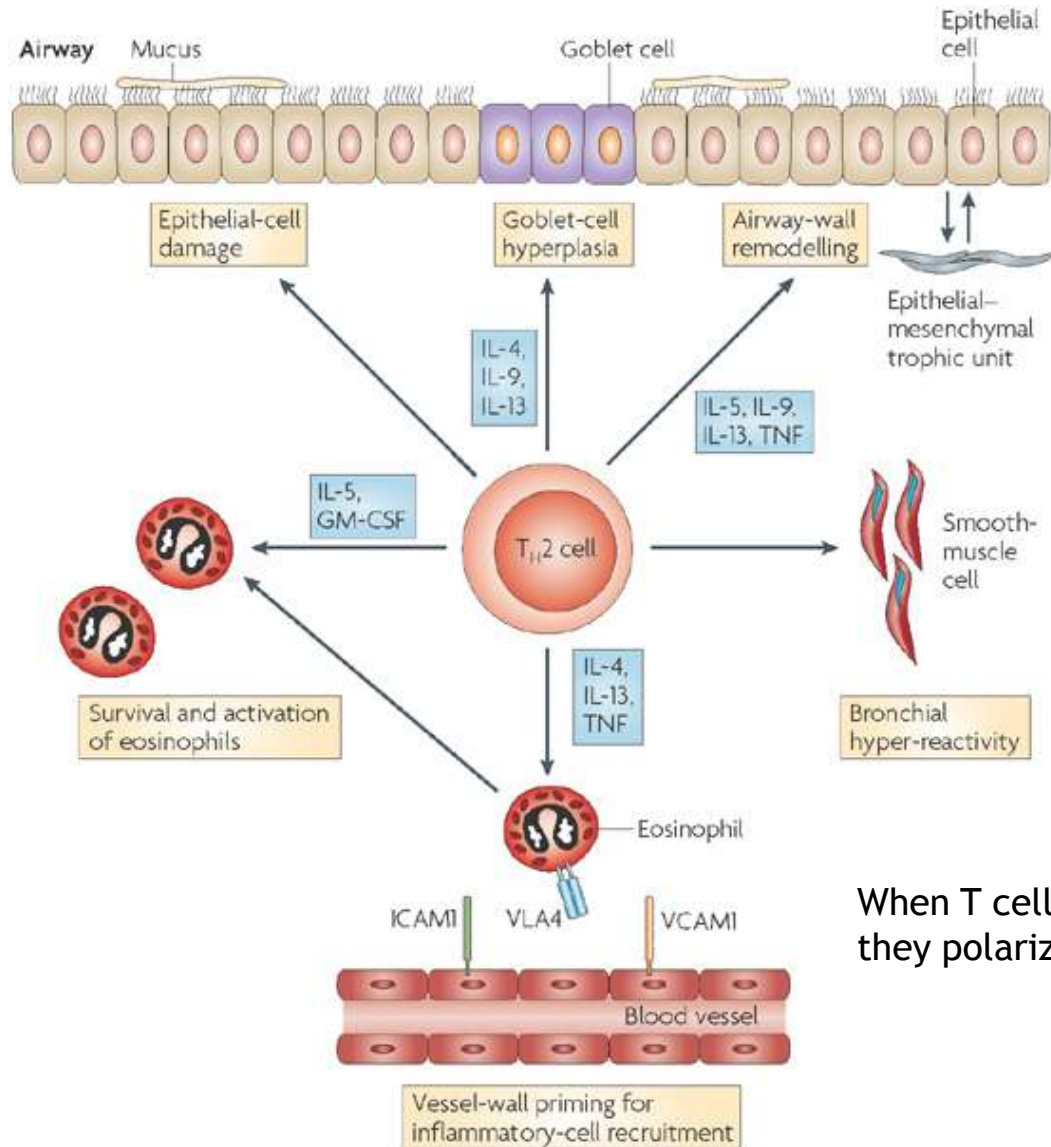




## Dendritic cells and osteopontin in allergic asthma

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# Allergic asthma is driven by allergen-specific Th2 cells



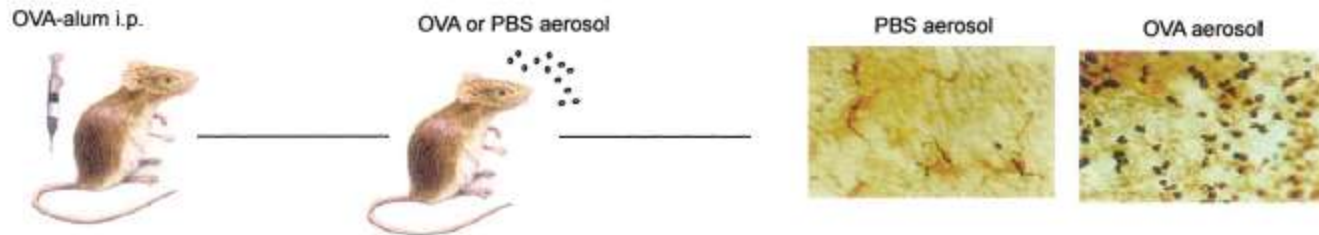
When T cells “see” allergen, they polarize to T helper 2 (Th2) cells

Dendritic cells are very important cells during the process of allergen-specific Th2 cell activation and polarization

1. They are the “professional” antigen-presenting cells with very high capacity
2. Experiments in allergic airway inflammation have demonstrated that .....

# Lung dendritic cells (DCs) are crucial for allergic airway inflammation

Koch I : number of DCs increased in mice with experimentally induced asthma



Koch II : administration of DCs to the airways of naive mice induces features of asthma

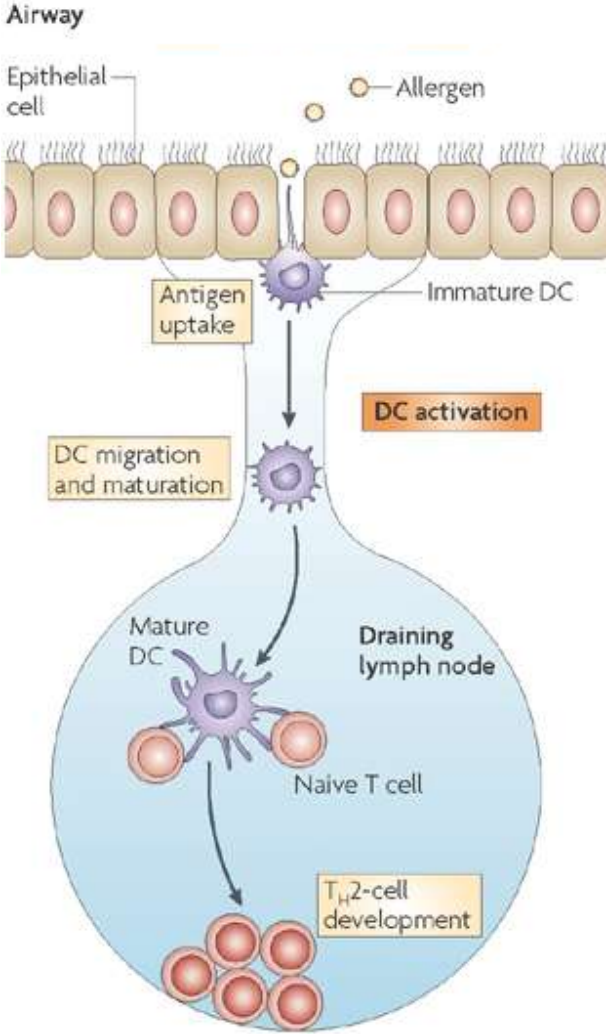


Koch III : removing DCs from the airways of sensitized mice reduces features of asthma

