



Osteopontin in asthma

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Research group

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Osteopontin

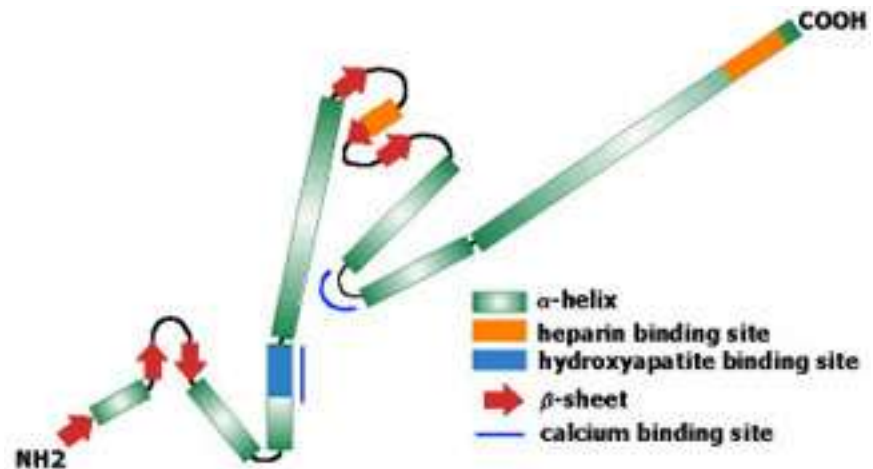
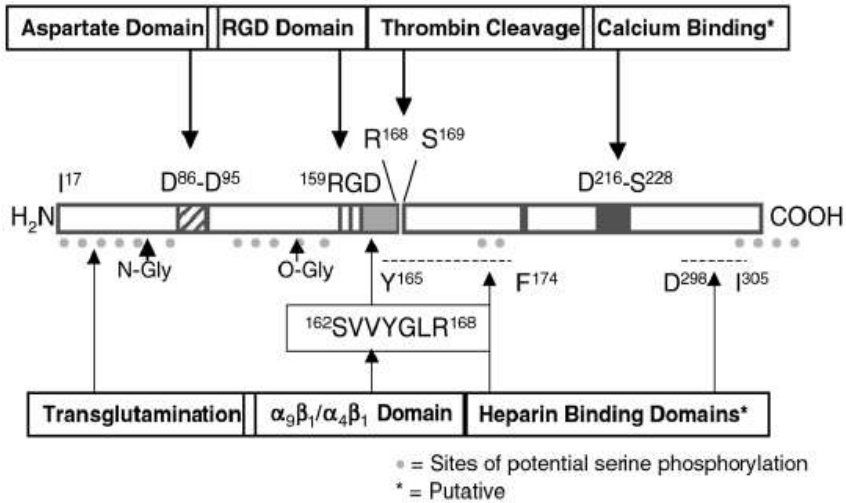
- Also known as bone sialoprotein, urinary stone protein, secreted phosphoprotein 1 SPP-1, nephropontin, uropontin and ETA-1 (T-Lymphocytes)
- Initially reported in 1979 as a secreted glycoprotein associated with malignant transformation
- Later on research focused on its role as an important [extracellular structural protein](#) secreted by osteoblasts (Oldberg et al, Proc Natl Acad Sci U S A. 1986)
- ETA-1 was then identified as the predominant transcript expressed by activated T cells and involved in Th1 immune responses (Patarca et al, J Exp Med. 1989)
- Recognized as an important early component of Type-1 (Cell-Mediated) Immunity (Cantor et al, Science, 2000)

- Osteopontin (OPN) is expressed in a range of immune cells, including macrophages, neutrophils, dendritic cells, and T and B cells and non-immune cells such as SMCs, epithelial, endothelial cells, fibroblasts, osteoclasts, osteoblasts and certain tumor cells

- OPN is reported to act as an immune modulator in a variety of ways:

- chemotaxis
- adhesion
- cell activation and cytokine production
- apoptosis

OPN Structure



OPN & Lung Diseases

- Granulomatous and cell mediated immune lung diseases
- Lung Fibrosis
- Lung Carcinoma
- Pulmonary Vascular disease

*The role of Opn in lung disease
Cytokine & Growth Factor Reviews, 2003 (Review)*

What about the role of O_{pn} in
Allergic Airway diseases?
(Th2 mediated inflammation)

Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets

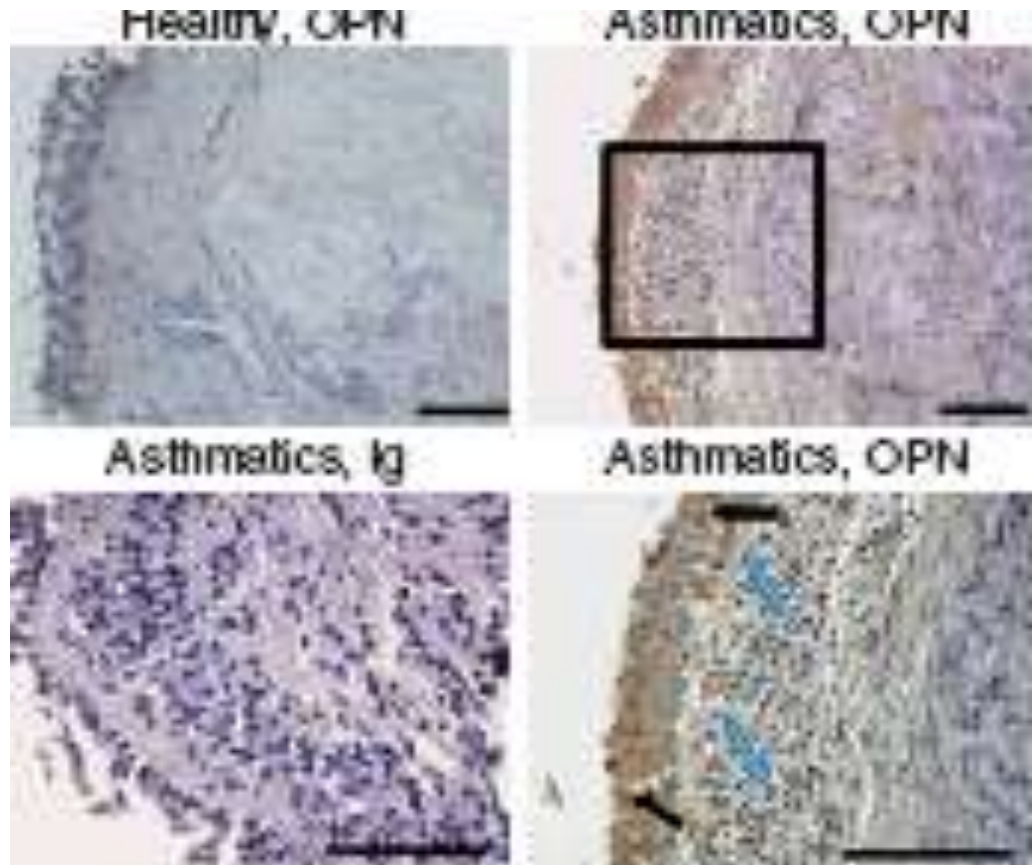
Georgina Xanthou¹, Themis Alissafi¹, Maria Semitekolou¹, Davina C M Simoes², Erasmia Economidou³, Mina Gaga³, Bart N Lambrecht⁴, Clare M Lloyd⁵ & Vily Panoutsakopoulou¹

Nat Med. 2007;13:570-8

Dual role of Opn

- pro-inflammatory at primary systemic sensitization
- anti-inflammatory during secondary pulmonary antigenic challenge
- mediated by the regulation of **TH2-suppressing plasmacytoid dendritic cells (DCs)** during primary sensitization and
- **TH2-promoting conventional DCs** during secondary antigenic challenge.

Opn is expressed in human bronchial tissue in asthma



Current research

Aim

- To investigate the expression pattern of Osteopontin in bronchial tissue, serum and BAL fluid in asthmatic patients
- Correlation to severity and control

Patient characteristics

Serum samples collected in steady state and during exacerbation from

- 55 asthmatics (35 MMA and 20 SA)
- 31 healthy controls

Fiberoptic bronchoscopy with endobronchial biopsies and BAL fluid collection

- 29 asthmatics (17 SA and 12 MMA)
- 13 healthy controls

Characteristics of patients who underwent bronchoscopy

Subjects undergone bronchoscopy	Mild - Moderate Asthmatics [MMA]	Severe Asthmatics [SA]	Healthy Controls [Ctrls]
Number	12	17	13
Sex (M / F)	4/8	4/13	8/5
Age (range) yr	46 (29-65)	54 (33-71)	48 (35-70)
Atopic	9	7	0
FEV₁ % pred	80.1 +3	66.5 ± 4.2 ^{£¥}	91.6 ± 2.5

*FEV₁ is expressed as mean ± SEM

[£] p<0.05 compared to MMA

[¥] p<0.001 compared to healthy controls.

