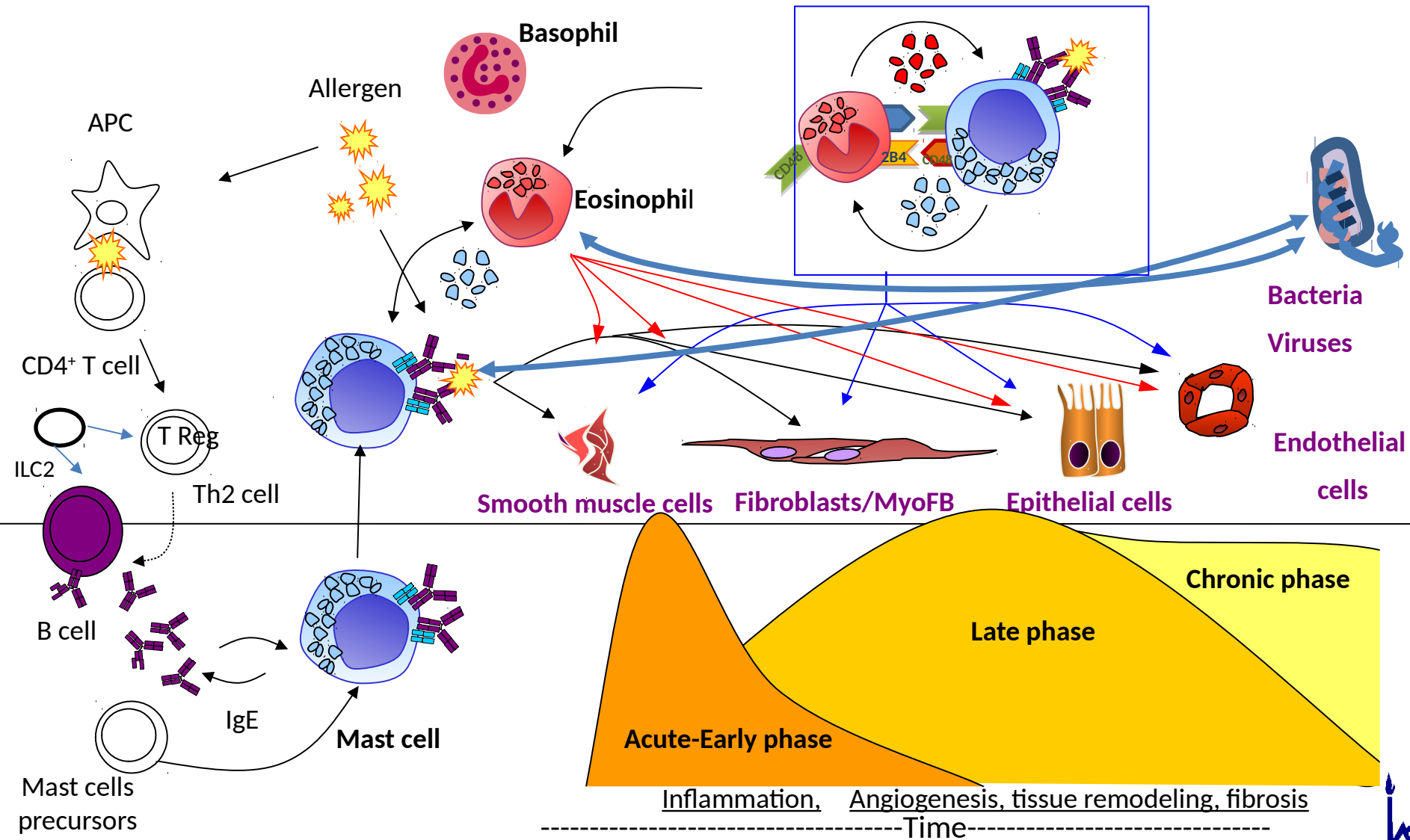
Two unmet clinical needs: severe asthma and atopic dermatitis

Our GOAL is to determine new immunopharmacological targets for the treatment of allergic diseases. This by focusing on the two main effector cells of allergic inflammation i.e. the mast cells (MCs), the allergy” primum movens “, and the eosinophils (Eos) the most common MC “companions”, and their allergic effector unit (MCs/Eos interactions)

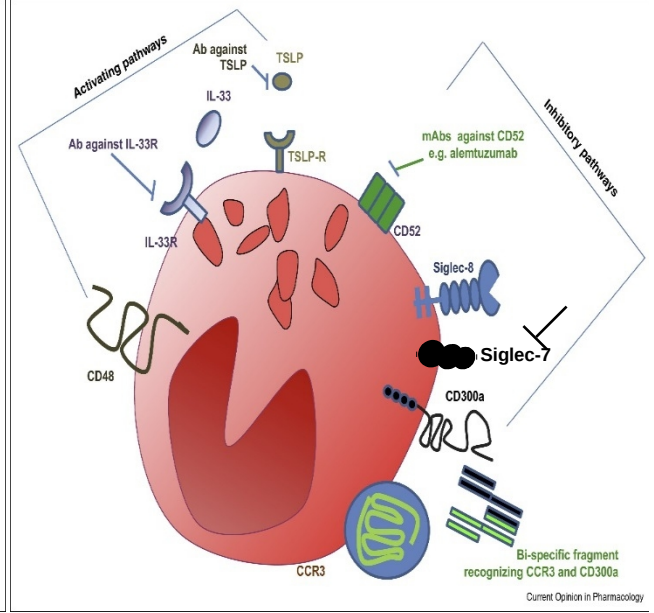
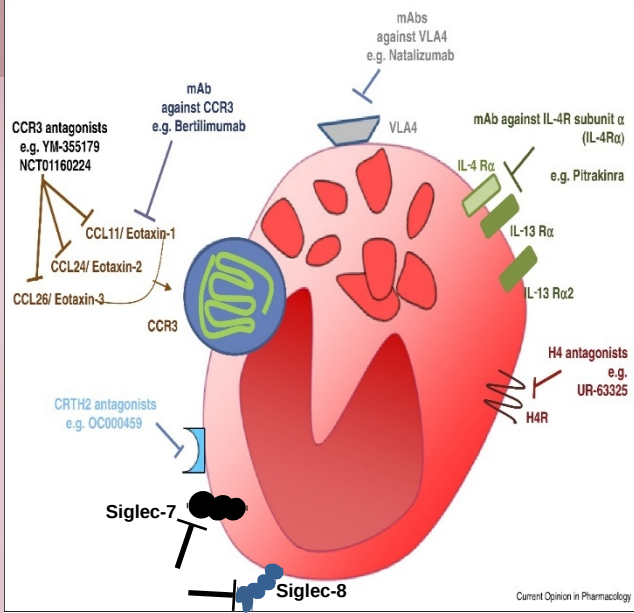
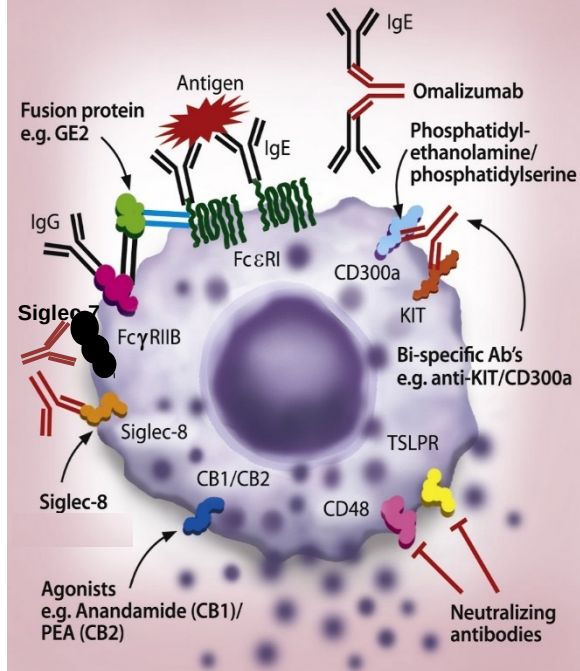
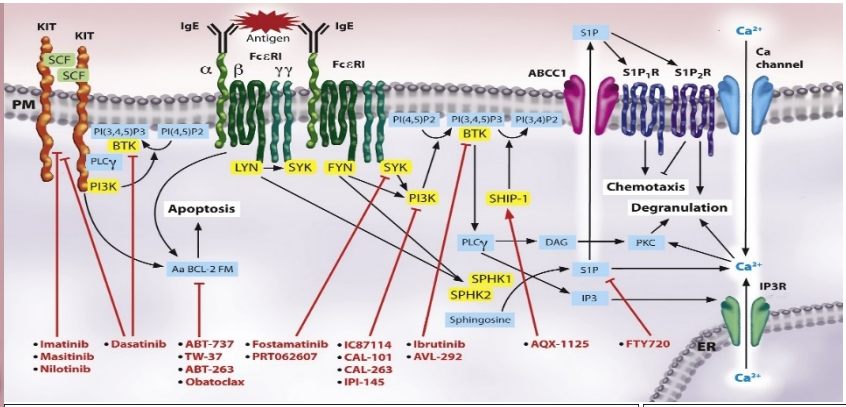
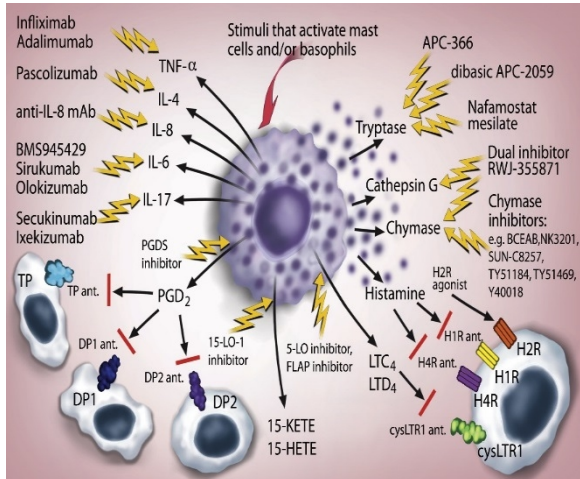


Our Oversimplified View of the Allergic Inflammatory Reaction

The "Allergic Effector Unit"



MC and Eos Soluble and Cellular Targets for Novel Anti-Allergic Therapy



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

Our findings in mast cell and eosinophil allergy related research

1. Human MCs express the functional activating receptors **CD48**,

DNAM-1 and PAR-2. And the death receptor TRAIL

2. Human Eos express the functional activating receptors **CD48**, **2B4**

and Nectin-2.

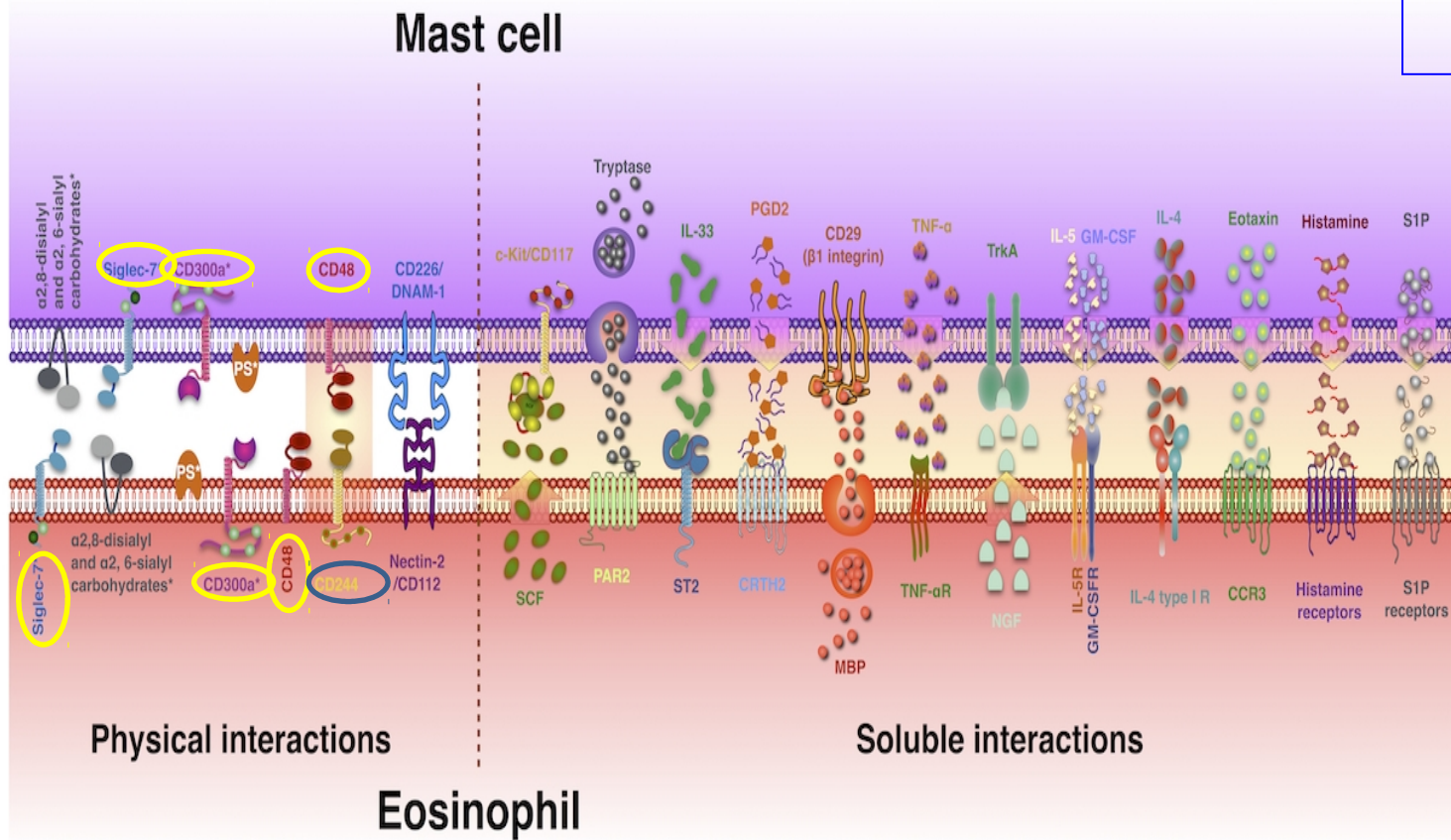
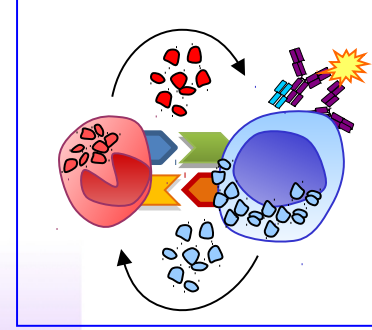
3. MCs and Eos have a soluble and physical cross-talk :the Allergic Effector Unit (**AEU**).