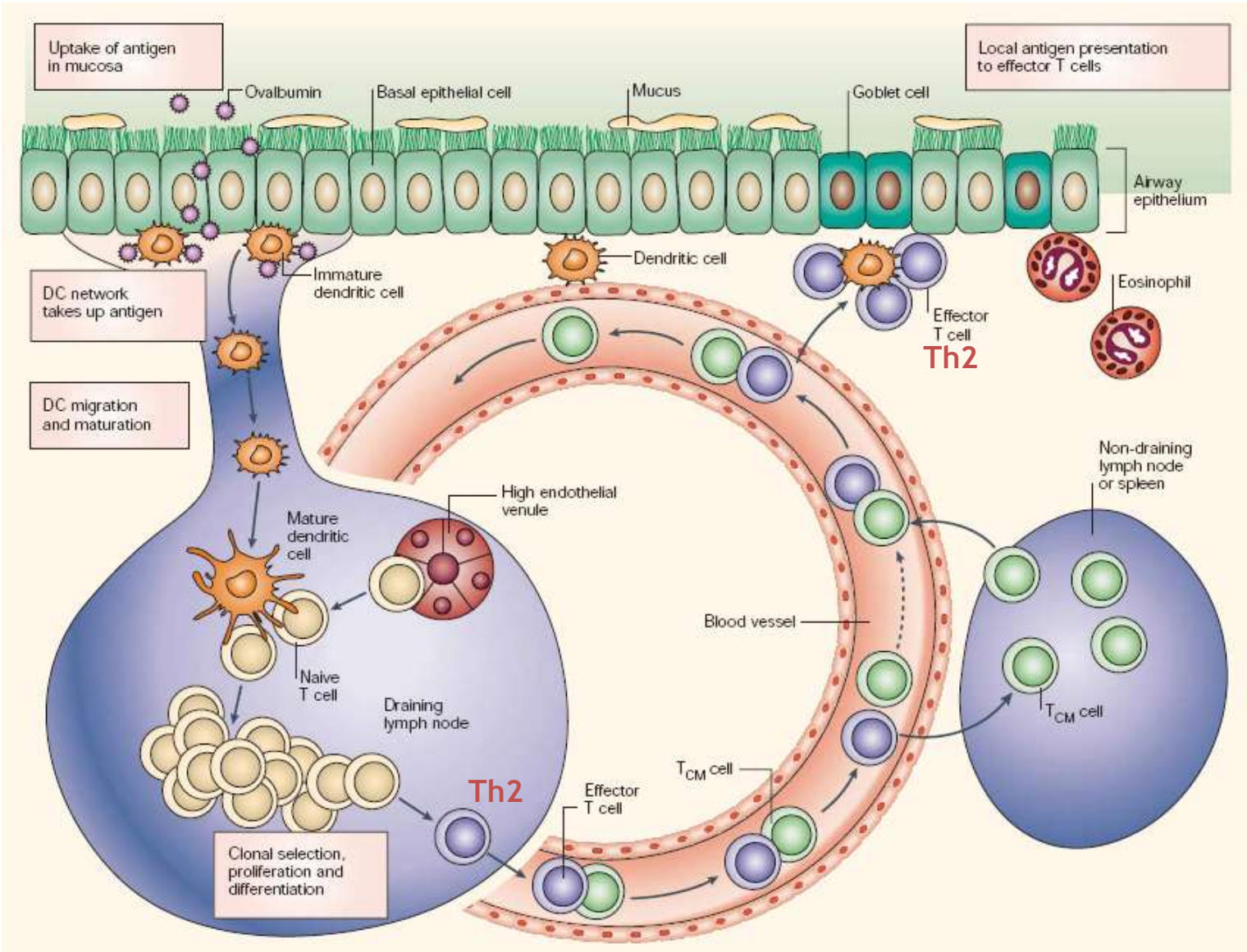


or *in vivo* depletion of DCs in the CD11c-DT Tg mouse

# Lung dendritic cells (DCs) are crucial for the activation/polarization of Th2 cells



# Activated, polarized Th2 cells travel from the draining lymph node to the lung



DCs are affected by several factors one of which is the cytokine milieu.

## Osteopontin (Opn)

First described in 1979 (secreted by **malignant** epithelial cells)

Identified as a bone matrix protein in 1985 (**bone** formation)

Cloned in 1986 (*Spp1*)

Described as a **cytokine** 1989 (Eta-1)

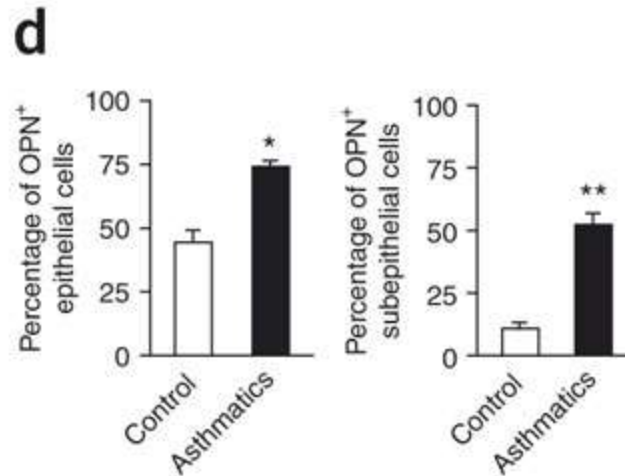
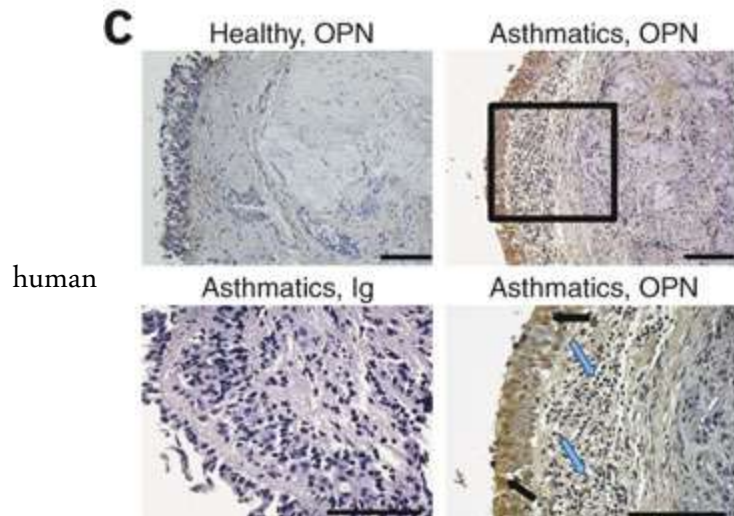
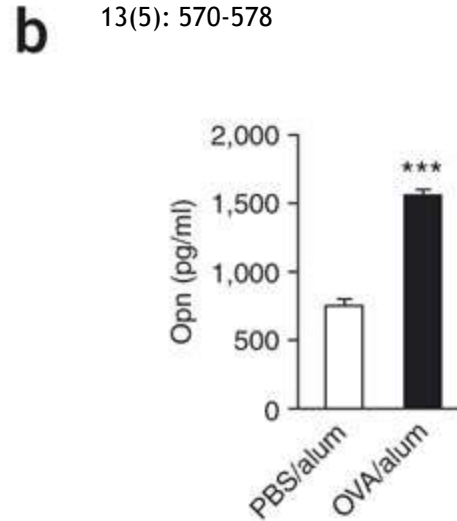
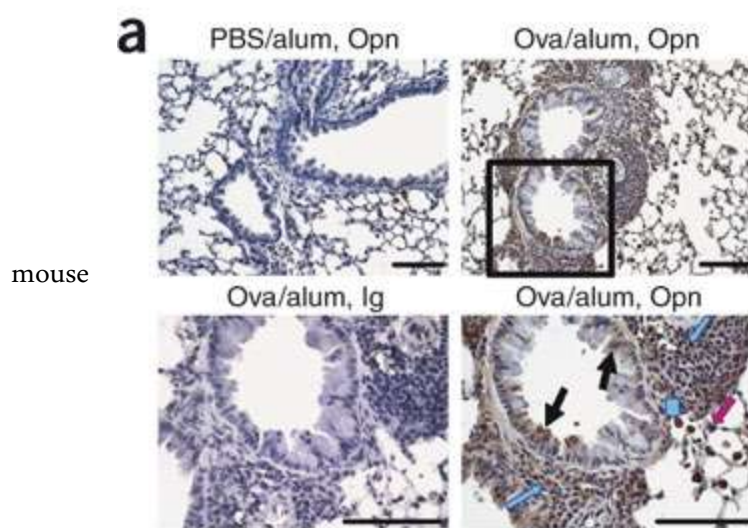
- expressed by activated T cells, macrophages, DCs and inflamed tissue cells (i.e. epithelial) **Receptors include CD44 and integrins ( $\alpha_v\beta_3$ )** (*Weber et al, 1996*)

Opn important cytokine for **Th1** immunity/autoimmunity 2000  
EAE/MS etc.(reviewed Nat Rev Immunol, 2009)



# Increased lung expression of Opioid Receptor (Opn) during mouse allergic airway inflammation and asthma

Xanthou G., Alissafi T., Semitekolou M., Simoes D., Economidou E., Gaga M., Lambrecht B.N., Lloyd C.M., Panoutsakopoulou V. (2007) *Nature Medicine* 13(5): 570-578



Samitas *et al.*, in press; Takahashi *et al.*, Clin Exp Allergy. 2009 ; Lu *et al.*, Allergy. 2009; Simoes *et al.*, Am J Respir Crit Care Med. 2009; Kohan *et al.*, Am J Respir Cell Mol Biol. 2009; Hansel *et al.*, Allergy. 2008; Kohan *et al.*, Clin Exp Allergy. 2007, etc

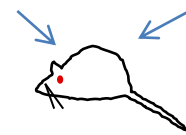


allergen  
OVA/alum



anti-Opn

allergen  
OVA



Primary immune response  
Sensitization  
Lymphoid organs

Secondary immune responses  
Challenge  
Tissue - Lung

Dendritic cells (DCs)



Activation of Th lymphocytes



Th naive cell polarization



Th2  
immunity

Dendritic cells (DCs), epithelial  
cells



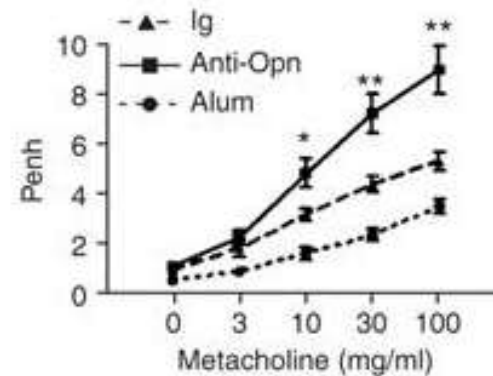
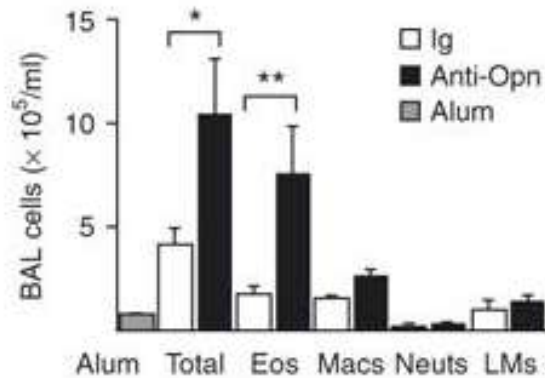
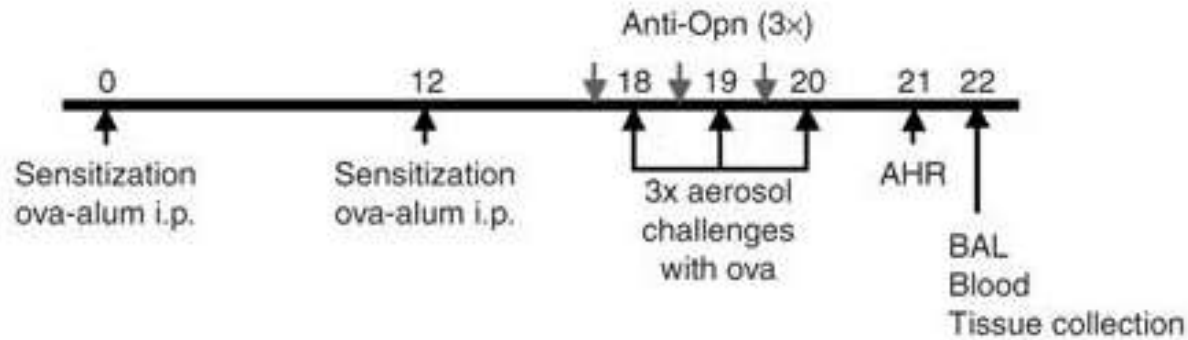
Th2 memory  
Newly-activated Th2