Methodology and Statistics

Serum and BAL measurements: ELISA

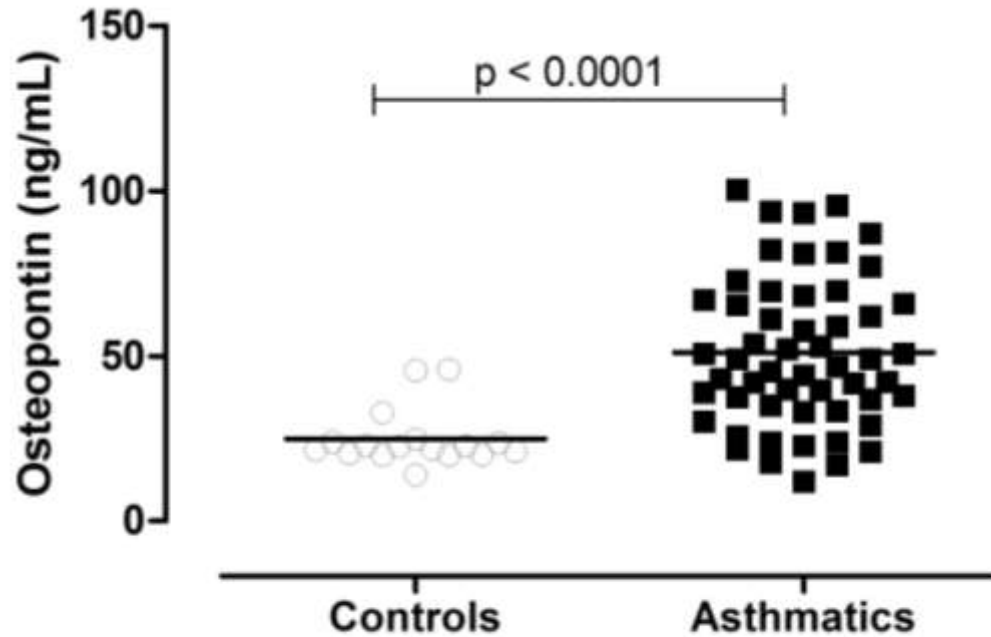
Bronchial biopsies: IHC

- Formalin-fixed, paraffin-embedded 5 μm sections, incubated overnight with
- Opn primary monoclonal antibody (RnD Systems)
- Matched isotype controls were used

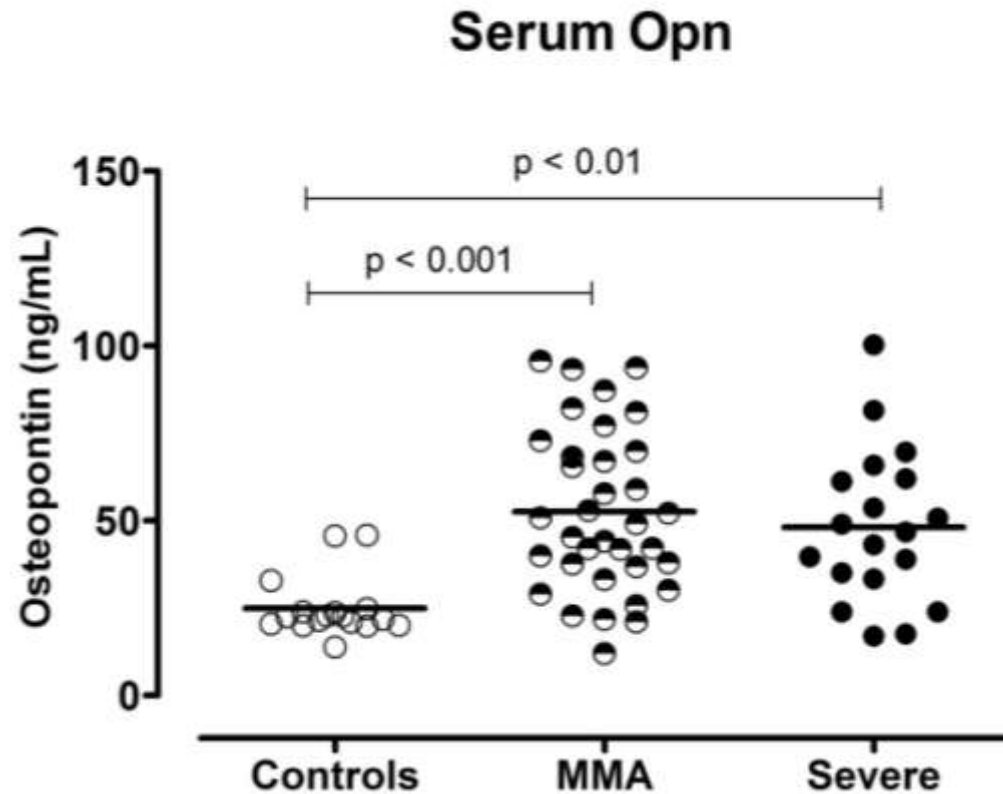
- epithelial cells were counted along the entire basement membrane
- sub-mucosal cells were determined by counting at least 5 fields
- expressed as % of positive cells/field