4. Both human MCs and Eos express the functional inhibitory receptors **CD300a** and **Siglec-7**.

5. The activity of the *pro-resolving lipid mediators (SPMs)* LXB4 and LXA4 on MCs and Eos and in mice models of allergic inflammation.

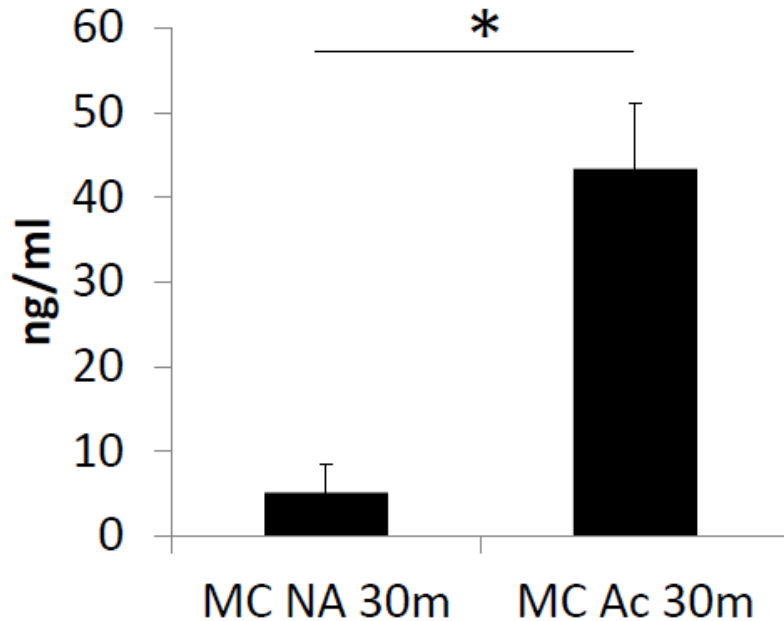


The Allergic Effector Unit (AEU): a Strong Pro-inflammatory Cross-talk

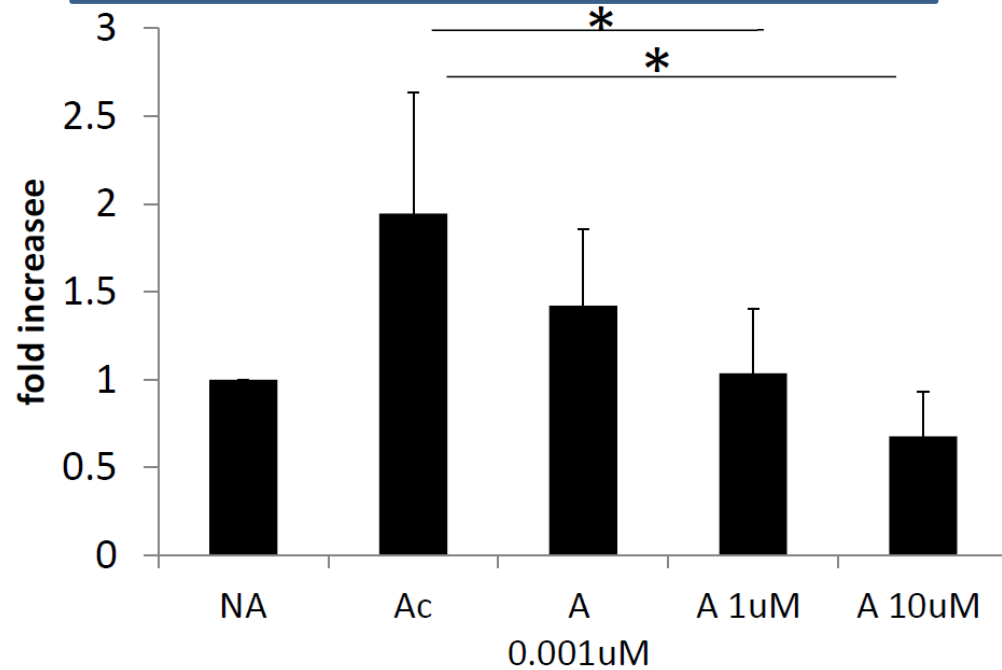


Mast cell derived PGD2 is a component of the AEU: Fevipiprant, a selective DP2 antagonist inhibits eosinophil chemotaxis towards IgE-activated mast cells

IgE-activated CBMC release PGD2



Fevipiprant inhibits human eosinophil chemotaxis towards IgE-activated mast cells



Our Immunomodulatory Strategies

We aim to target receptors that are shared by MCs and Eos and that are important in the AEU



Activating receptors:
CD48 (MC and EOS)



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

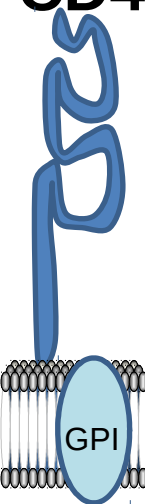
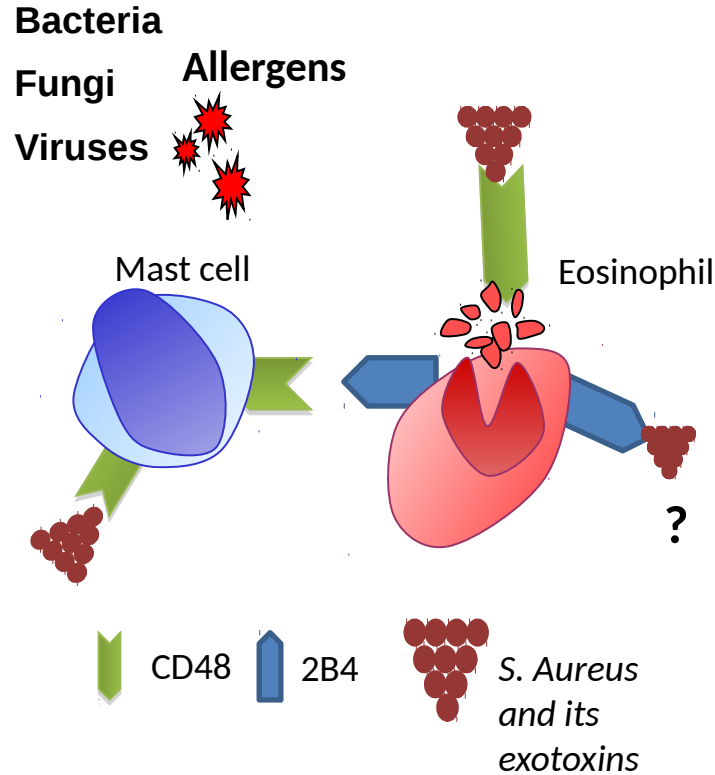


The Human AEU

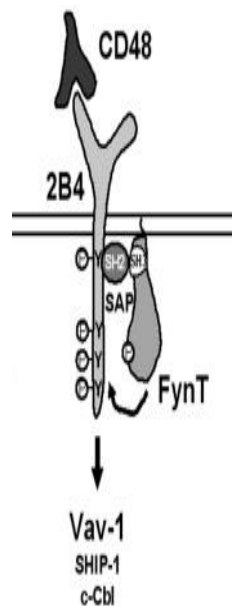
CD48 and 2B4 (CD244) (CD2 family)

CD48 GPI (glycosylphosphatidylinositol)

- Membrane bound form on leukocytes
- Soluble form
- 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
- In the mouse on MCs and NKs it is an inhibitory receptor

CD48

2B4

SH2

SH1

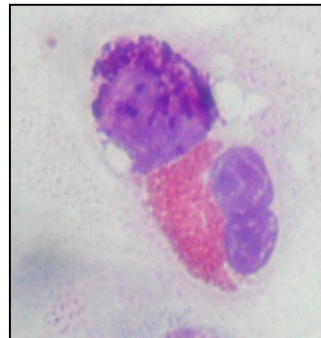
SAP

FynT

Vav-1

SHIP-1

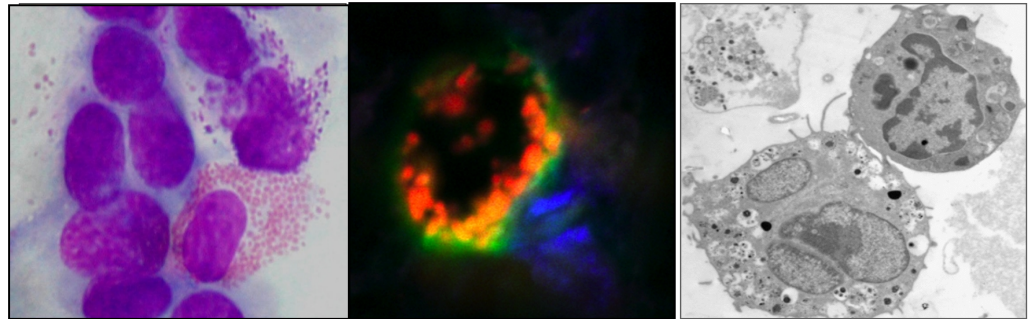
c-Cbl



The Human AEU: Soluble and Physical Cross-talk

Physical contact

- ✓ Takes place in inflammatory states.
- ✓ Occurs at significant rates.
- ✓ Is durable and stable.
- ✓ Partially involves 2B4 on Eos and - CD48 on MCs interactions.



Physical induced Cell Survival

- ✓ MC 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.
- ✓ It is not inhibited by dexamethasone.

Physical induced Cell Activation

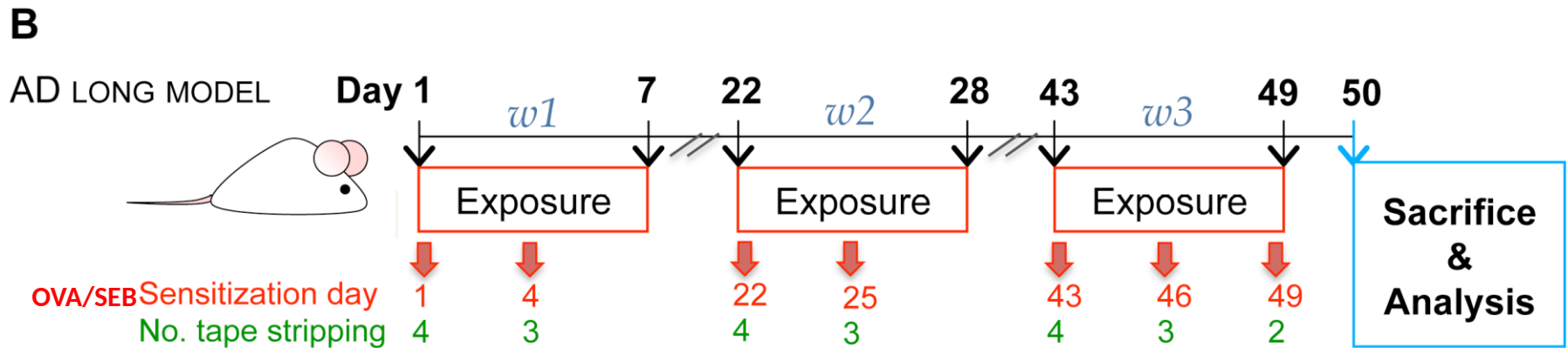
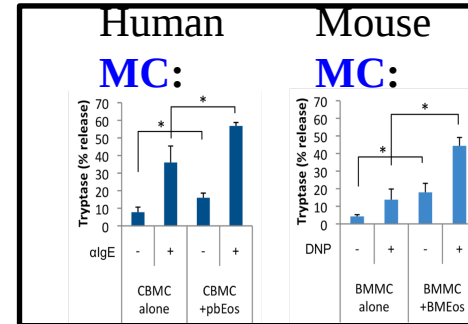
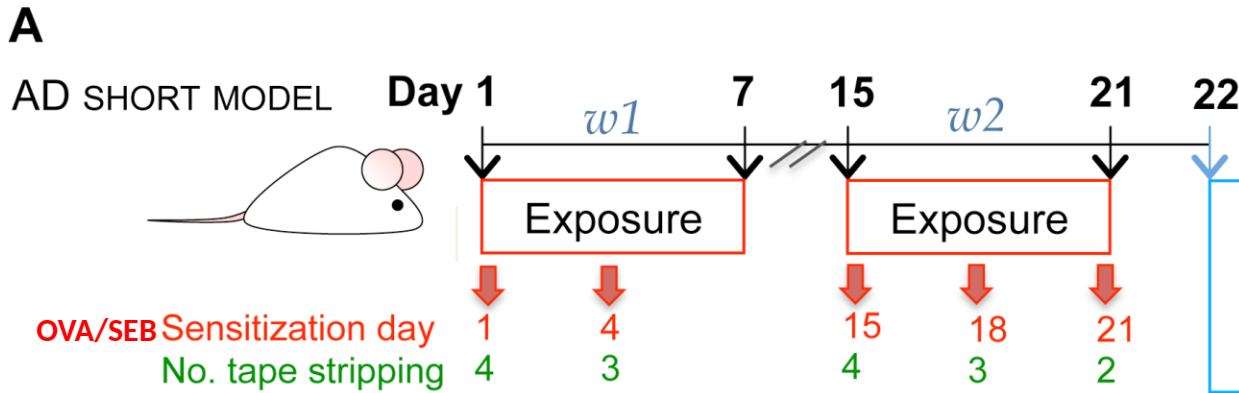
- ✓ MC activation (β -Hex release, tryptase) is induced by Eos via 2B4/CD48.
- ✓ Eos activation (EPO release) is induced by MC but it is not via 2B4/CD48.
- ✓ MC and Eos maintain an activated phenotype for up to 3 days :TNF α and IL-8 release; Syk and Lyn phosphorylation; activating receptors DNAM-1, Nectin2, LFA1 and CD49b expression stable and ICAM-1 on Eos is increased.