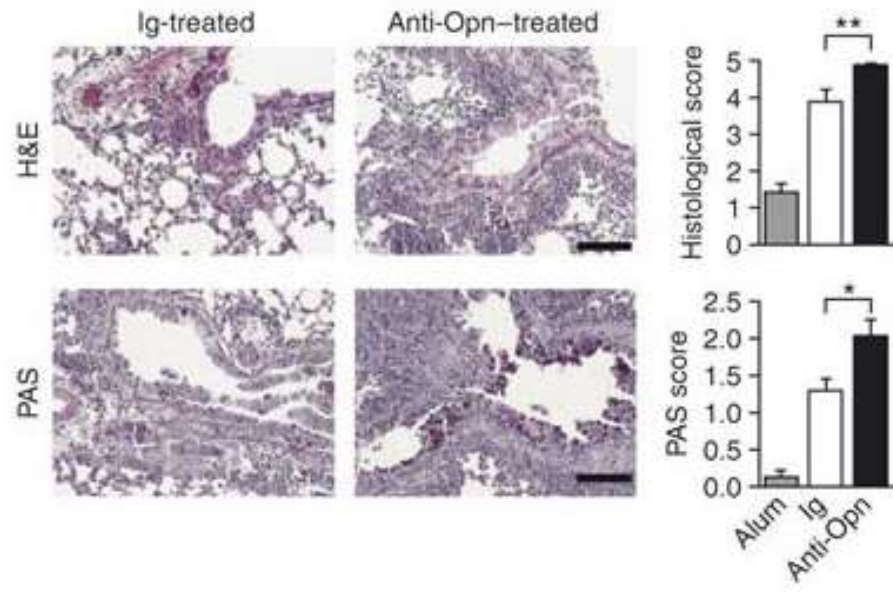
Allergic airway disease

- sustained inflammatory response (Th2 cytokines, chemokines, eosinophils, mast cells, IgE)
- airway hyperresponsiveness (AHR)
- mucus hypersecretion

**In vivo depletion of Opn-s during local antigenic challenge enhanced allergic airway disease**

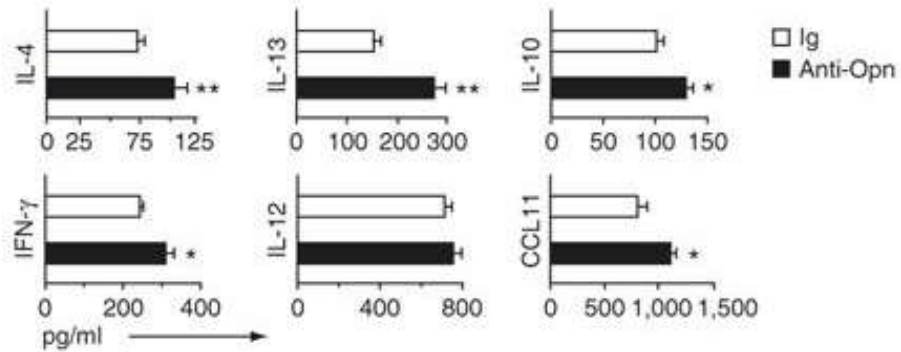


*In vivo* depletion of Opi-s during local antigenic challenge resulted in enhanced allergic airway inflammation