- non-parametric unpaired T test (Controls vs asthmatics), non-parametric 1-way ANOVA (Kruskal-Wallis test) was performed followed by Dunns' post-hoc analysis (subgroup analysis between CTRLs, MMA and SA).

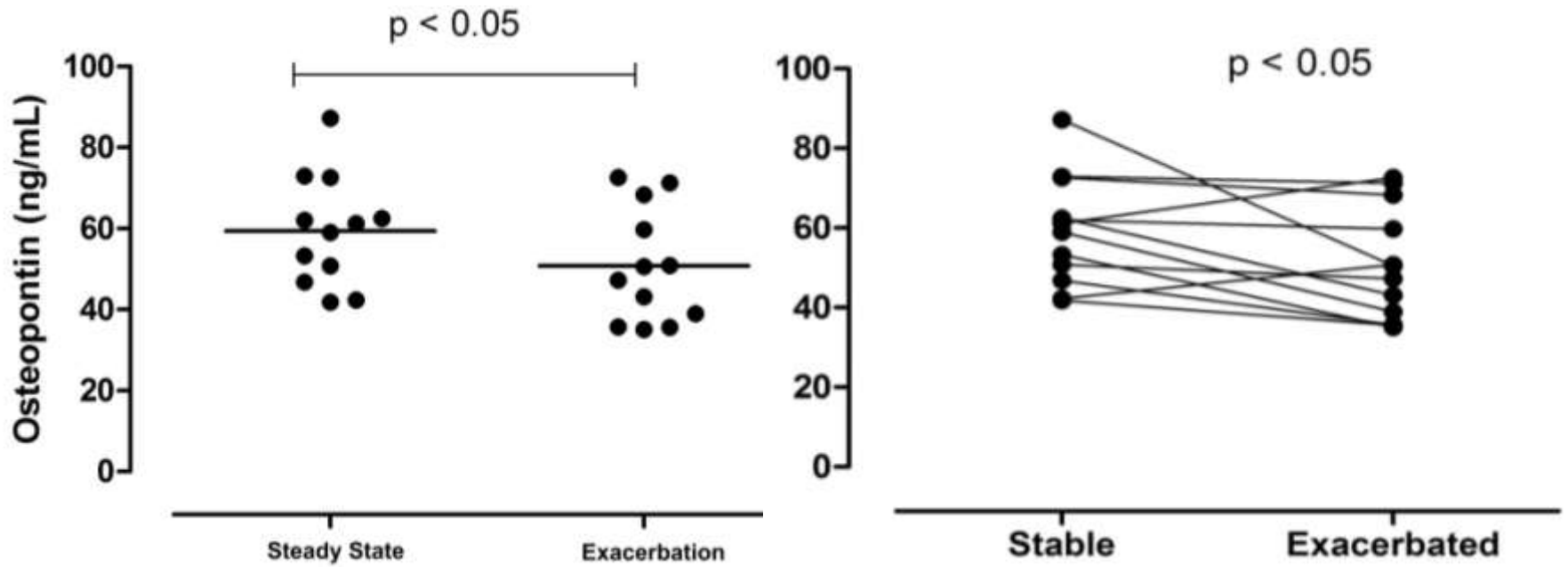