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



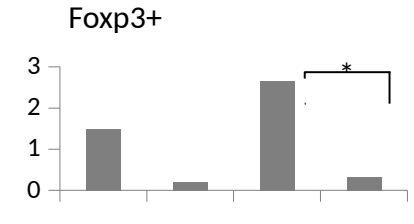
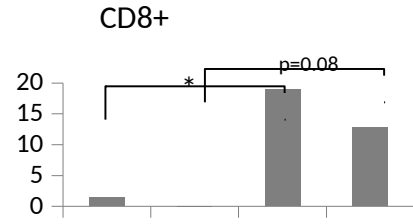
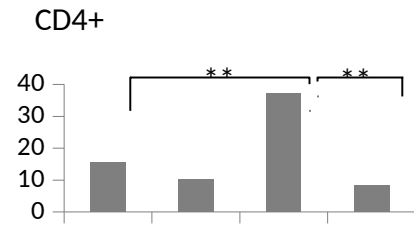
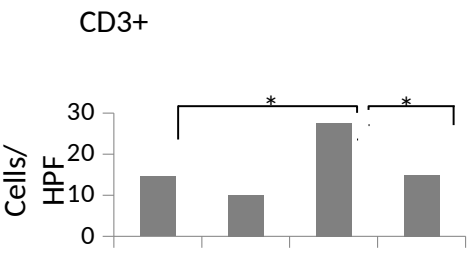
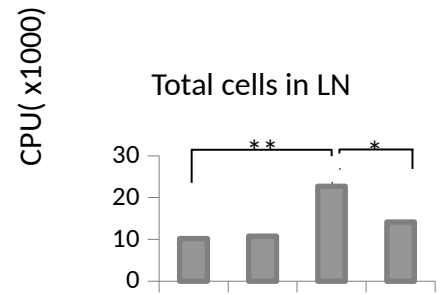
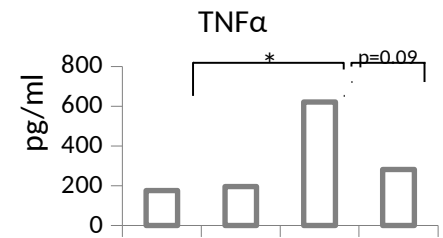
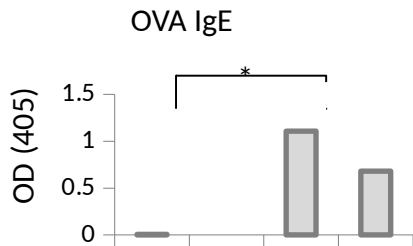
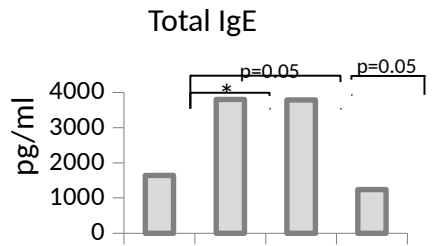
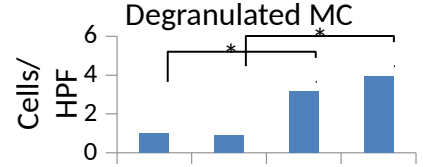
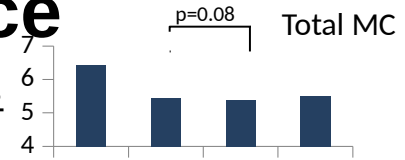
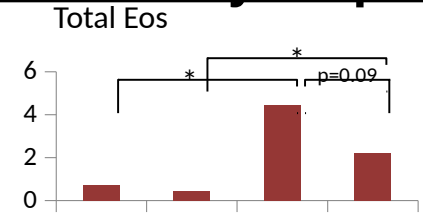
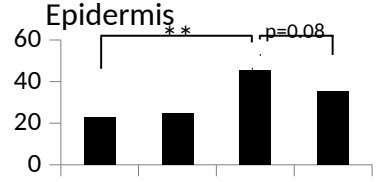
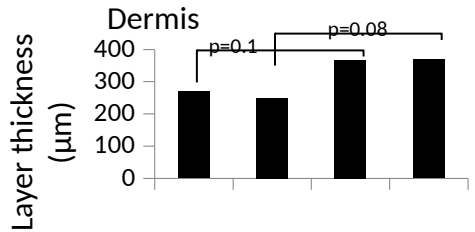
Murine Model of Atopic Dermatitis (AD)

The role of MCs, Eos, AEU and *S. aureus*



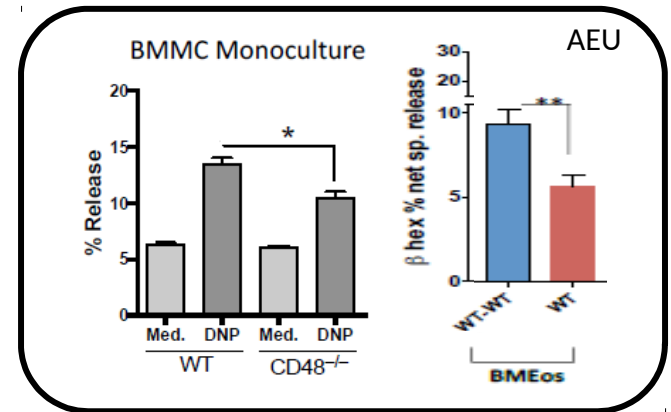
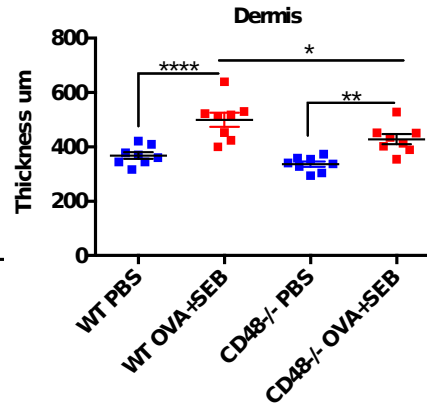
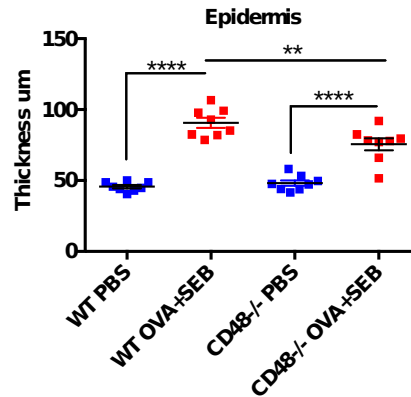
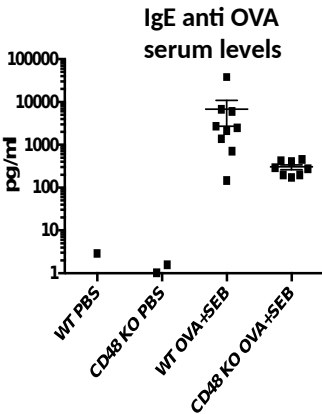
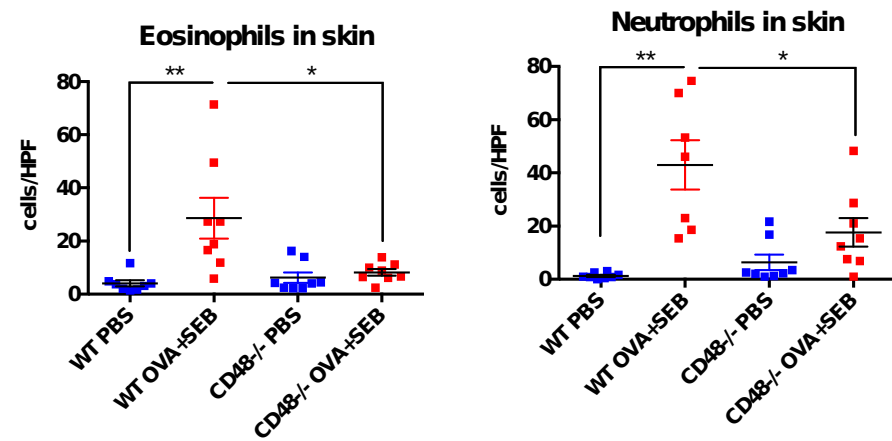
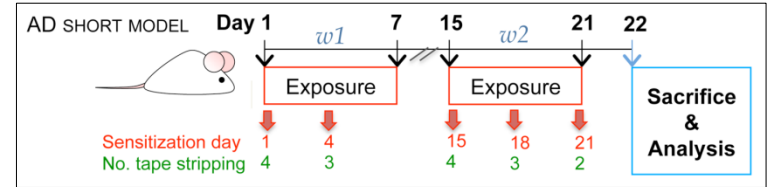
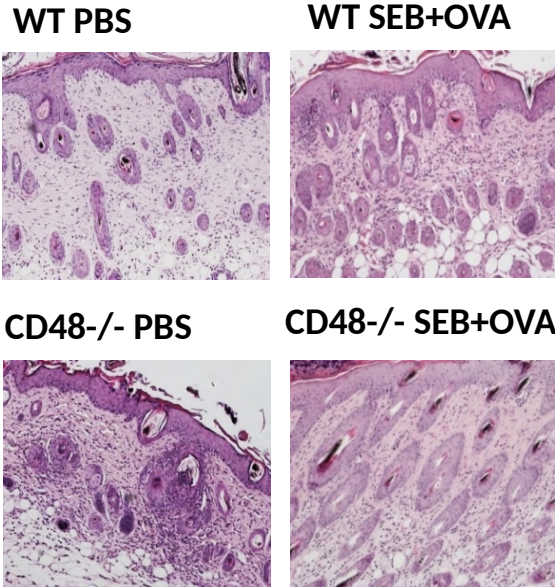
Short Term AD in **2B4^{-/-}** Mice

Decreased inflammatory responses



Short Term AD in **CD48^{-/-}** Mice

Decreased skin inflammatory responses



CD48 as Target for Anti-Allergy/Anti-Inflammation Intervention

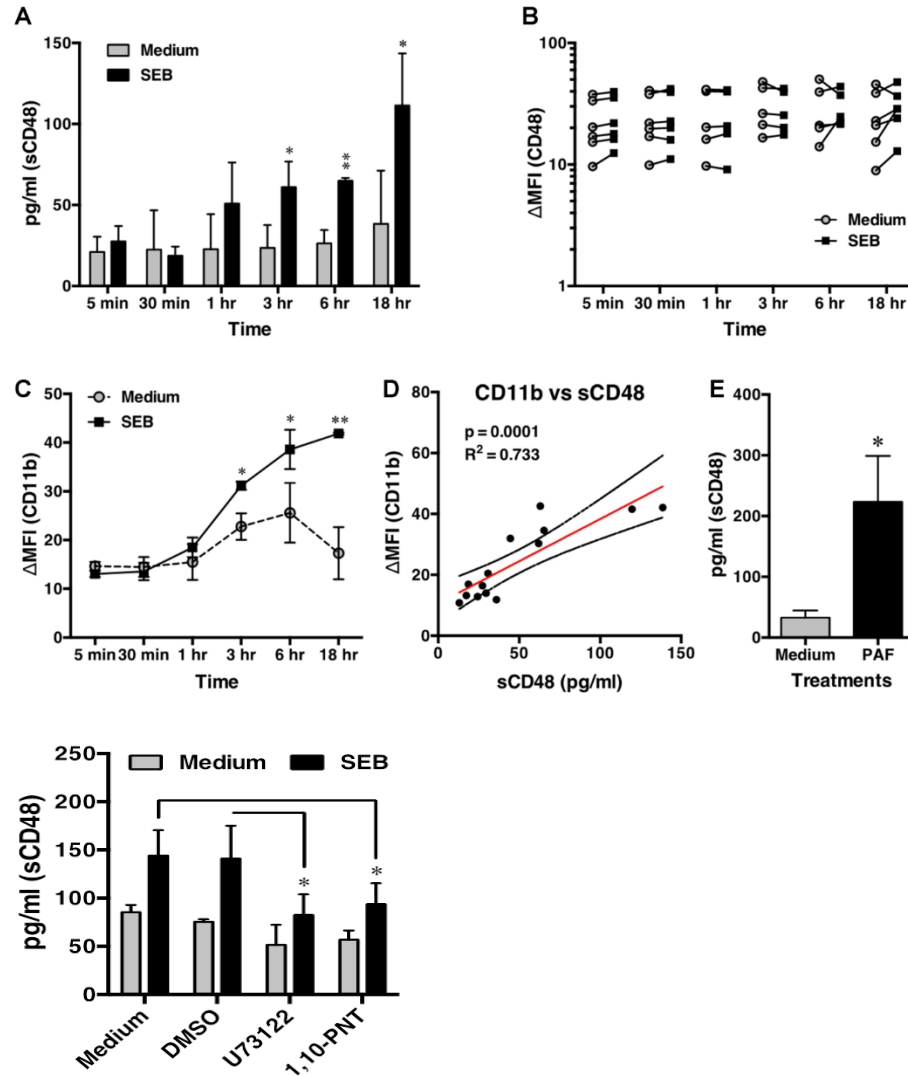
“Inhibit Activation”

- CD48 is one of the 291 mouse asthma signature-genes (Zimmerman N *et al.*, *JCI* 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, 2014, *J Invest Dermatol* 2014).
- Both MCs and Eos express CD48 that is 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, *Allergy* 2016).
- 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).
- 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. Reserch*, in press)



The Importance of CD48: *S.aureus*-Eos

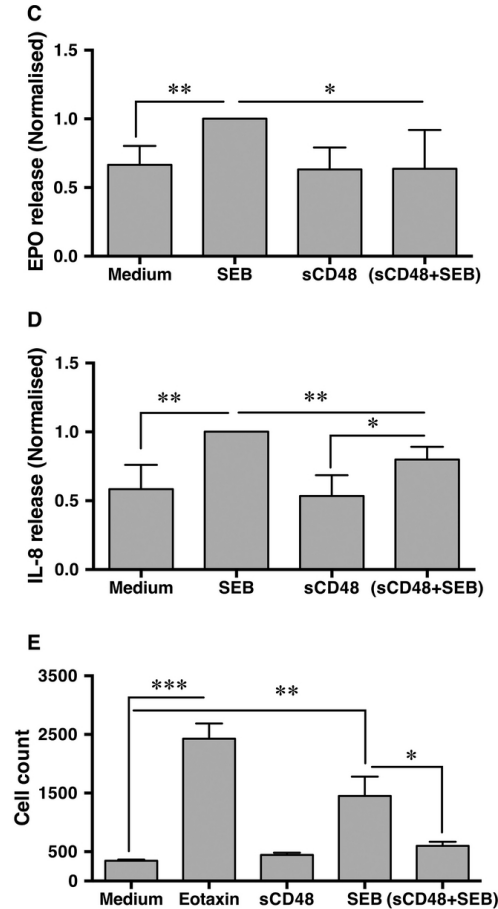
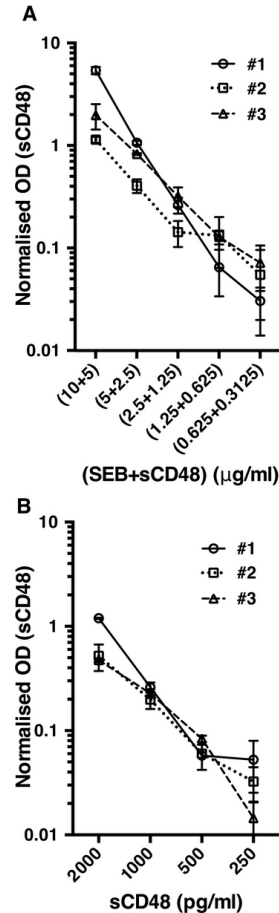
SEB Regulates CD48 on Eos and sCD48 Formation via a Phospholipase Mechanism