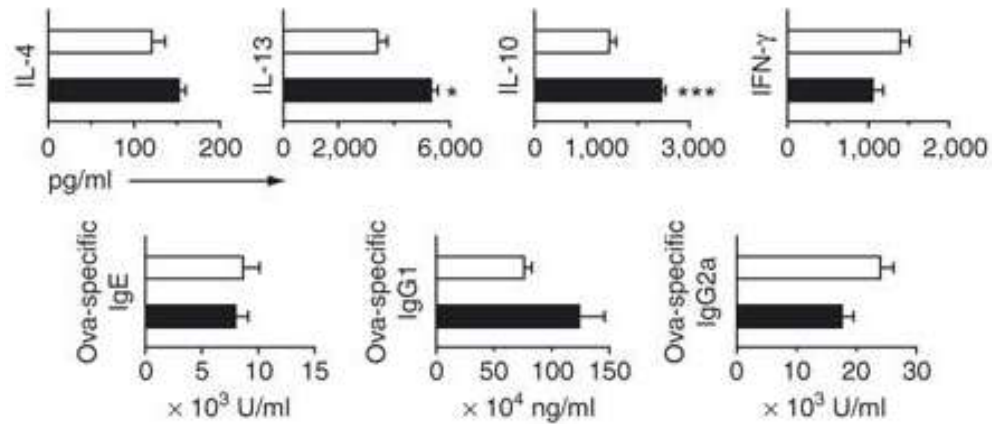


# *In vivo* depletion of Opn-s during local antigenic challenge increased Th2 cytokines and allergic responses

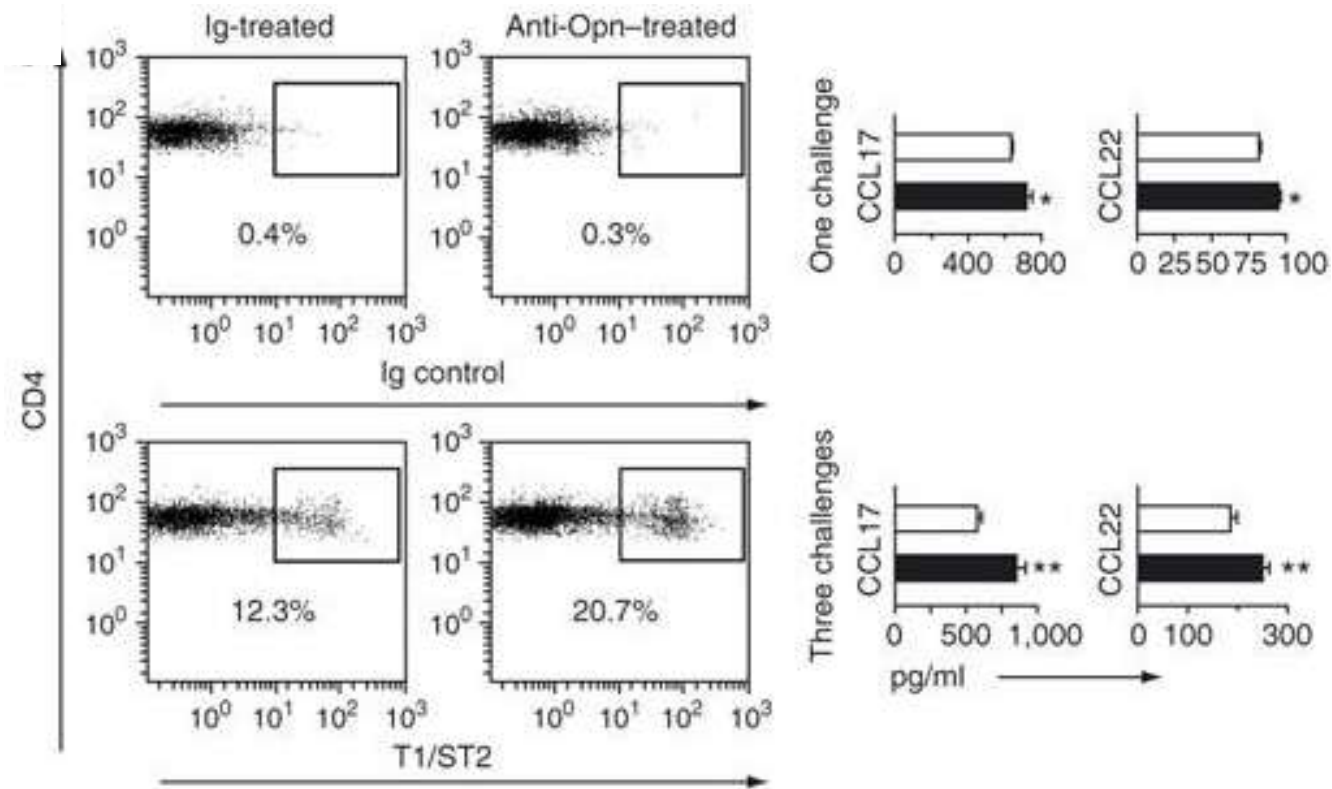
Lung Homogenates



Antigen-specific  
T and B cell responses

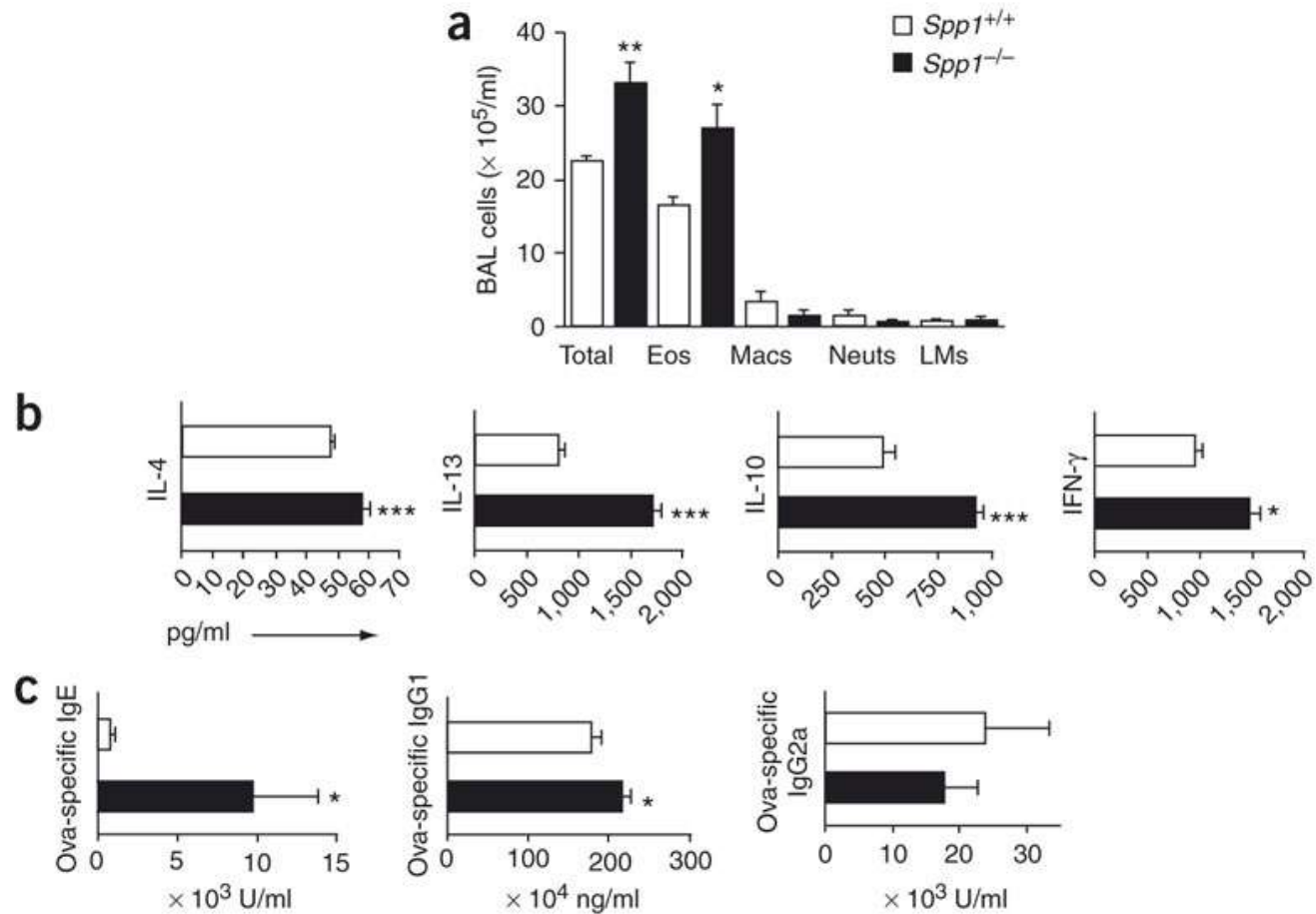


*In vivo* Opn-s depletion during primary antigenic sensitization increased Th2 cell recruitment and Th2 chemokine release

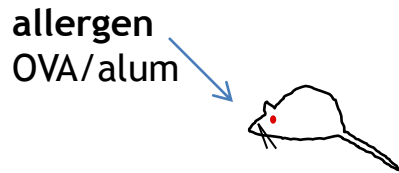


Therefore, depletion of Opn-s during pulmonary antigenic challenge enhanced Th2 secondary allergic responses and exacerbated airway disease, suggestive of an **anti-inflammatory/anti-allergic** role for Opn-s.

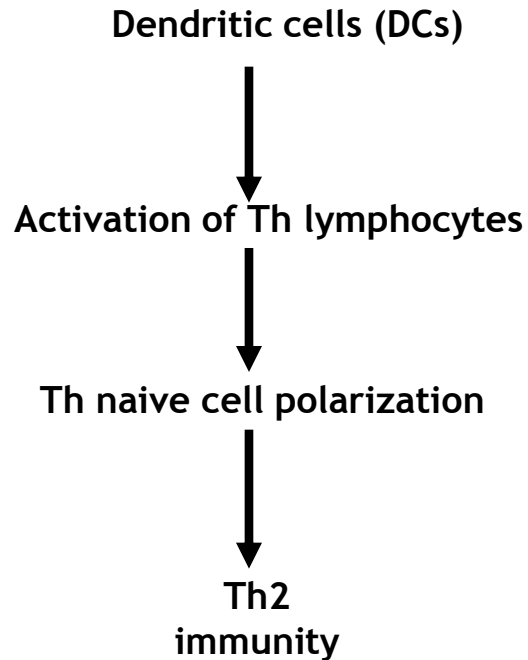
# Opn-deficient mice had enhanced Th2-mediated allergic airway inflammation





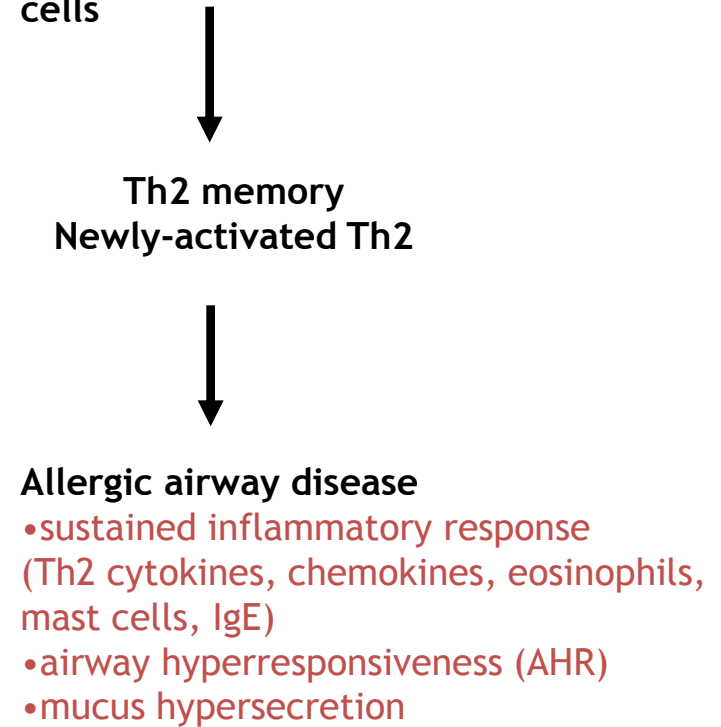


**Primary immune response**  
**Sensitization**  
*Lymphoid organs*

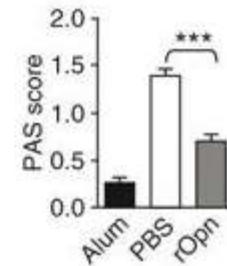
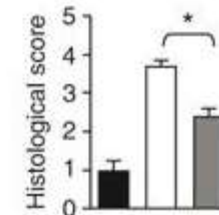
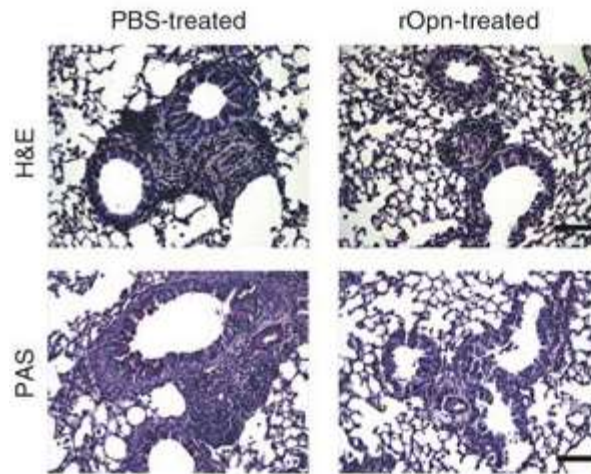
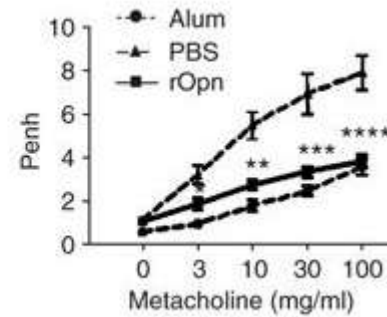
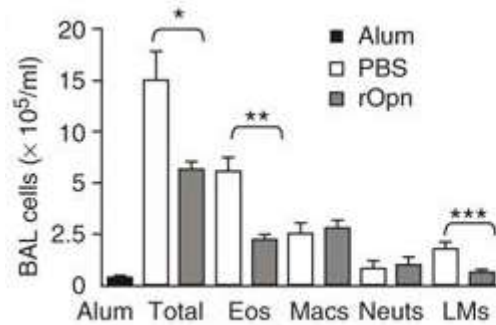