Serum Opn levels in asthmatics



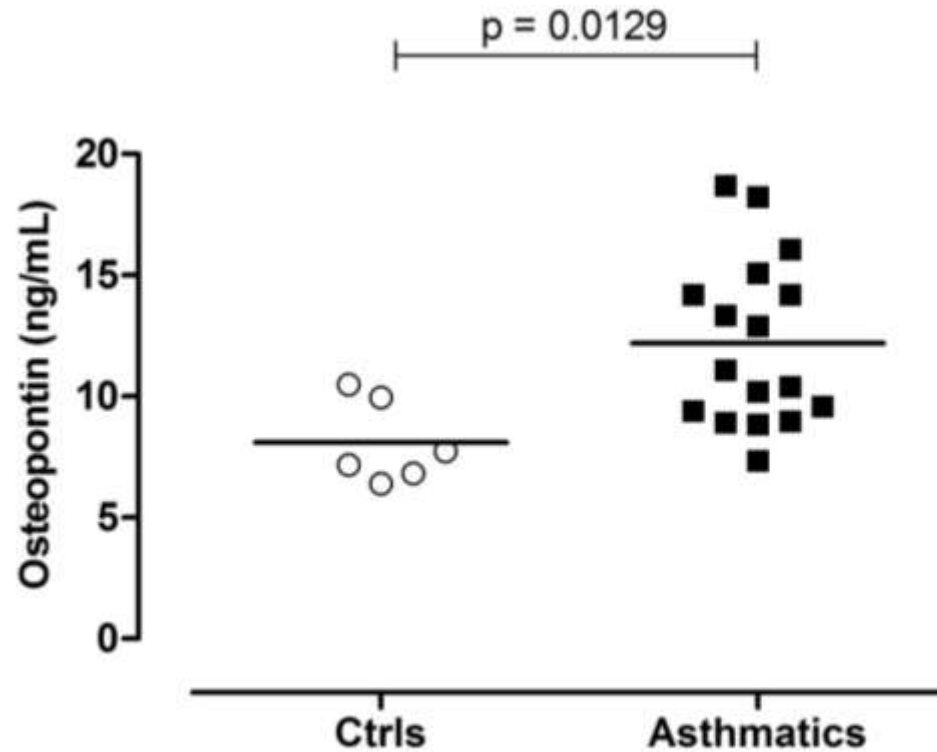
Serum Opn levels in asthmatics (Subgroup analysis)



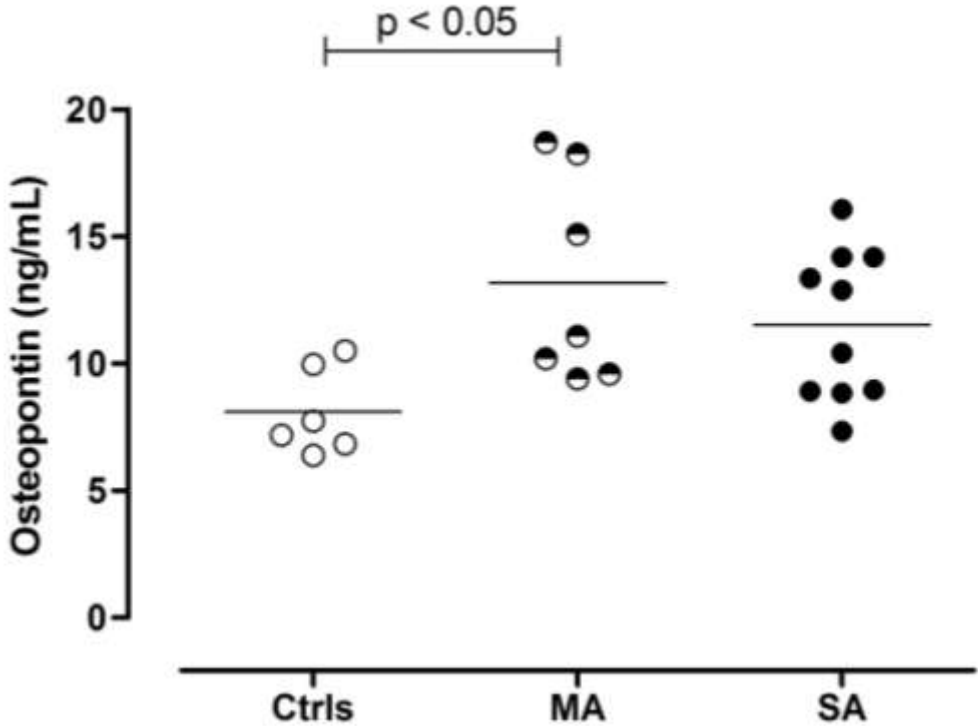
Serum Opn levels in exacerbated asthmatics