The Importance of CD48: S.aureus-Eos

sCD48 binds to SEB and acts as a decoy receptor on Eos

sCD48 binding with SEB



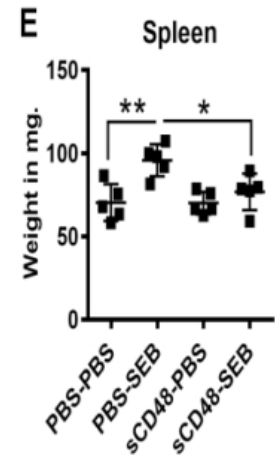
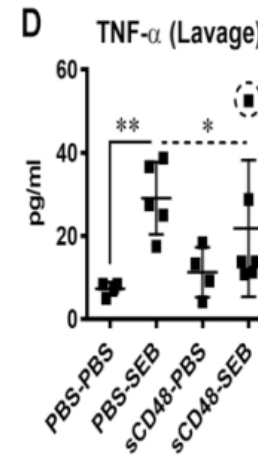
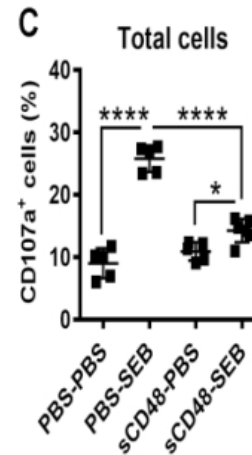
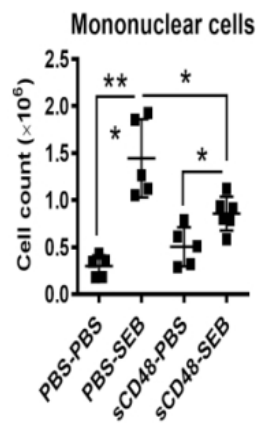
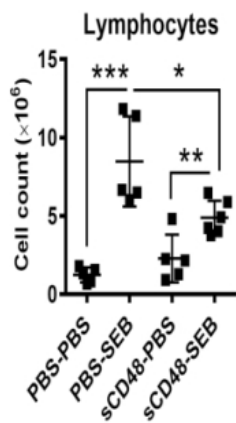
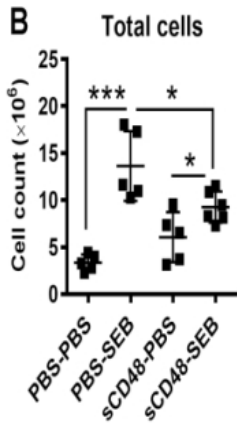
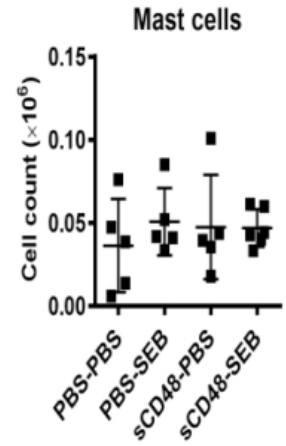
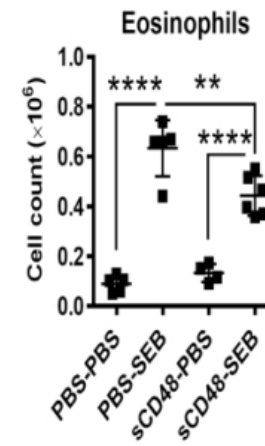
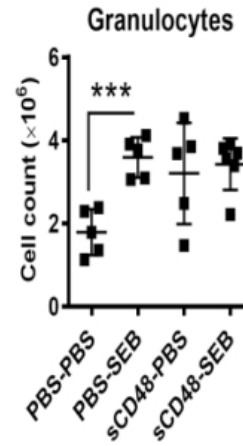
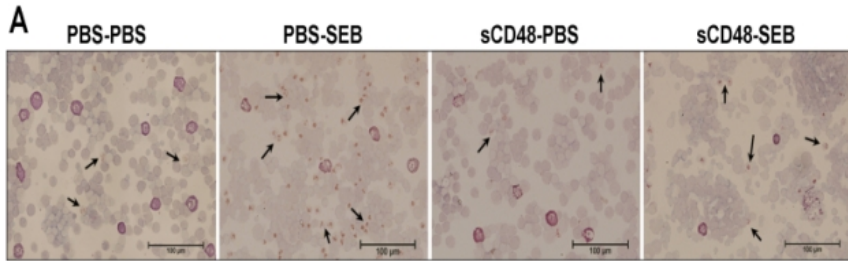
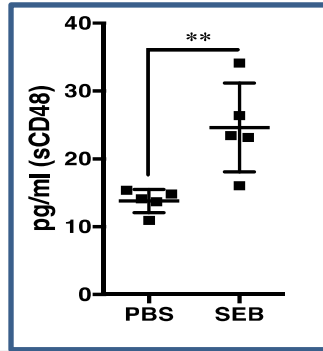
Anti-inflammatory effects of sCD48 *in vitro*



The Importance of CD48: S.aureus-Eos

sCD48 is anti-inflammatory in SEB induced peritonitis

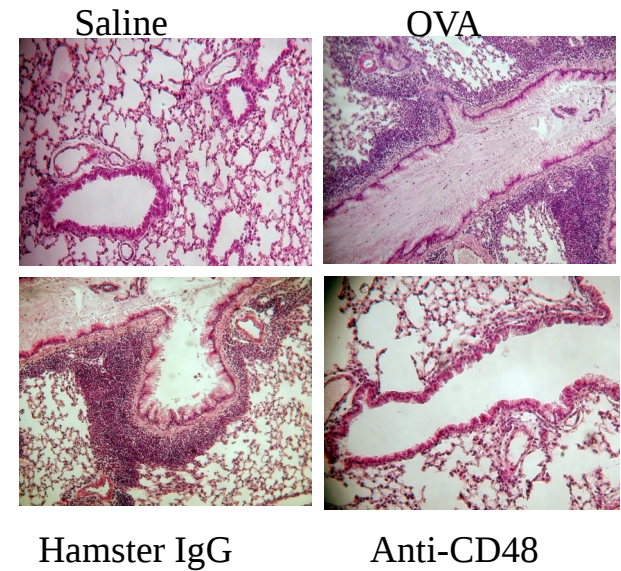
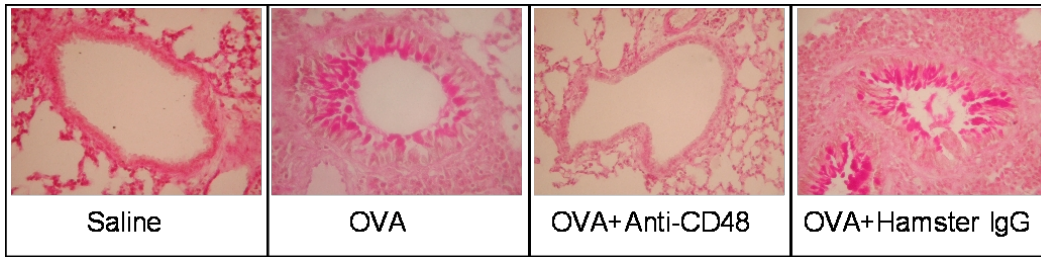
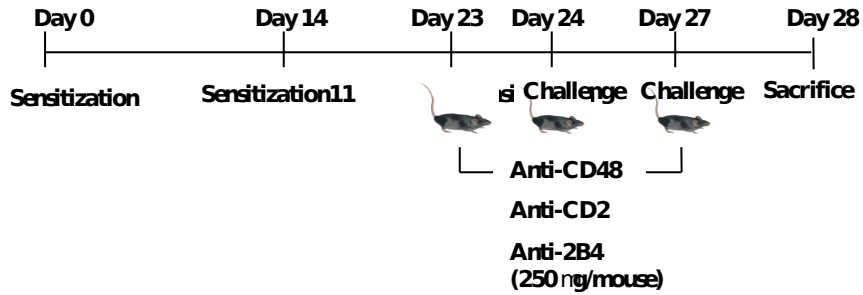
SEB induced peritonitis in mice



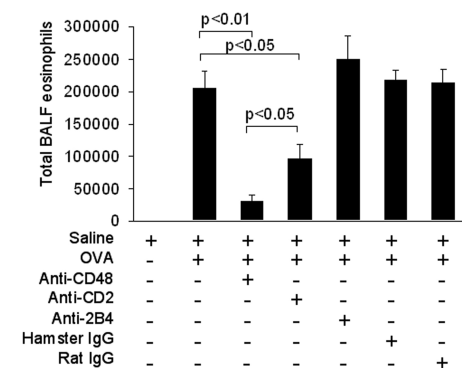
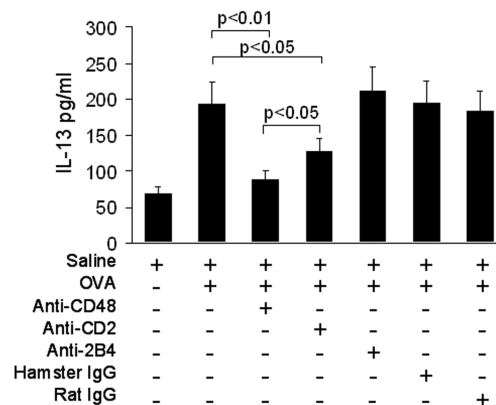
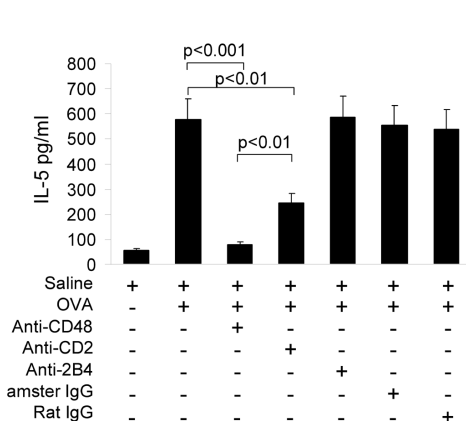
$n=3$. *, $p<0.05$. **, $p<0.01$. ***, $p<0.001$ and ****, $p<0.0001$.



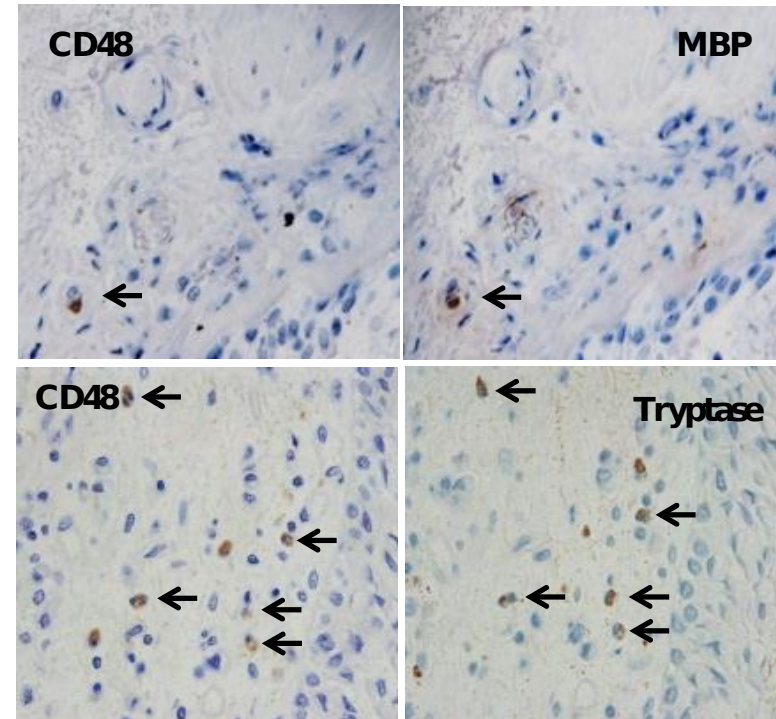
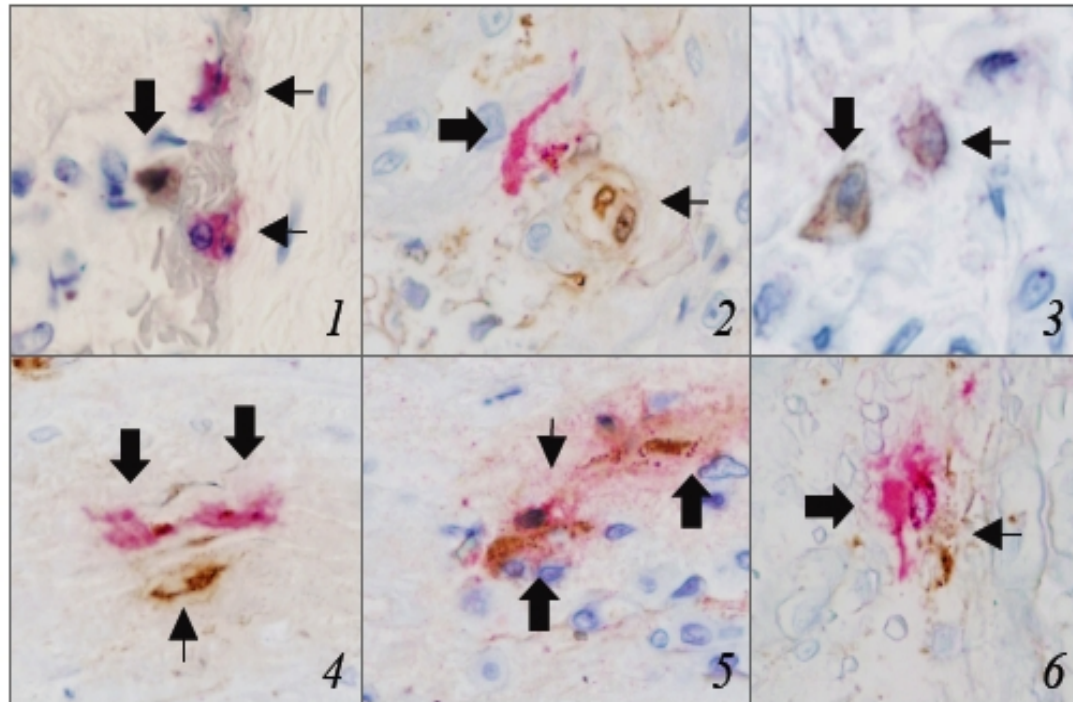
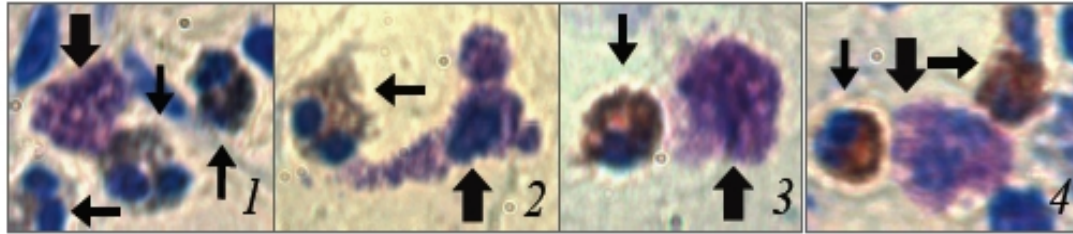
Neutralization of CD48 Inhibits Mouse Asthma