**Secondary immune responses**  
**Challenge**  
*Tissue - Lung*

Dendritic cells (DCs), epithelial  
cells

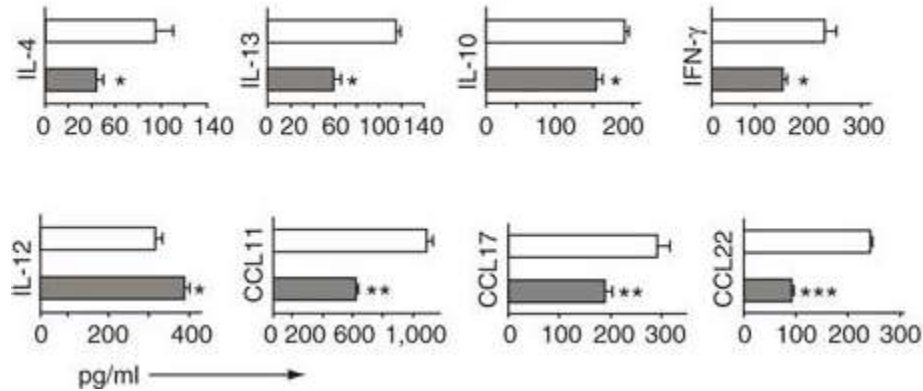


# Administration of recombinant Opi during challenge *in vivo* protected from allergic airway disease



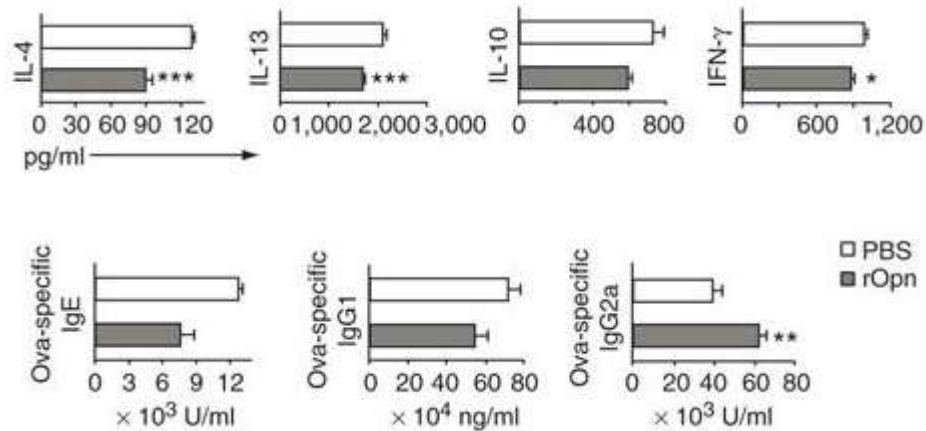
Administration of recombinant Opn during challenge *in vivo* suppressed established Th2 allergic responses

Lung Homogenates

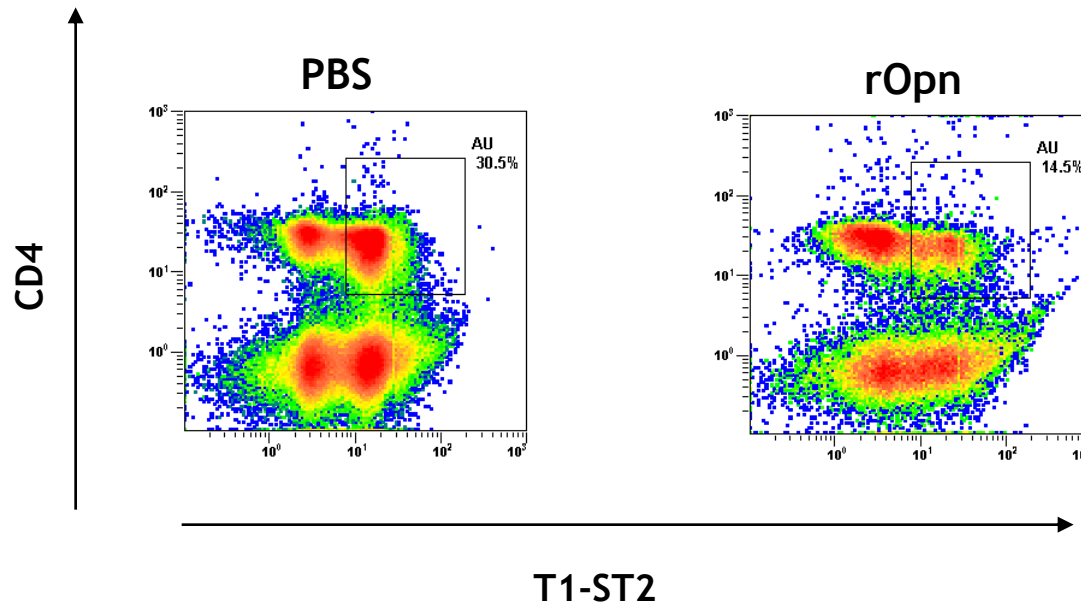


Important therapeutic implications

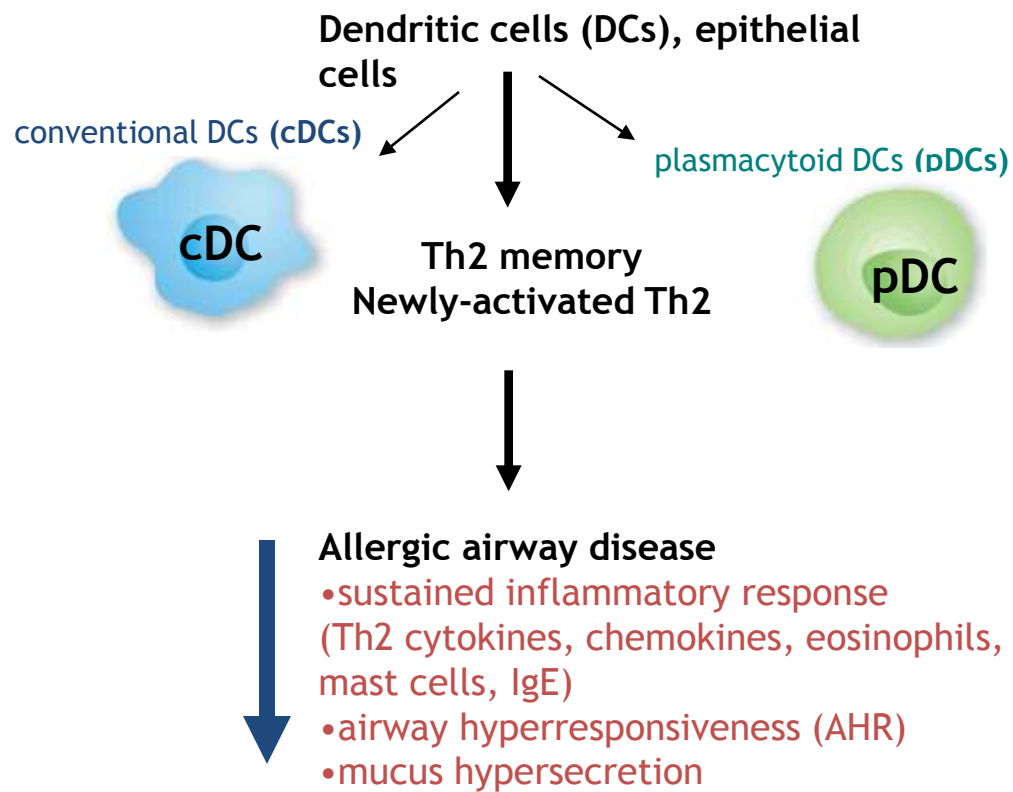
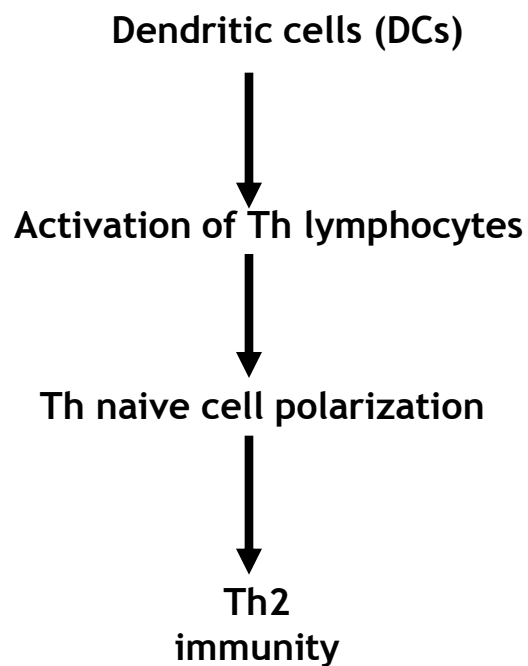
Antigen-specific T and B cell responses



Administration of recombinant Opn during challenge *in vivo* reduces Th2 effector cell recruitment

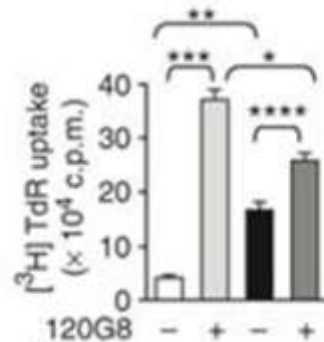
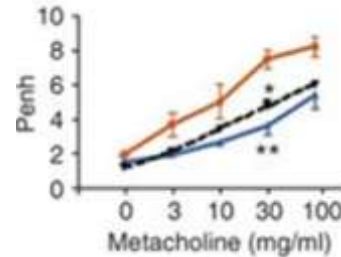
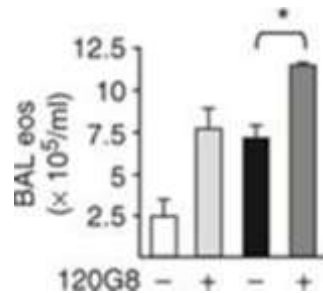


What is the mechanism of rOpn-mediated suppression??



