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 ethiopathogenesis) **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"

- APC
- CD4+ T cell
- Th2 cell
- B cell
- IgE
- Mast cell
- Mast cells precursors
- Basophil
- Allergen
- Eosinophil
- Smooth muscle cells
- Fibroblasts/MyoFB
- Epithelial cells

- Bacteria
- Viruses
- Endothelial cells

Time:
- Acute-Early phase
- Late phase
- Chronic phase

Inflammation, Angiogenesis, tissue remodeling, fibrosis

Time
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
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

Shamri T et al: under revision
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

CD48
- S. Aureus and its exotoxins
- Bacteria
- Fungi
- Viruses
- Allergens

Mast cell

Eosinophil

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

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**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: TNFα 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.

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

A

AD SHORT MODEL

Day 1 7 15 21 22

Exposure Exposure Exposure

OVA/SEB Sensitization day
No. tape stripping
1 4 15 18 21

4 3 4 3 2

Sacrifice & Analysis

B

AD LONG MODEL

Day 1 7 22 28 43 49 50

Exposure Exposure Exposure Exposure

OVA/SEB Sensitization day
No. tape stripping
1 4 22 25 43 46 49

4 3 4 3 2

Sacrifice & Analysis

Adapted from Wang.G et al, CEA 2007

Elishmereni M et al, Allergy, 2013

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Short Term AD in 2B4-/- Mice

Decreased inflammatory responses

**Elishmereni M et al, JID 2014**
Short Term AD in CD48-/- Mice

Decreased skin inflammatory responses

WT PBS  WT SEB+OVA

CD48-/- PBS  CD48-/- SEB+OVA

Eosinophils in skin

Neutrophils in skin

IgE anti OVA serum levels

Thicknes um

Grey cells/HPF

BMMC Monoculture

Gangwar, R.S, et al. unpublished data
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).


• 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 el 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

Gangwar RS and Levi-Schaffer F, Allergy 2016
The Importance of CD48: S. aureus-Eos

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

Anti-inflammatory effects of sCD48 in vitro

Gangwar RS and Levi-Schaffer F, Allergy 2016
The Importance of CD48: S.aureus-Eos
sCD48 is anti-inflammatory in SEB induced peritonitis

Neutralization of CD48 Inhibits Mouse Asthma

Decrease of eosinophilia, cytokine and chemokine production
Human Asthma, Human Atopic Dermatitis the AEU and CD48

Elishmereni M et al, Allergy 2013; Gangwar R et al, Allergy 2017
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

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

Gangwar RS et al. *Allergy* 2017
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

Breuer O et al, J Immunol Research, in press
**“Our” Inhibitory Receptors**

**CD300a**

- 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 phosphatidylserine (PS) and phosphatidylethanolamine (PE) on apoptotic cells

**Siglec7**

- 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)

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The inhibitory receptor CD300a is upregulated 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

Journal of Allergy and Clinical Immunology
Volume 118, Issue 5, November 2006, Pages 1082-1089

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

Ido Bachelet†, Ariel Munitz†, Beeta Berent-Mooz†, David Mankuta† and Francesca Levi-Schaffer²†
CD300a expression is differentially increased in the lesional skin of AD patients.
Skin thickness and inflammation are modulated in an AD model in CD300a−/− mice

Karra L et al, under revision
SPM promote the resolution of tissue inflammation and limit further leukocyte recruitment

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

Karra L. et al., Journal of Immunology: in press
CD300a\(^{-/-}\) mice present a delayed resolution of inflammation in the AP model

Karra L. et al, Journal of Immunology: in press
ALX/FPR2 is down-regulated on Eos while LXA$_4$ is increased in the peritoneum of CD300a$^{-/-}$ mice in the AP model.

Karra L. et al., Journal of Immunology: in press.
CD300a activation modulates ALX/FPR2 expression on BMMC

Karra L. et al., Journal of Immunology: in press
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

Karra L. et al., Journal of Immunology: in press
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.
Francesca Levi-Schaffer’s group  

Thanks!

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

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<tr>
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<th>WT OVA</th>
<th>CD300a KO OVA</th>
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<tr>
<td>LPS 1ug/ml</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IL-4 10 ng/ml</td>
<td>-</td>
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**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.

Puzzovio PG, Levi-Schaffer F, Unpublished results
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

Puzzovio PG, Levi-Schaffer F, Unpublished results
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.

Puzzovio PG, Levi-Schaffer F, Unpublished results
Macrophages from peritoneal lavage of WT and CD300a KO Balb/c, AP 96 hrs, Expression of iNOS and Arg1

Puzzovio PG, Levi-Schaffer F, Unpublished results
Macrophages from peritoneal lavage of WT and CD300a KO Balb/c, AP 96 hrs, cytokines release

Puzzovio PG, Levi-Schaffer F, Unpublished results