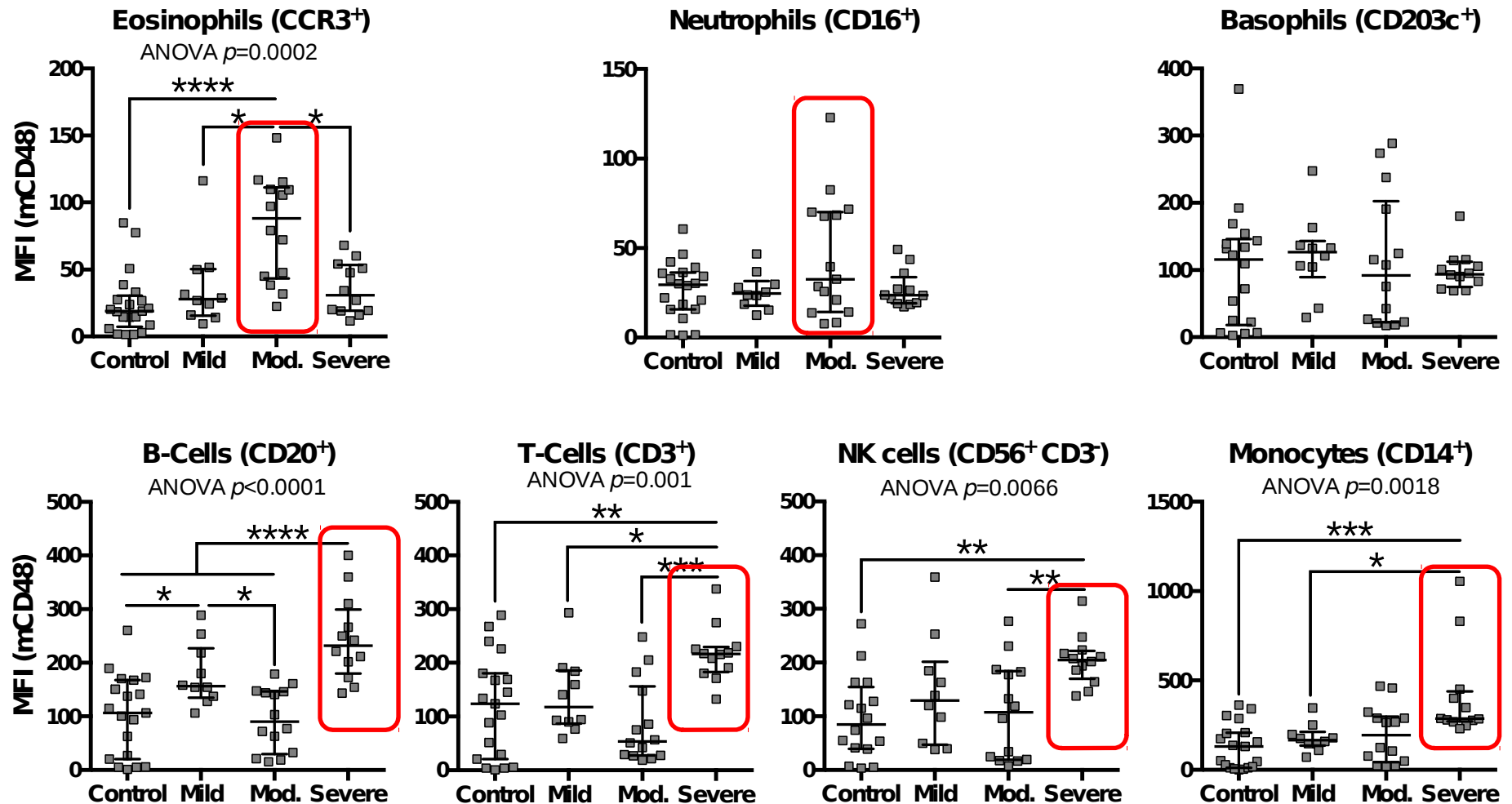
Decrease of eosinophilia, cytokine and chemokine production



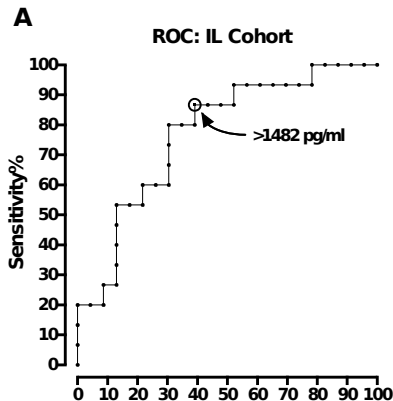
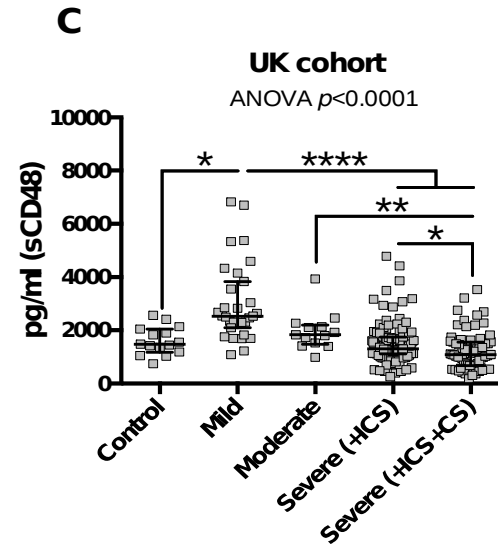
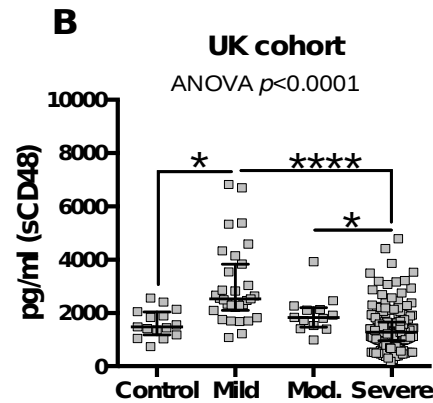
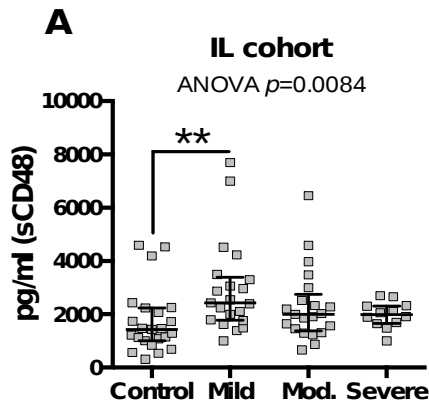
Human Asthma, Human Atopic Dermatitis the AEU and CD48



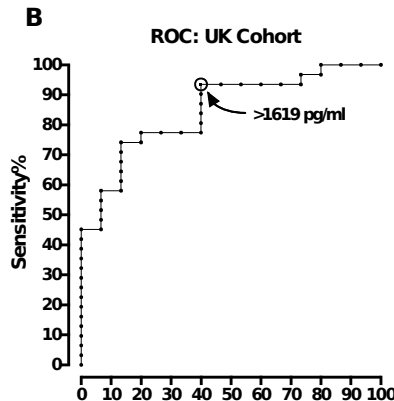
mCD48 is Differentially Expressed on Blood Leukocytes of Asthma Patients with Varying Severity



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



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



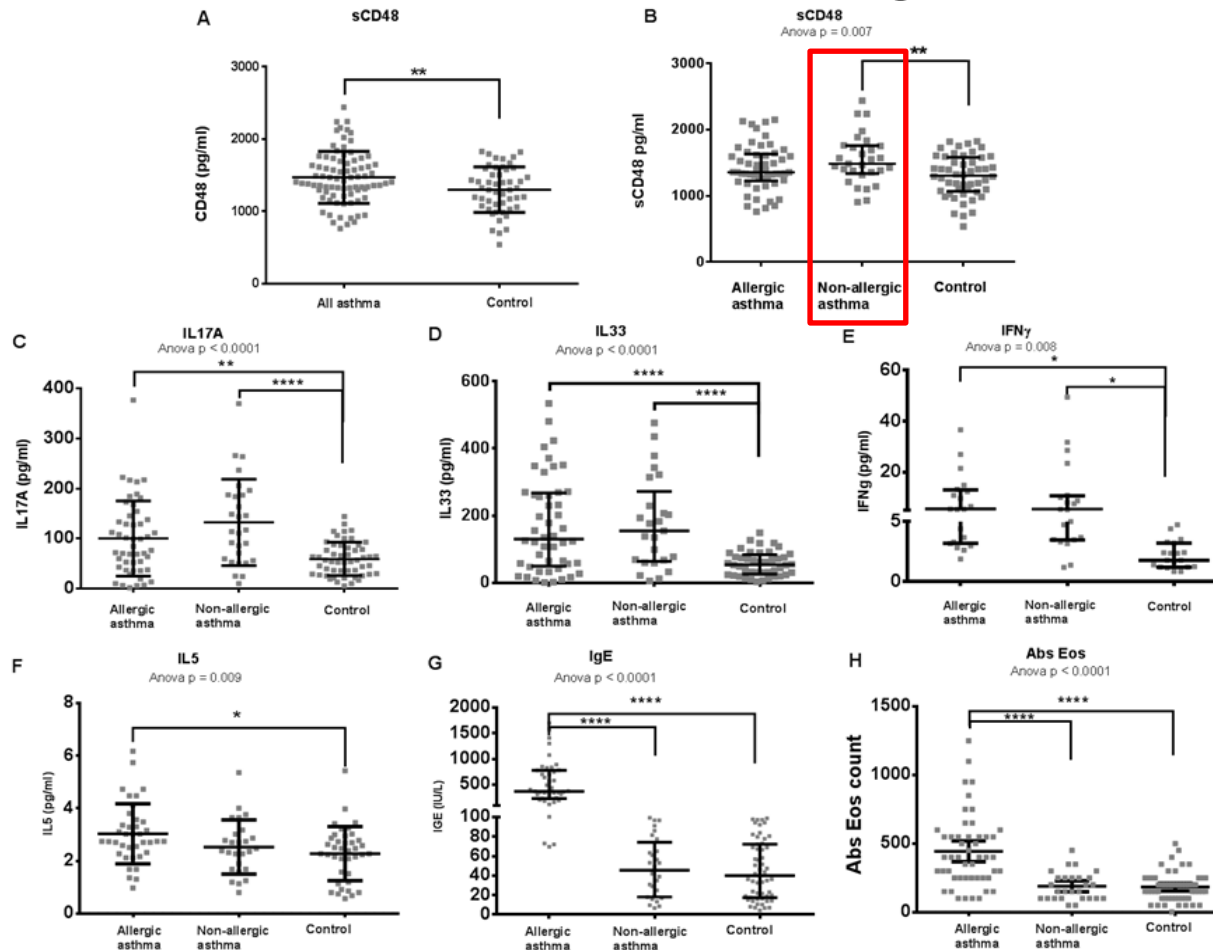
Results	
Area under the ROC curve	Area
Area	0.8495
Std. Error	0.05729
95% confidence interval	0.7371 to 0.9618
P value	0.0001

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

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



Is CD48 a New Independent Biomarker for Airway Inflammation and Non-allergic Asthma?



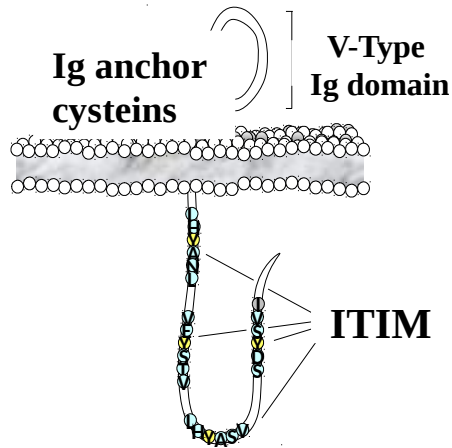
sCD48 in volunteers with asthma and control (A); sCD48 (B), cytokines (C-F), IgE (G) and absolute eosinophil numbers (H) in volunteers with allergic asthma, non-allergic asthma and control.