Some dendritic cells are not there to drive the Th2 effector response:

pDCs are suppressive during secondary allergic responses!!!



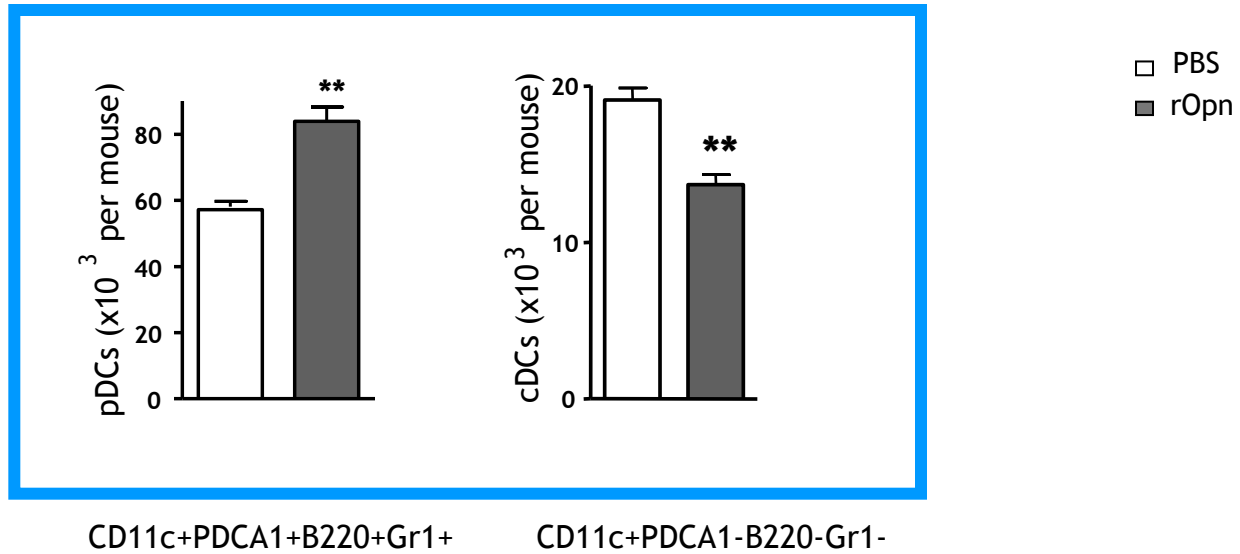
While,  
cDCs are immunogenic/pro-allergic

***In vivo* pDC-depletion experiments showed that pDCs are suppressive (de Heer *et al.*, *J Exp Med* 2004; Xanthou, Alissafi, *et. al.*, *Nat Med* 2007)**