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Abs – absolute, Eos - eosinophil

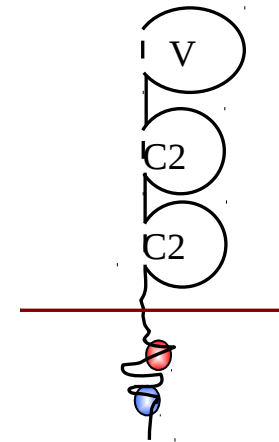


“Our” Inhibitory Receptors

CD300a



Siglec7

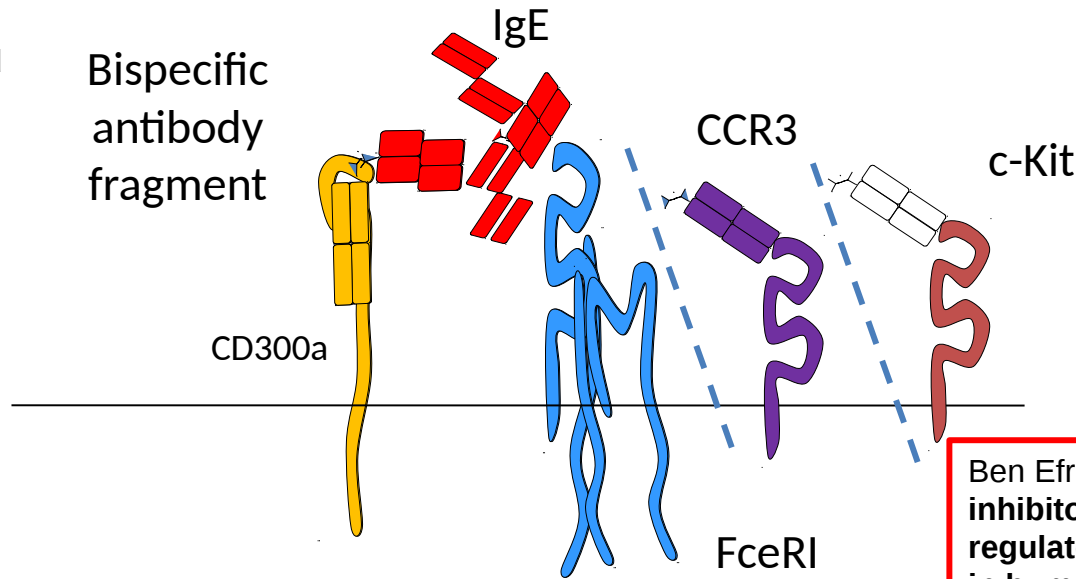


- It belongs to the Ig superfamily
- 3 classical and one non classical ITIMs
- Expressed on NK cells, neutrophils, T and B lymphocytes, **mast cells**, **eosinophils**, **basophils**. Expressed on malignant cells
- CD300a recognizes phosphatidyl serine (PS) and phosphatidylethanolamine (PE) on apoptotic cells

- It belongs to the Ig superfamily
- 1 classical ITIM and 1 ITIM like
- Expressed on NK cells, monocytes, subset of CD8 T cells, **mast cells**, **eosinophils**, **basophils**. Expressed on malignant cells
- Siglec-7 recognizes sugars with sialic acid *N*-acetylneuraminic acid (Neu5Ac)



Karra et al , Submitted
"CD300a expression and
role in atopic dermatitis"



Ben Efraim *et al.*, *Allergy* 2013 The inhibitory receptor **CD300a** is up-regulated by **hypoxia** and **GM-CSF** in human peripheral blood eosinophils.

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

Journal of Allergy and Clinical Immunology

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



ELSEVIER





THE JOURNAL OF
IMMUNOLOGY

HOME | ABOUT | SUBMIT | AUTHOR INFO | ARCHIVE | SUBSCRIBE | PERMISSIONS | ADV

Institution: Berman National Medical Library, Hebrew University

Mechanisms of asthma and allergic inflammation

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

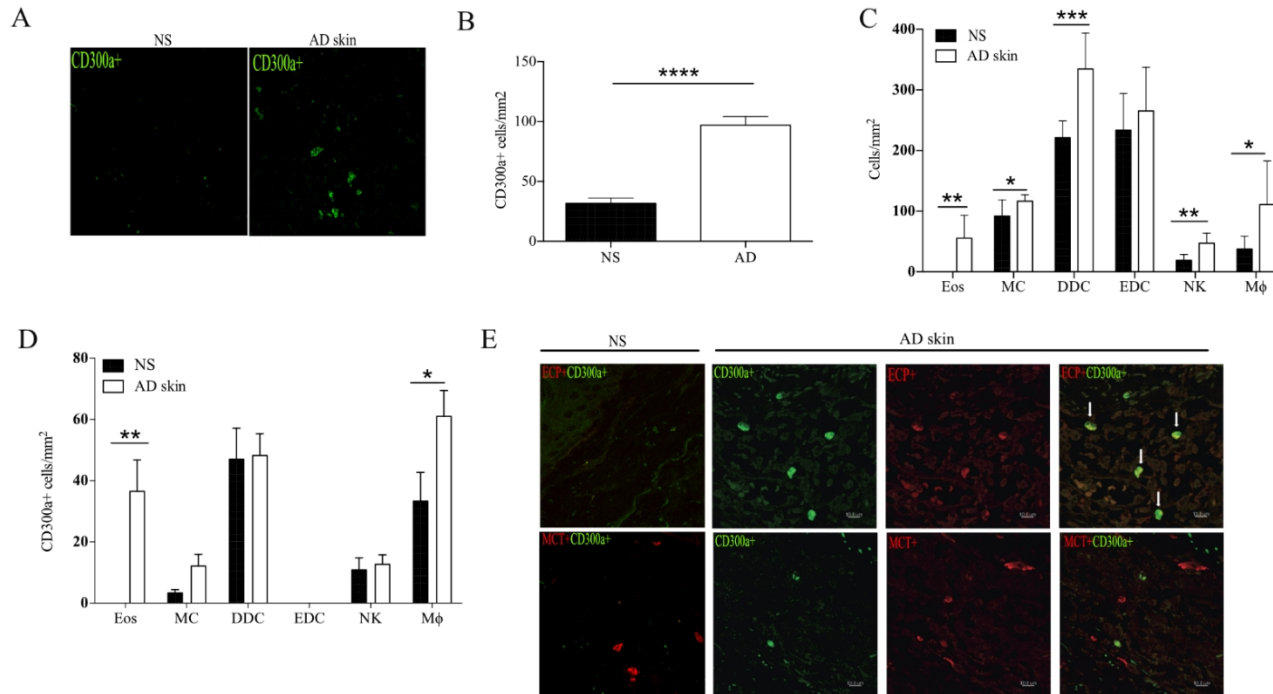
Ariel Munitz, MSc¹, Ido Bachelet, MSc¹, Francesca Levi-Schaffer, PhD  

Suppression of Normal and Malignant Kit Signaling by a Bispecific Antibody Linking Kit with CD300a¹

Ido Bachelet¹, Ariel Munitz¹, Beata Berent-Maoz², David Mankuta¹ and Francesca Levi-Schaffer²



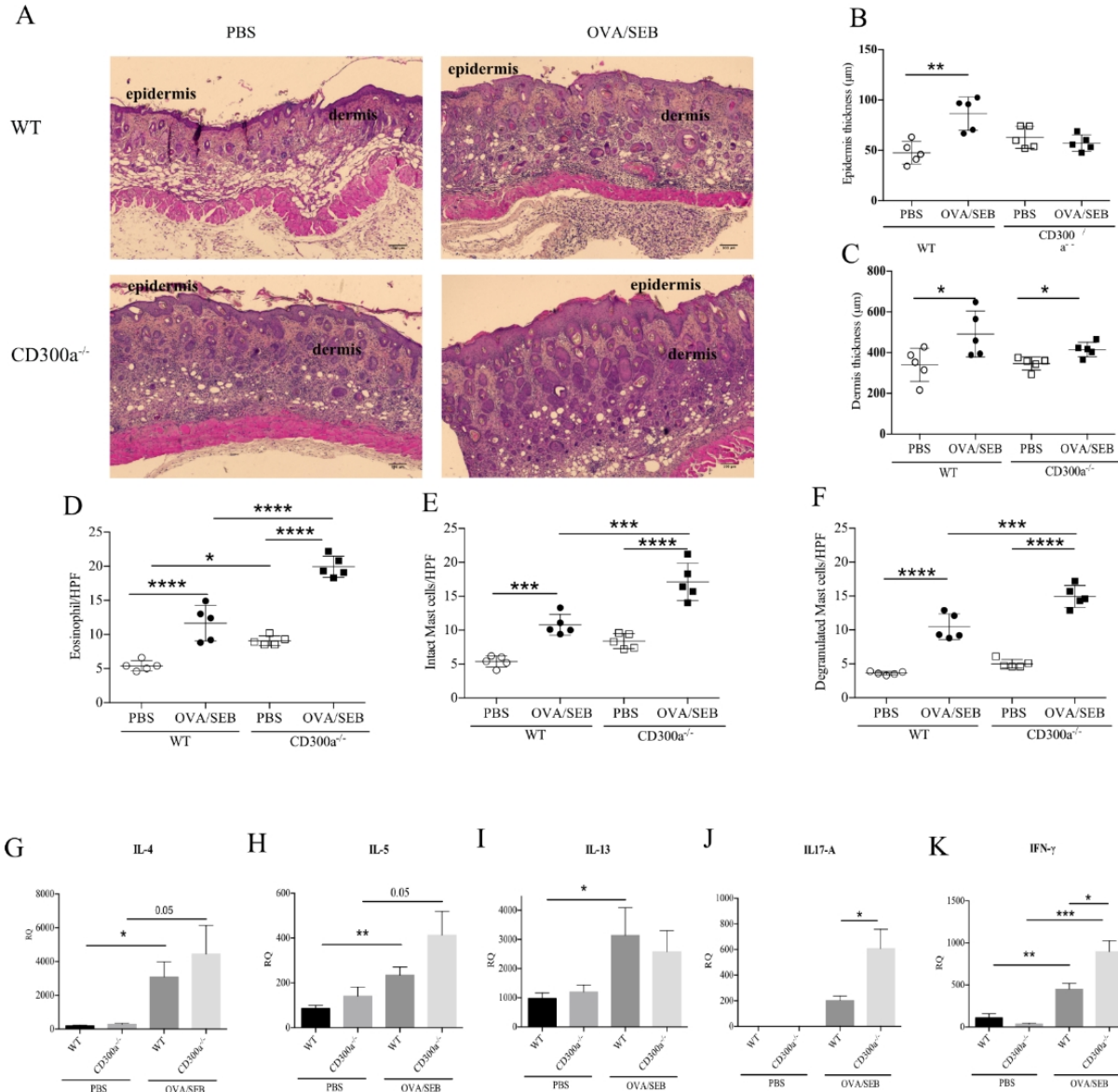
CD300a expression is differentially increased in the lesional skin of AD patients.



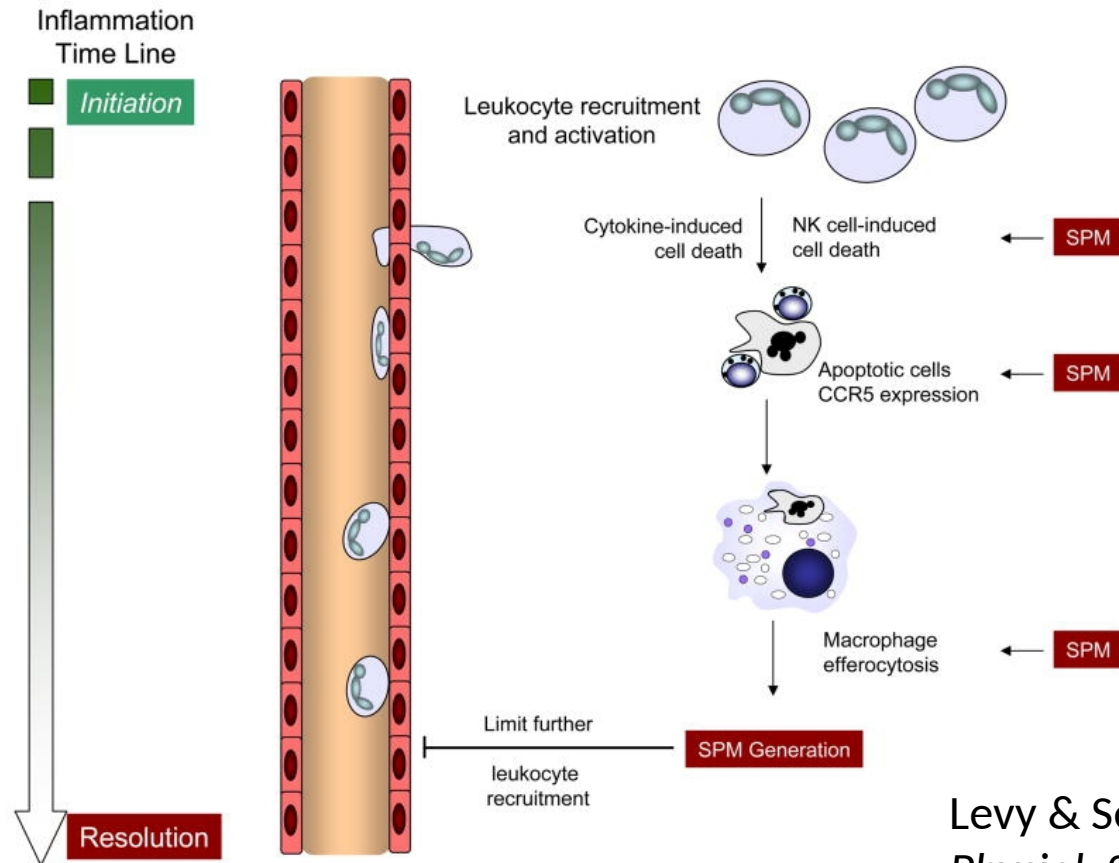
Karra L et al ,under revision



Skin thickness and inflammation are modulated in an AD model in CD300a^{-/-} mice



SPM promote the resolution of tissue inflammation and limit further leukocyte recruitment