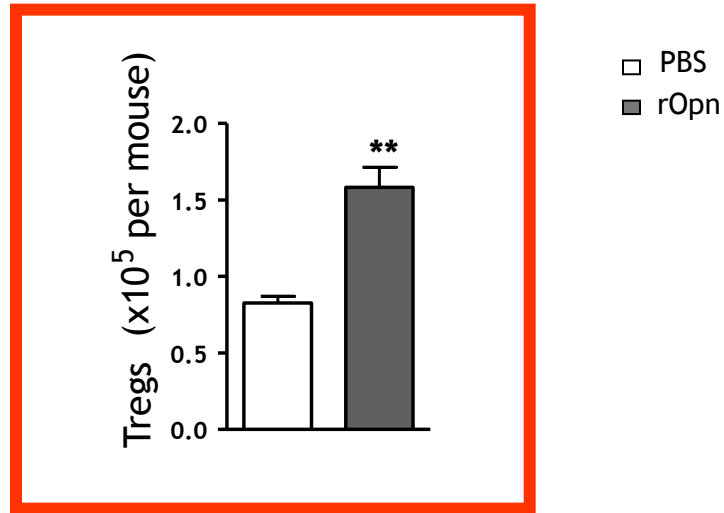


pDC numbers are increased in mice treated with rOpn

Antigen-loaded DC subsets

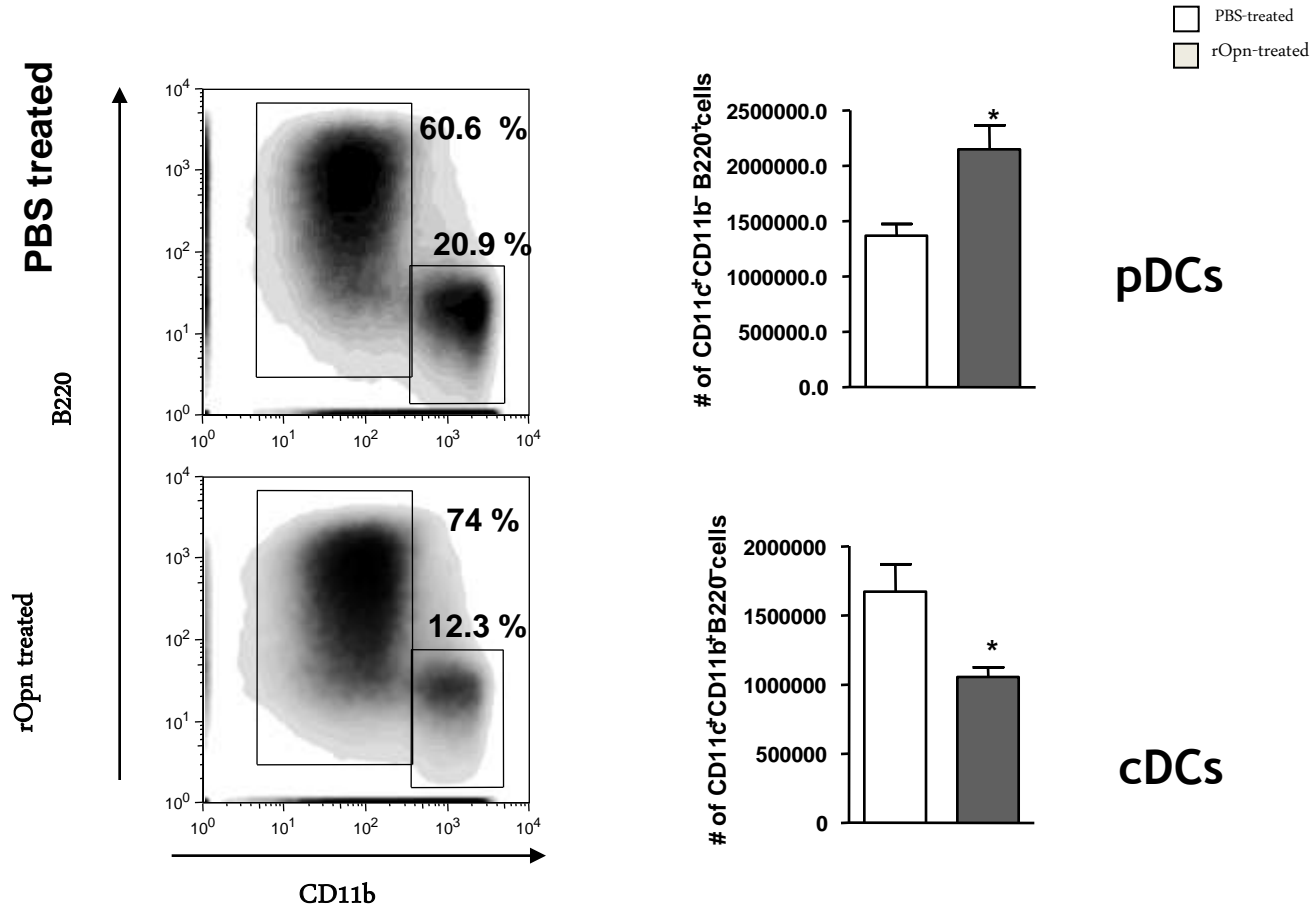


FoxP3+ Treg cell numbers are increased in mice treated with rOpn

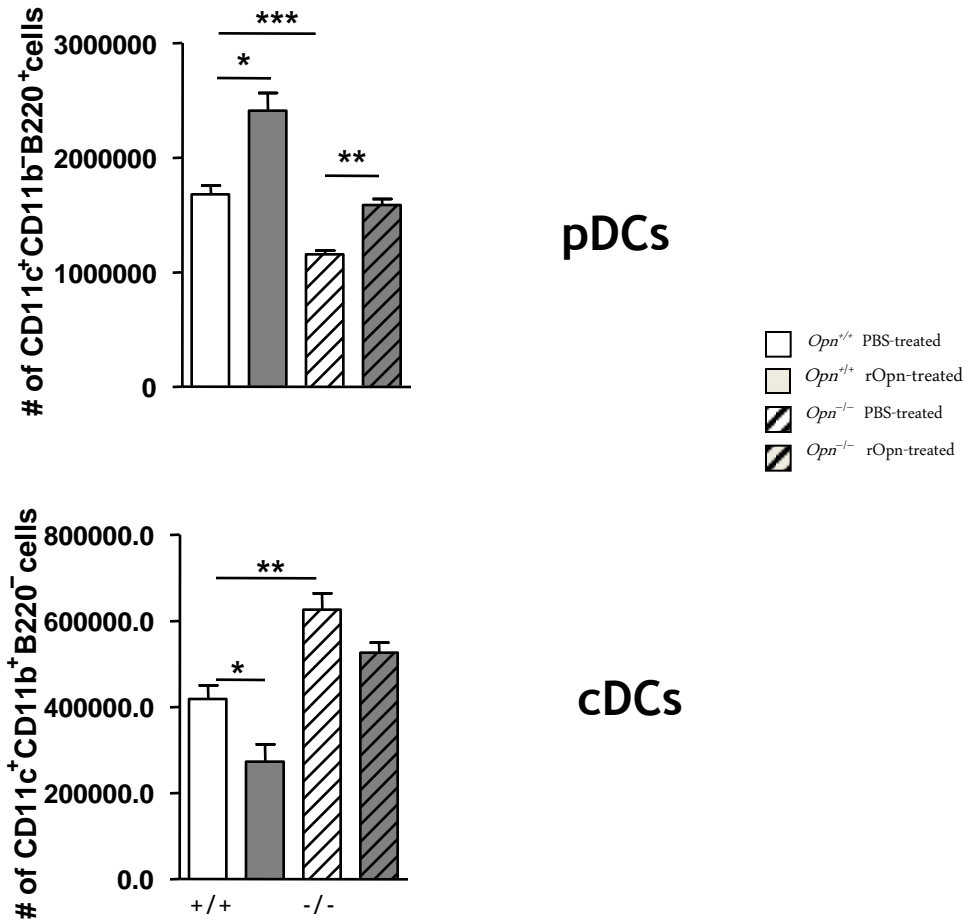


# rOpn can switch Flt3L-driven cultures towards enhanced pDC polarization.

Gated on 7AAD<sup>-</sup>CD11c<sup>+</sup>

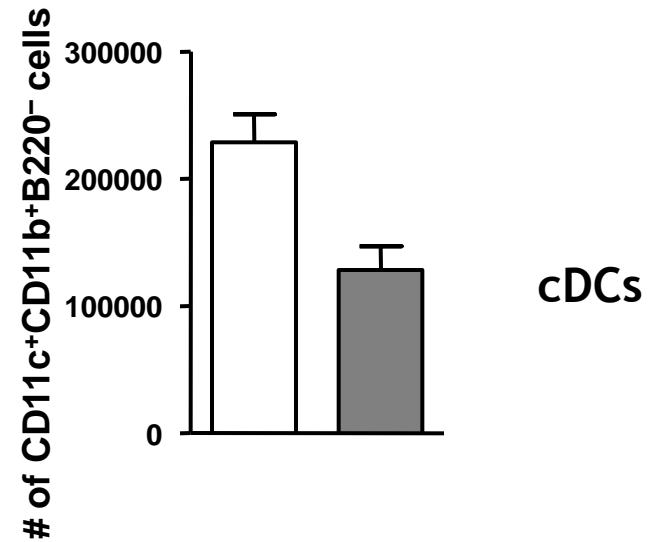
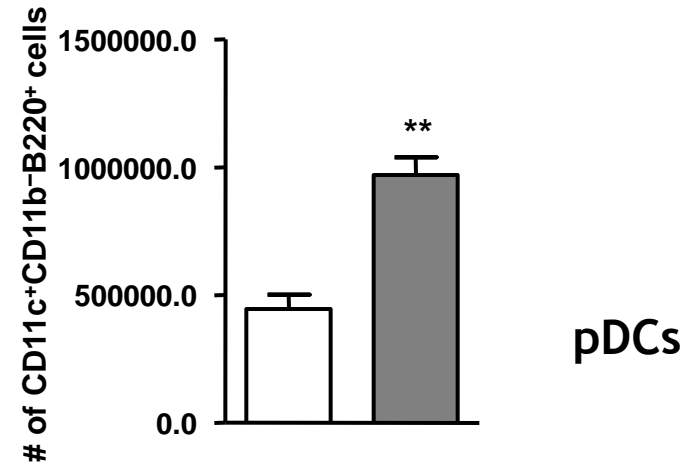
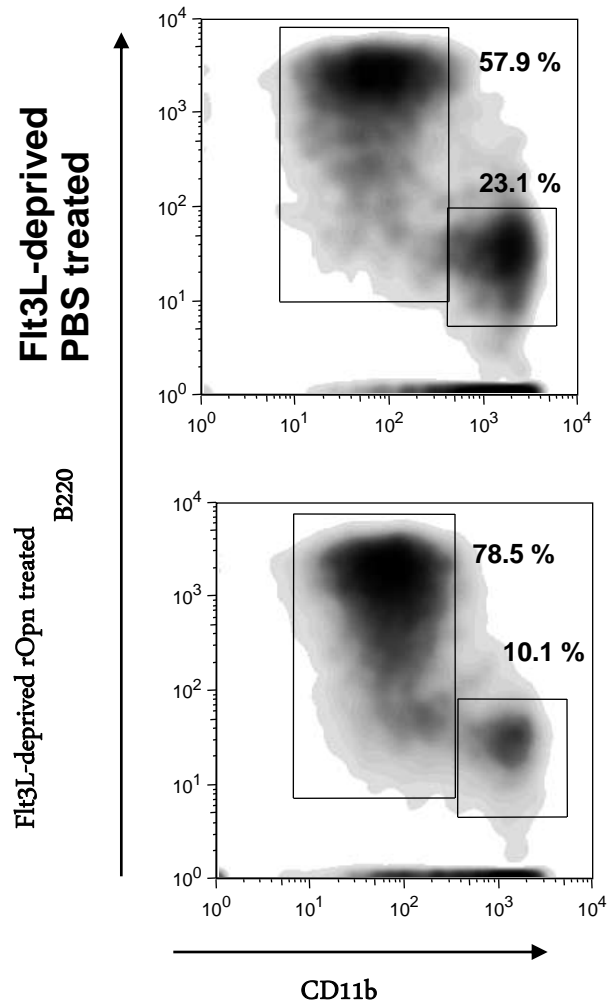


Flt3L BMDC *Opn*<sup>-/-</sup> cultures have significantly lower numbers of pDCs and correspondingly higher numbers of cDCs, compared to cultures from *Opn*<sup>+/+</sup> cells.



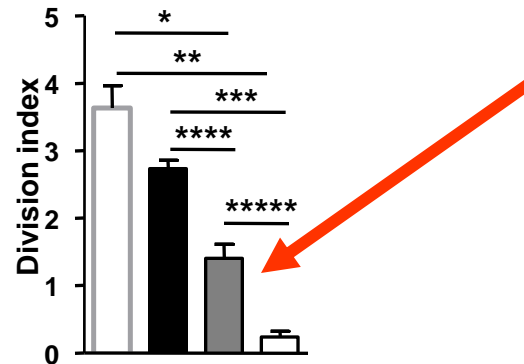
Under Flt3L deprivation conditions, rOpn leads to preferential survival of pDCs over cDCs.

□ Flt3L deprived PBS treated  
■ Flt3L deprived rOpn treated



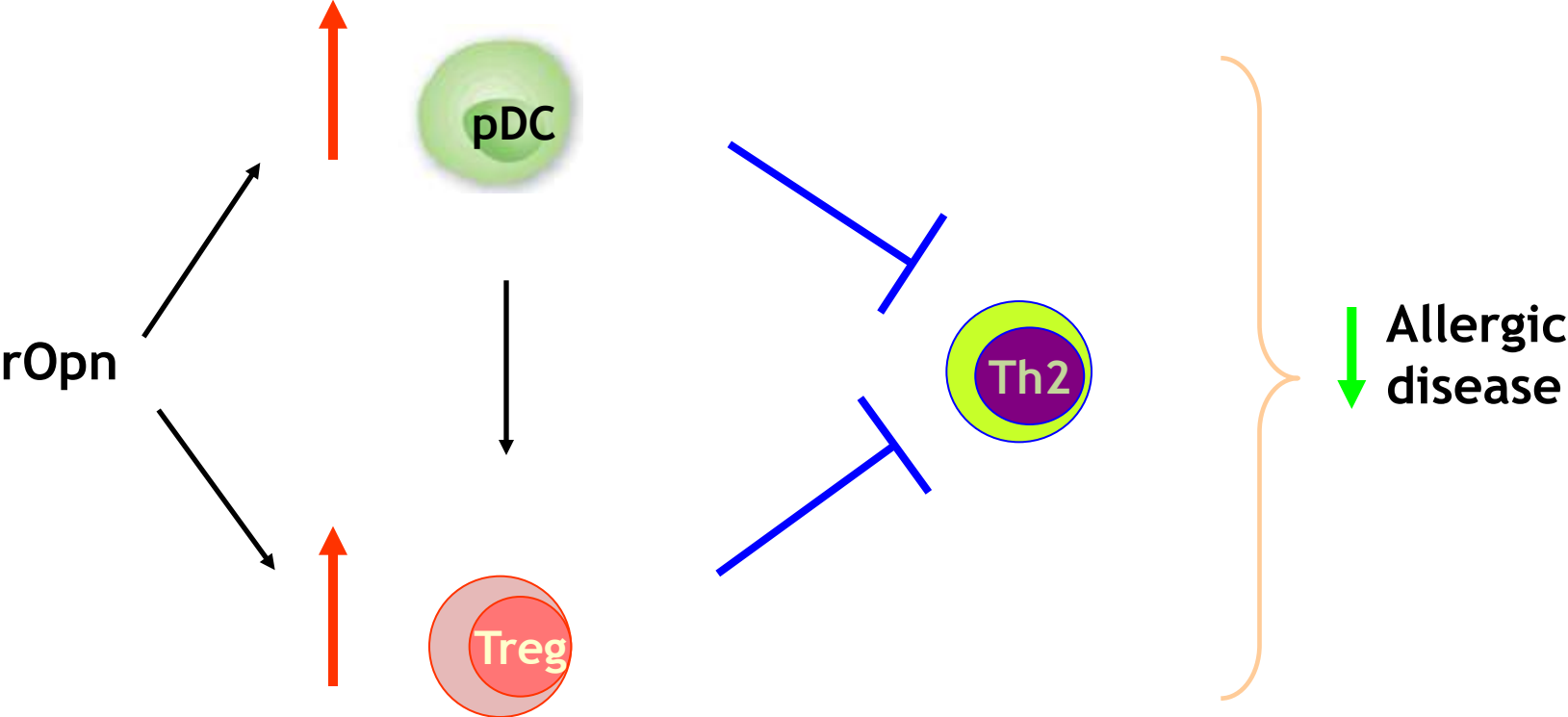
# Opn instructs pDCs to generate Treg cells

	Splenocytes	OVA	CFSE+ naive T	pDC-pretreated T cells	pDCS-rOpn pretreated T cells
□	+	+	+	-	-
■	+	+	+	+	-
■	+	+	+	-	+
□	+	-	+	-	-





Opn as a key immunoregulatory cytokine that controls Th2 responses.