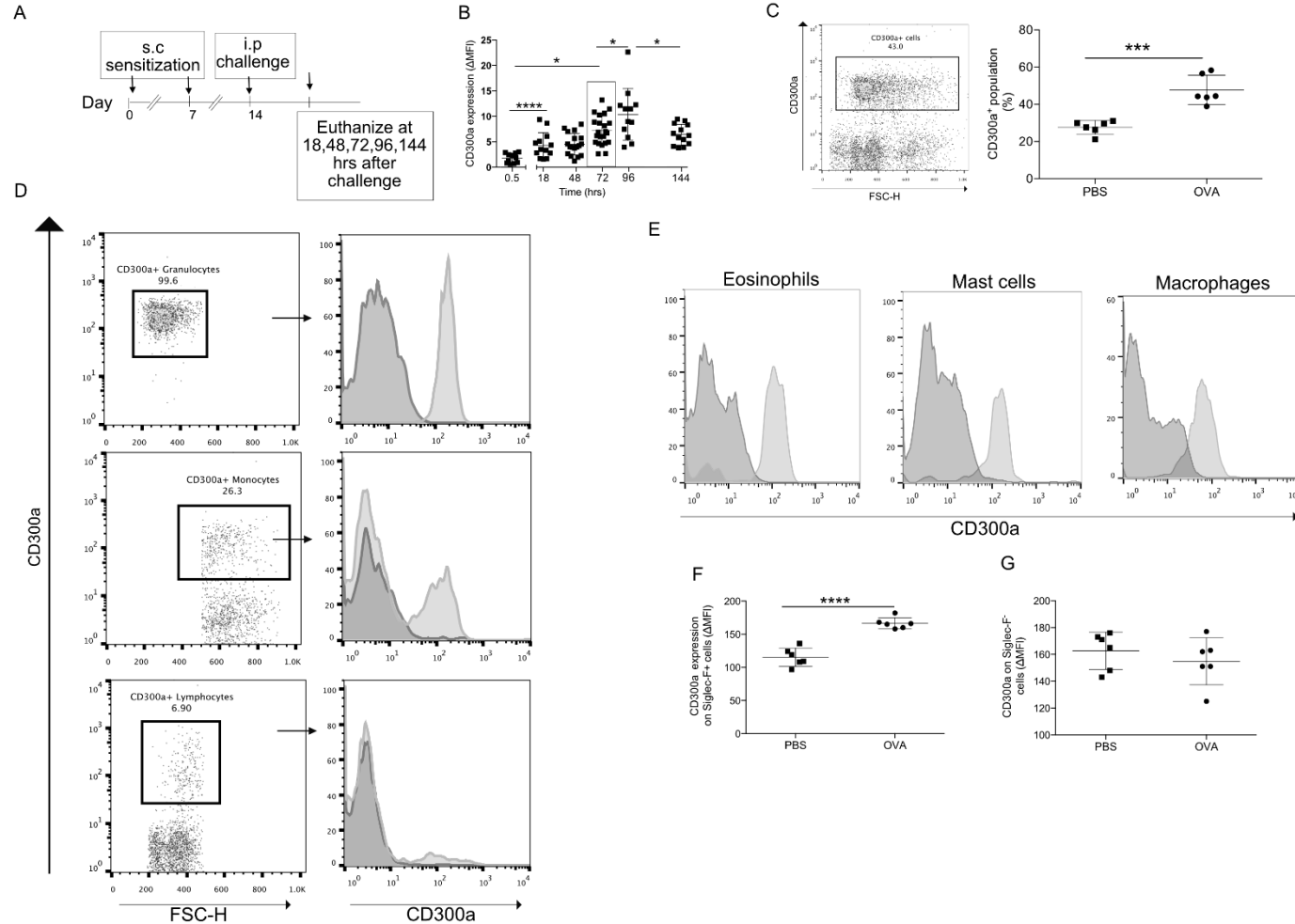


Levy & Serhan, *Annu Rev Physiol*, 2014

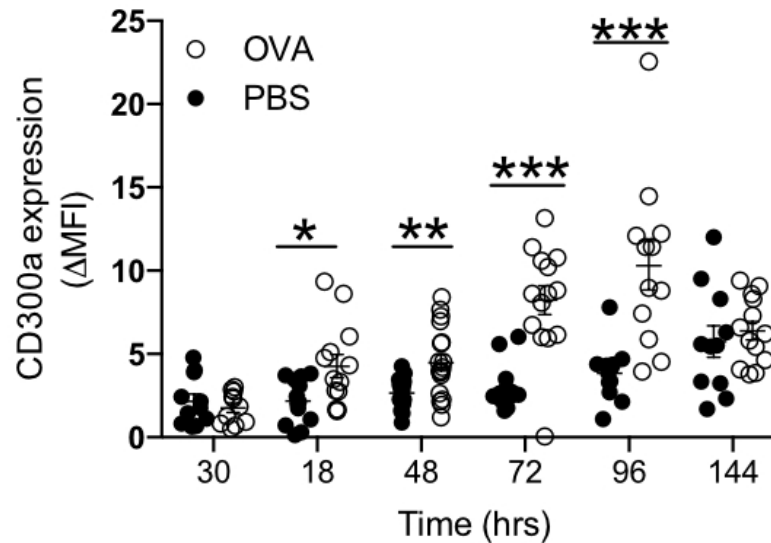
Are both CD300a and SPMs involved in resolution of allergic peritonitis ?



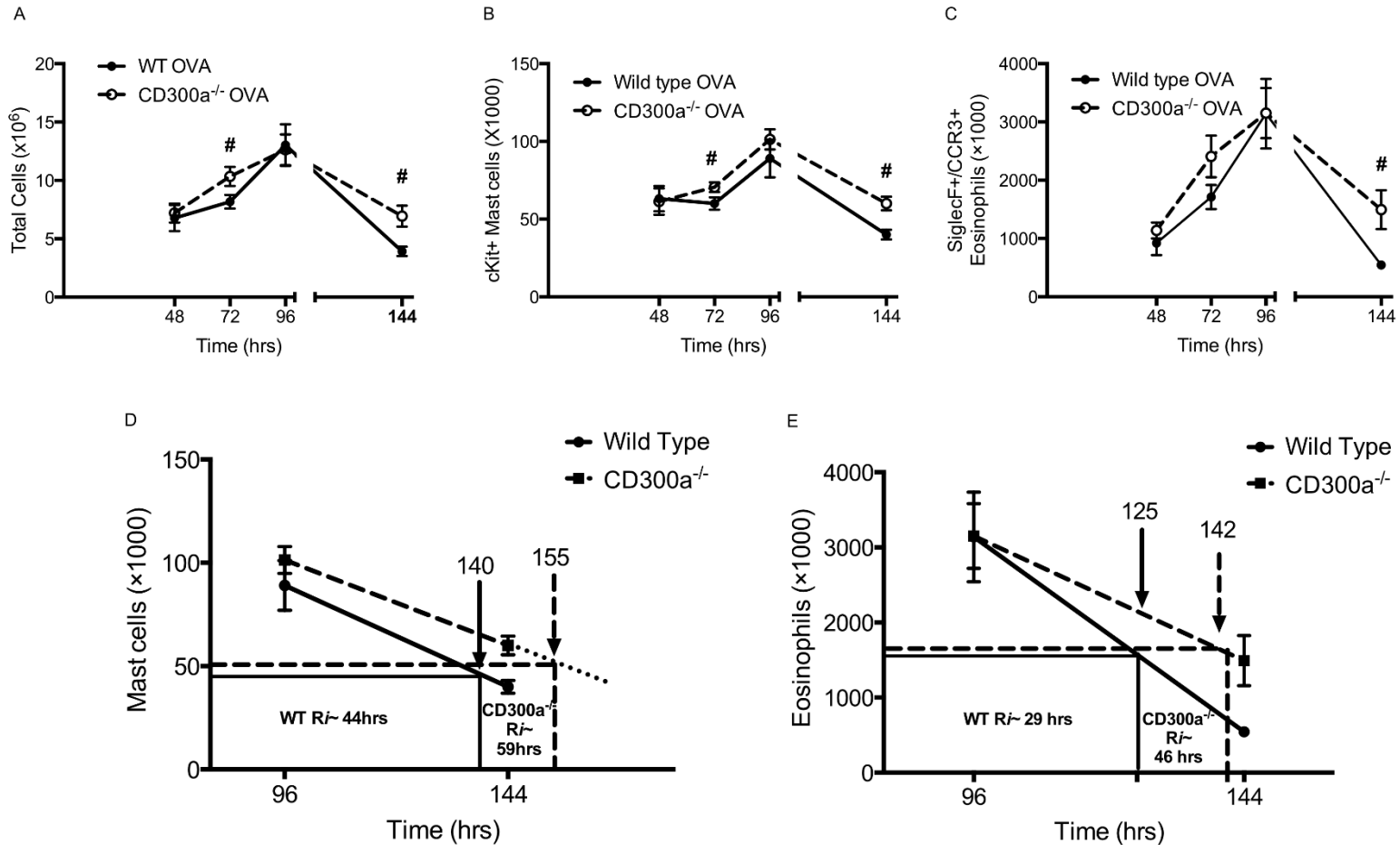
CD300a expression on peritoneal cells is modulated in an AP model



CD300a increased expression on peritoneal cells is allergen challenge-specific

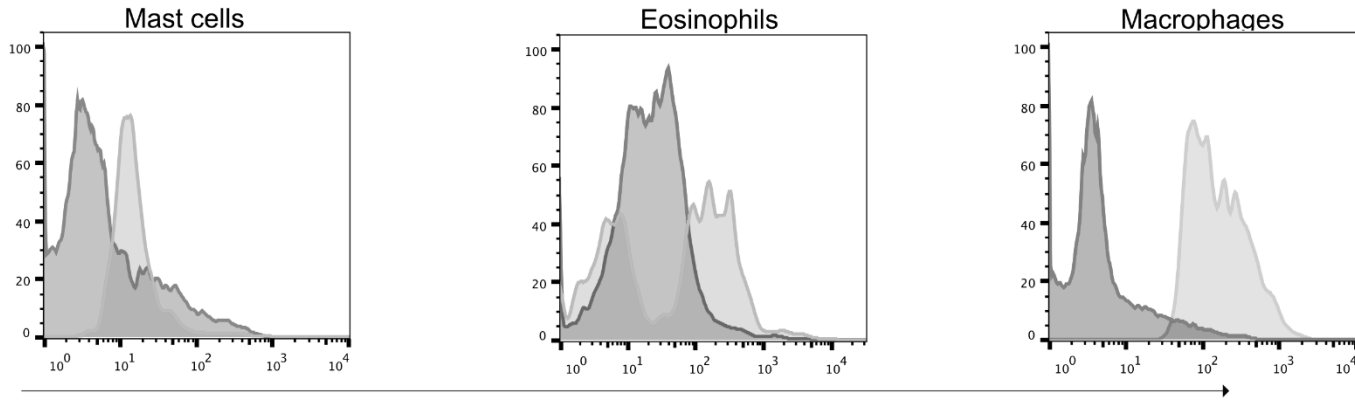


CD300a^{-/-} mice present a delayed resolution of inflammation in the AP model



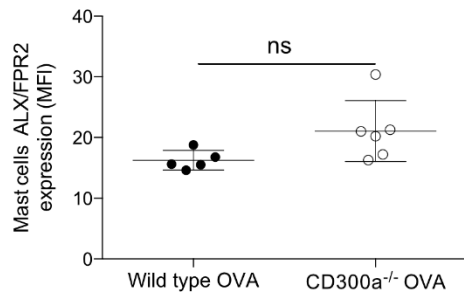
ALX/FPR2 is down-regulated on Eos while LXA₄ is increased in the peritoneum of CD300a^{-/-} mice in the AP model

A

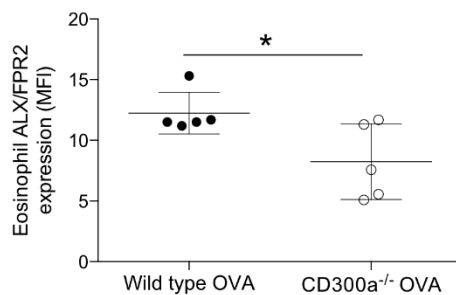


CD300a^{-/-}, 48 hrs AP

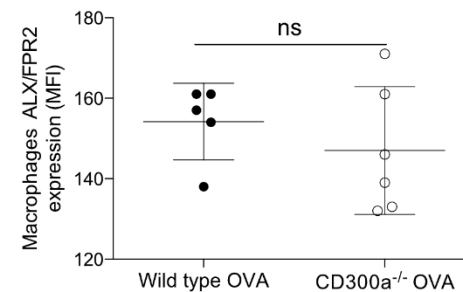
D



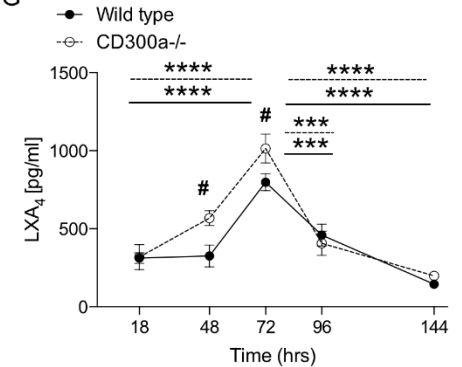
E



F

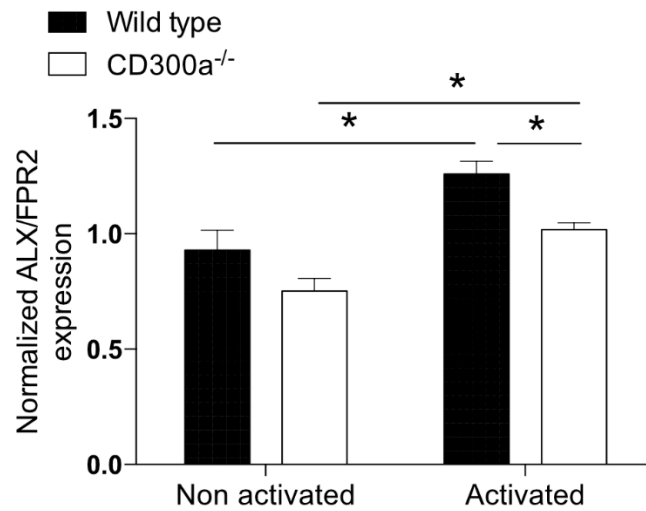


G

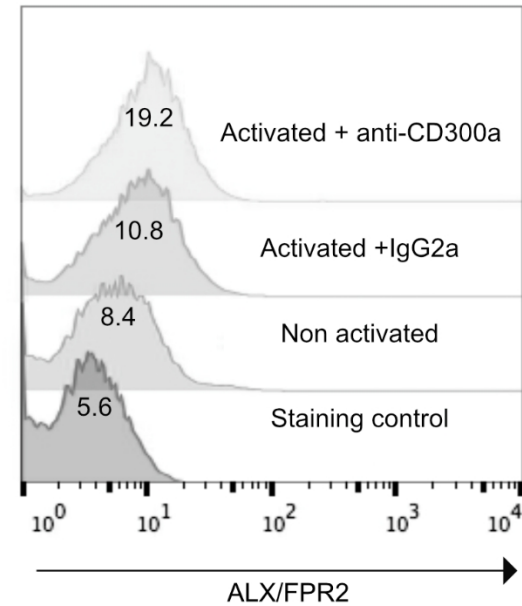


CD300a activation modulates ALX/FPR2 expression on BMMC

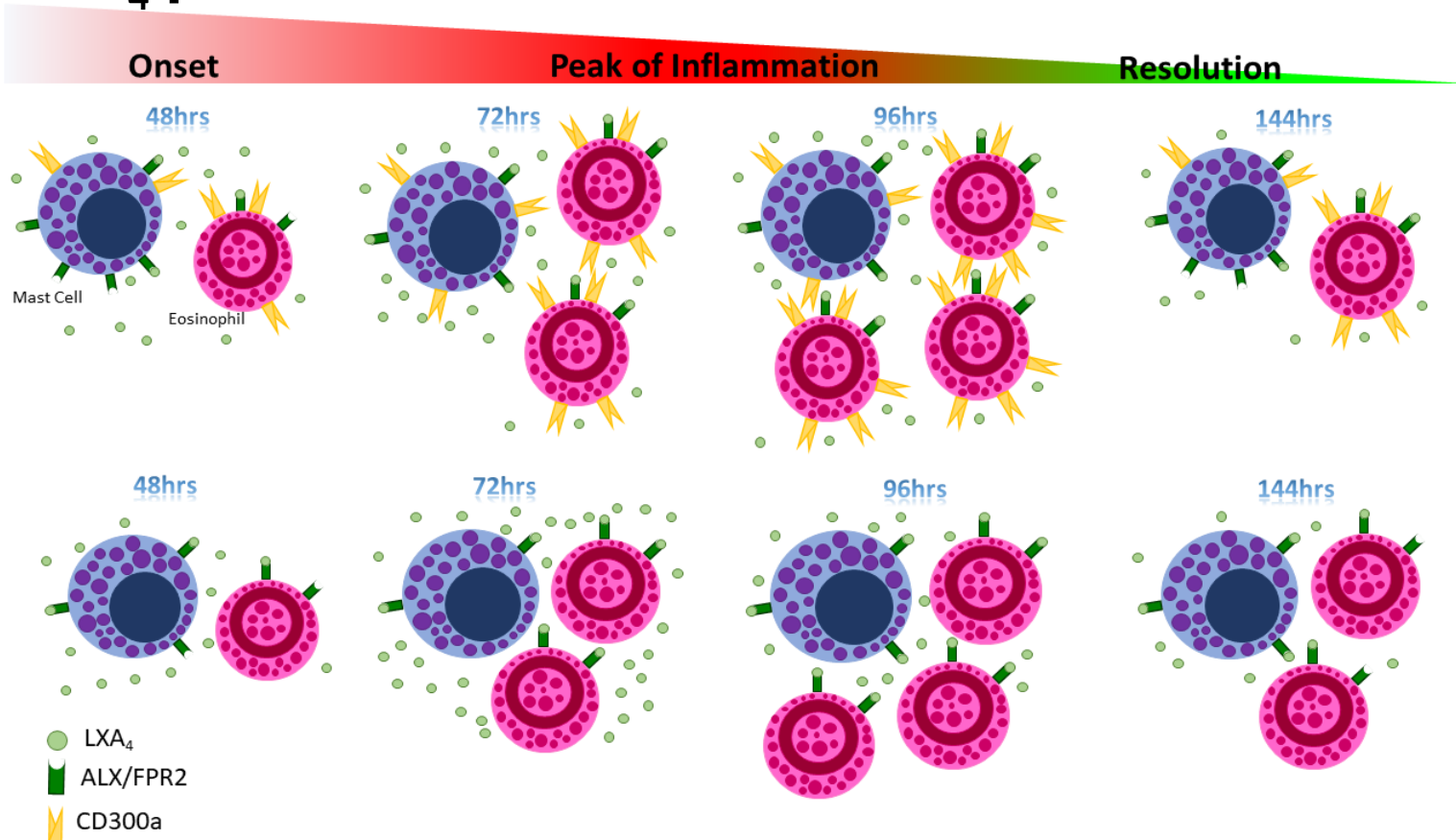
A



B



AP spatiotemporal expression of mast cell and eosinophil associated CD300a and ALX/FPR2, and LXA₄ production.



Conclusion :Leukocyte CD300a contributes to the resolution of murine allergic inflammation



Summary

We have demonstrated the important role of the MC and Eos shared receptors CD48 and CD300a.

Conclusions

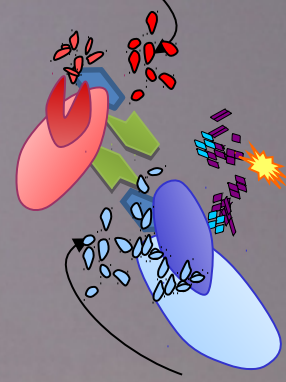
Allergic inflammation and other diseases in which mast cells and eosinophils have a role can be down-regulated by immunopharmacological modulation of these cells either by inhibiting the activating receptor **CD48** or by activating the inhibitory receptors **CD300a** (and **Siglec-7**).

What is the best strategy in the allergic patients? To personalize the treatment. For a subgroup of patients who display high CD48 expression and do not respond optimally to any of the currently available therapies, to block CD48. For all the subtypes of patients who display CD300a or Siglec-7 to activate these receptors.





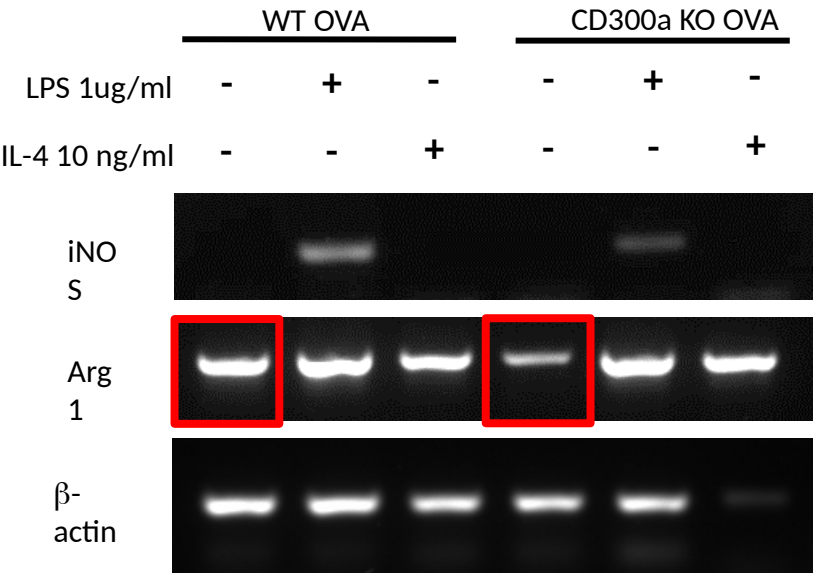
Thanks !



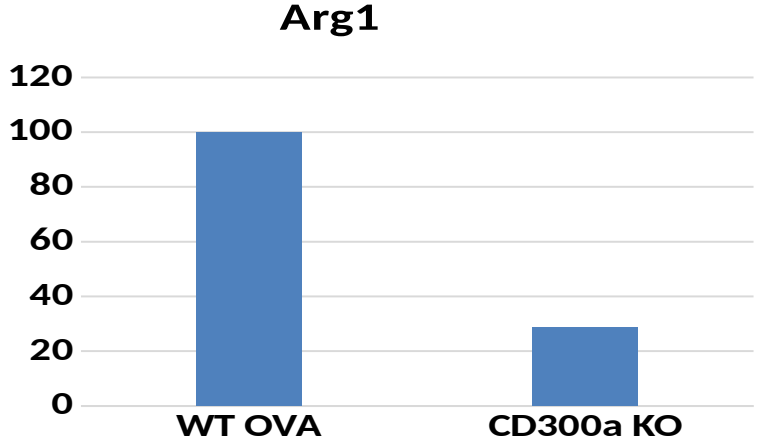
Micha Ben Zimra
Nadine Landolina
Hadas Pahima
Pier Giorgio Puzzovio
Mansour Seaf
Ilan Zaffran
Yaara Zoabi

Research grants: ISF (Israel Academy of Science), BSF (United States-Israel Binal Science Foundation), Aimwell Trust Foundation, A Gutmann Funds, Rosetrees Trust

Macrophages from peritoneal lavage of WT and CD300a KO Balb/c, AP 48 hrs, Expression of iNOS and Arg1



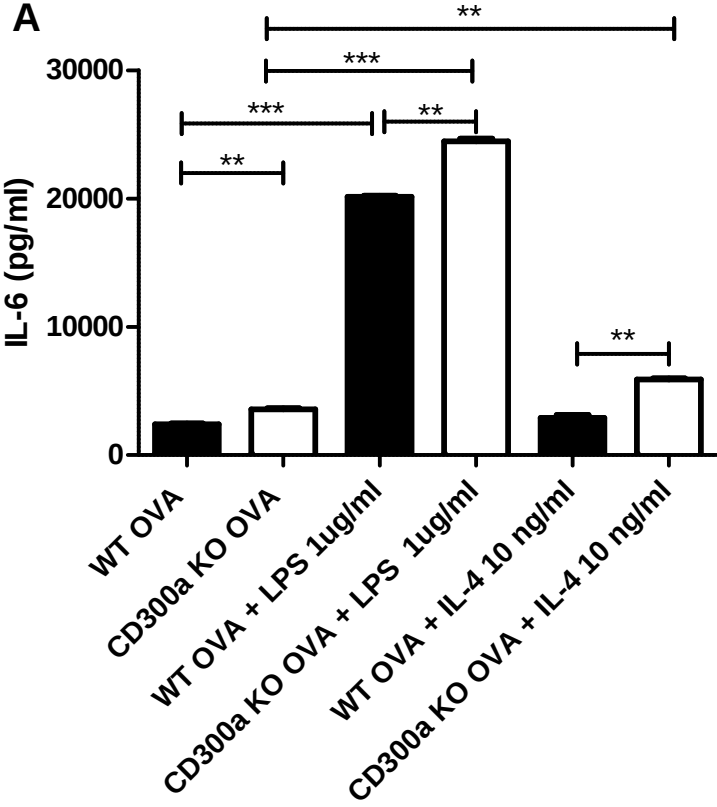
Level of expression of Arg1 (% in response to WT)



iNOS (marker for M1 Macrophages) mRNA appears only after treatment with LPS, but is reduced in CD300a KO macrophages. LPS is commonly used as inducer of M1 phenotype.

Arg1 (marker for M2 macrophages) mRNA is reduced in CD300a untreated macrophages (marked in red squares). IL-4 is used as inducer of M2 phenotype.

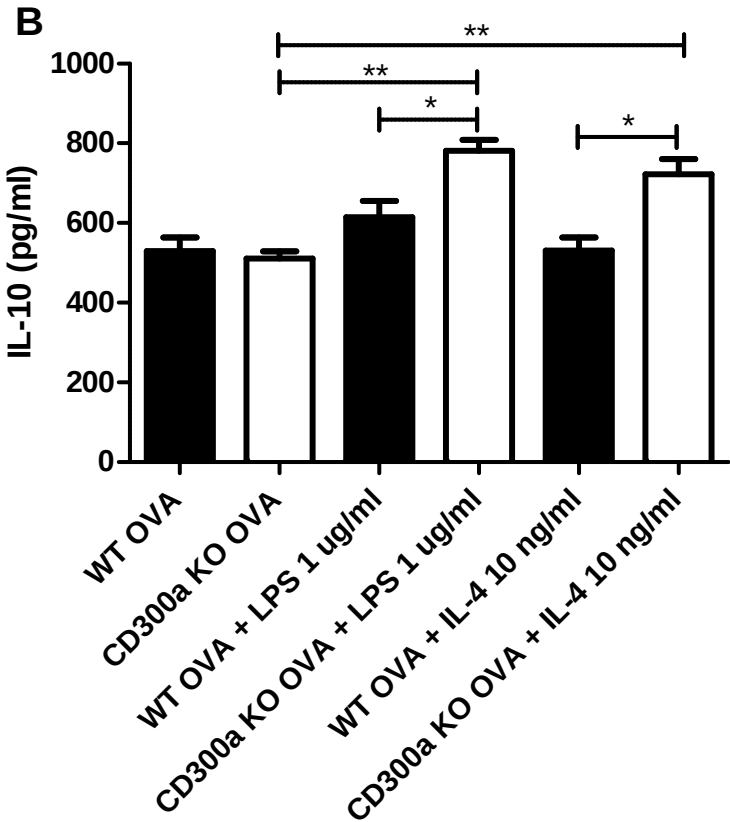
Macrophages from peritoneal lavage of WT and CD300a KO Balb/c, AP 48 hrs, IL-6 release



CD300a KO macrophages show significant increase in IL-6 production in respect to WT

Significant increases occur in IL-6 release after treatment with LPS and IL-4

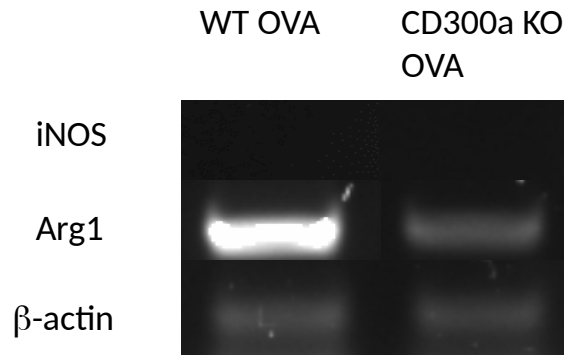
Macrophages from peritoneal lavage of WT and CD300a KO Balb/c, AP 48 hrs, IL-10 release



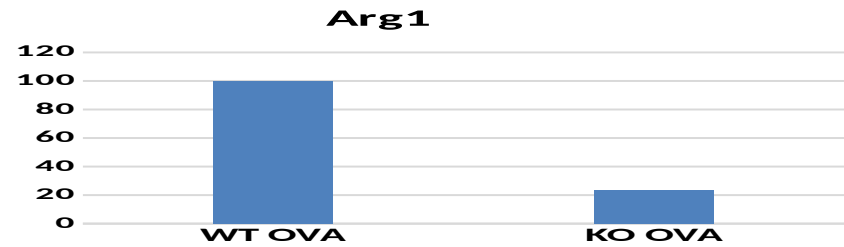
CD300a KO macrophages have no significant change in IL-10 release in respect to WT (maybe because of the early time point?).

After treatment with LPS or IL-4, IL-10 release increases significantly in CD300a KO macrophages.

Macrophages from peritoneal lavage of WT and CD300a KO Balb/c, AP 96 hrs, Expression of iNOS and Arg1



Level of expression of Arg1 (relative to WT)



Macrophages from peritoneal lavage of WT and CD300a KO Balb/c, AP 96 hrs, cytokines release