# Thank you!

## Cellular Immunology Lab BRFAA

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PENED (GGRT), ERS, EAACI

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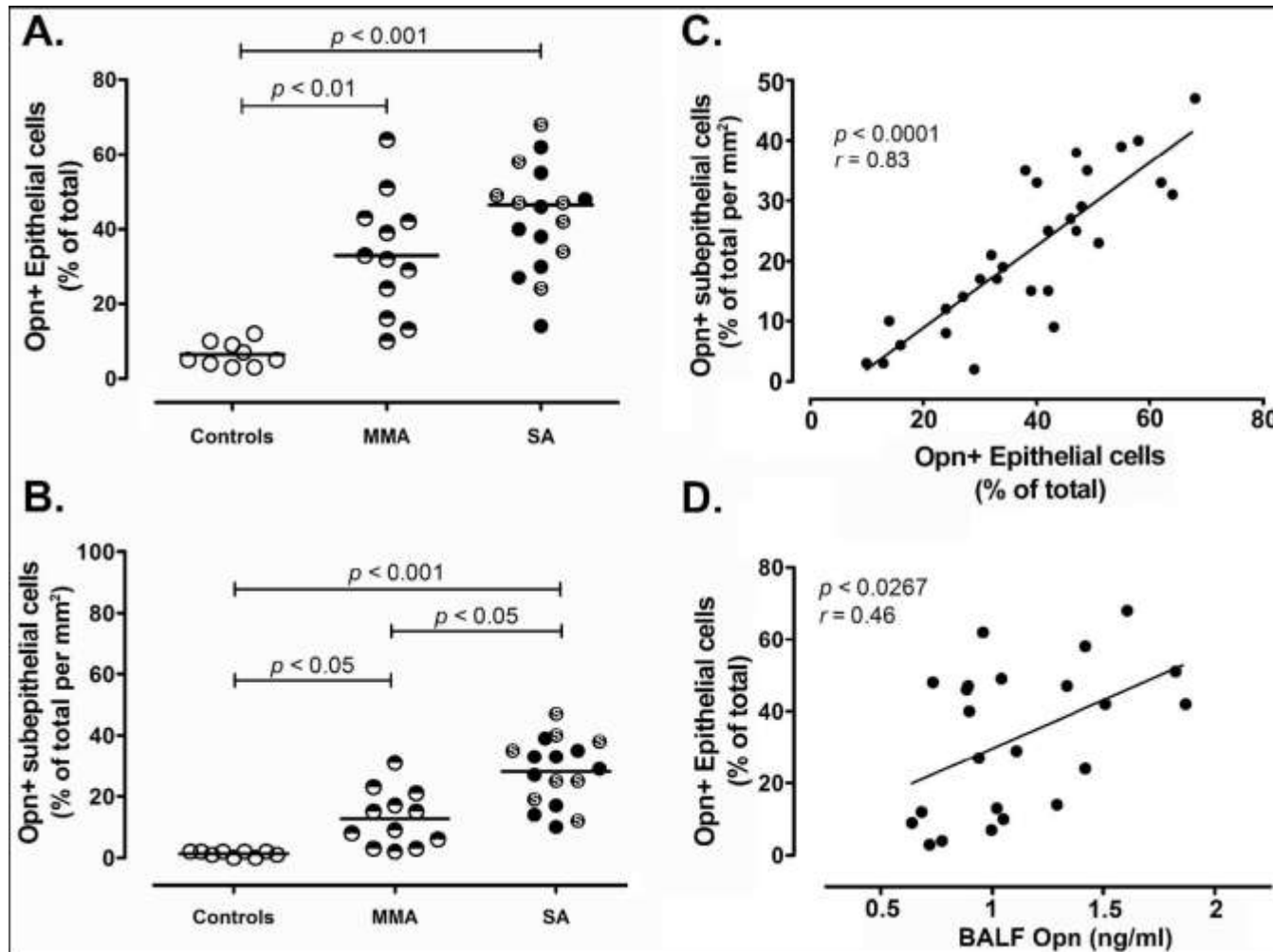
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Imperial College  
Leukocyte Biology Dept.  
Clare M. Lloyd

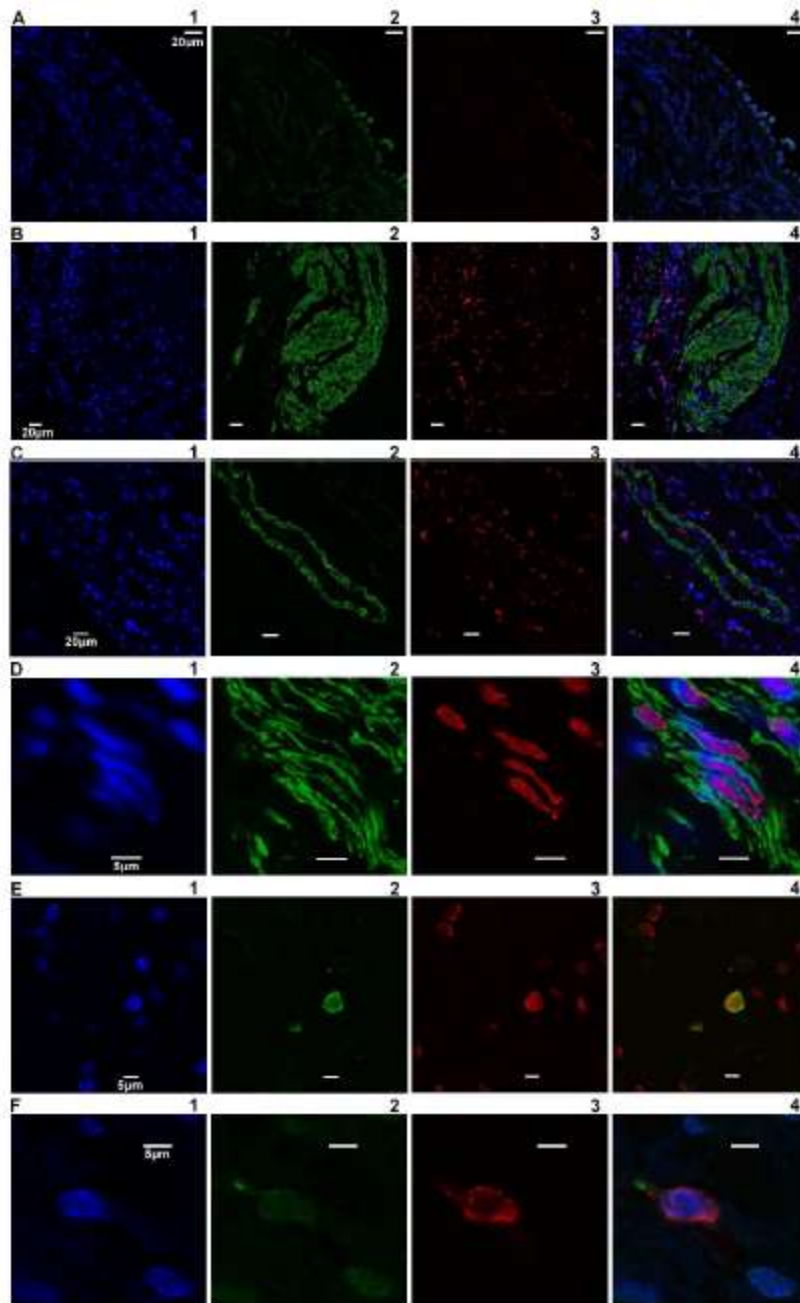
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Tim Sparwasser

Bioceros BV, Amsterdam, The  
Netherlands  
Louis Boon

Opn is upregulated in the bronchial tissue of patients with asthma.



Konstantinos Samitas, Eleftherios Zervas, Stelios Vittorakis, Maria Semitekolou, Themis Alissafi, Apostolos Bossios, Haris Gogos, Erasmia Economidou, Jan Lötval, Georgina Xanthou, Vily Panoutsakopoulou\* and Mina Gaga\* "Osteopontin expression and relation to disease severity in human asthma" In Press.



Airway smooth muscles  
(alpha-SMA)

Vascular smooth muscle  
and endothelial cells

Myofibroblasts

T lymphocytes  
(anti-CD3)

Mast cells  
(anti-mast cell  
tryptase)

Opn is expressed by many types of cells  
in bronchial biopsies from asthmatics