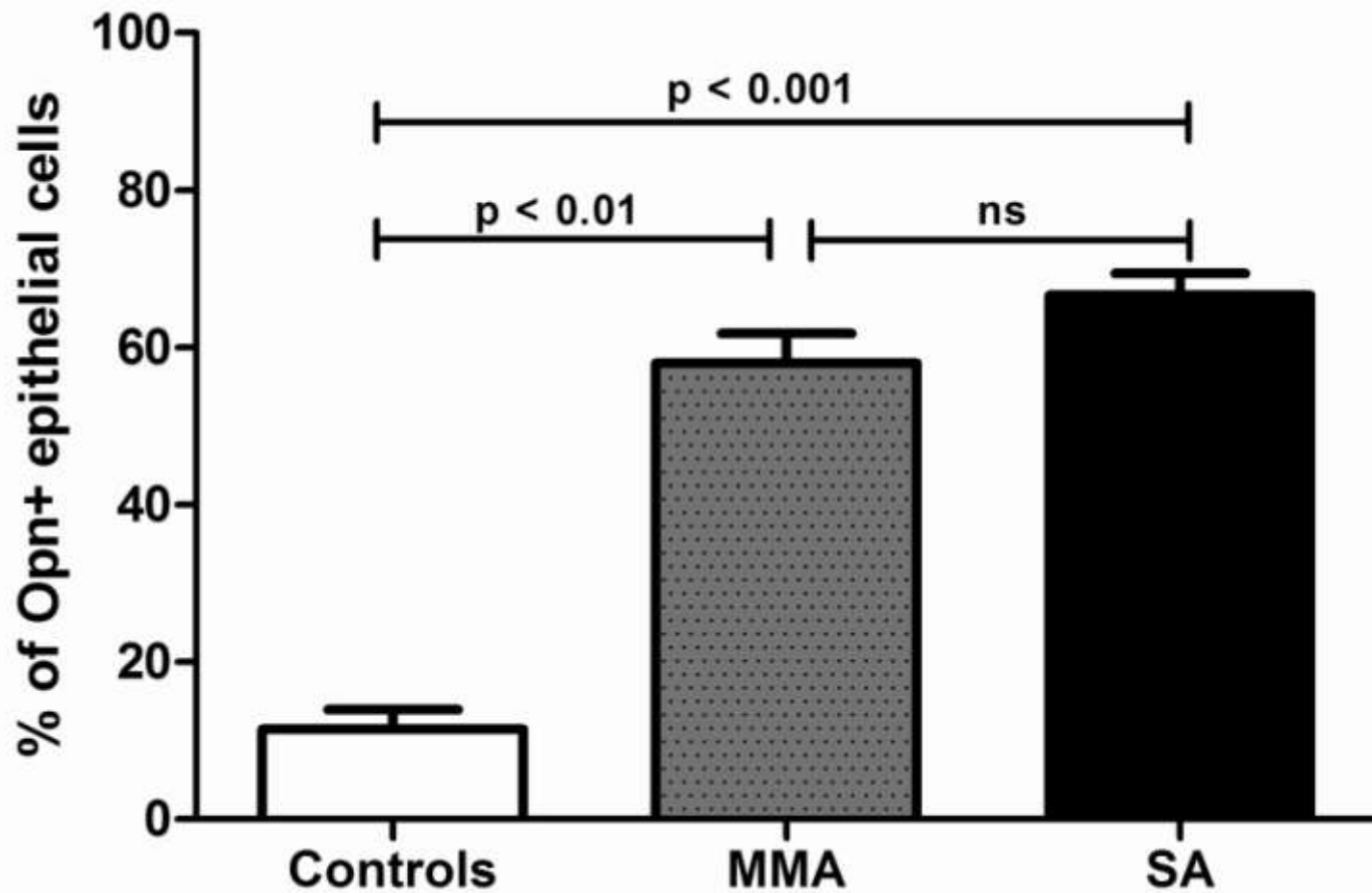
BALF levels of Opn in asthmatics



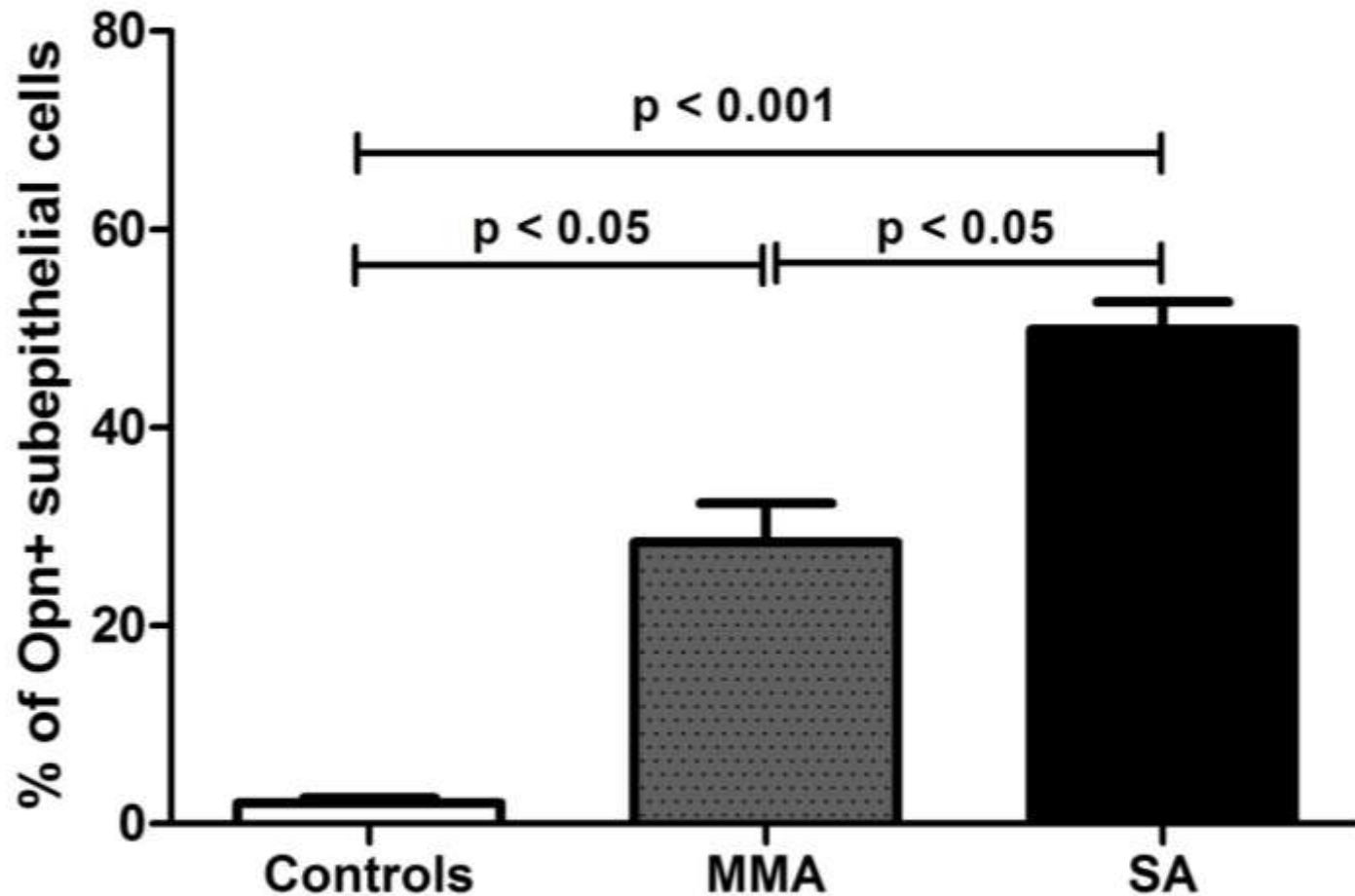
BALF levels of Opn in asthmatics (Subgroups analysis)



Expression of Opn in bronchial epithelial cells

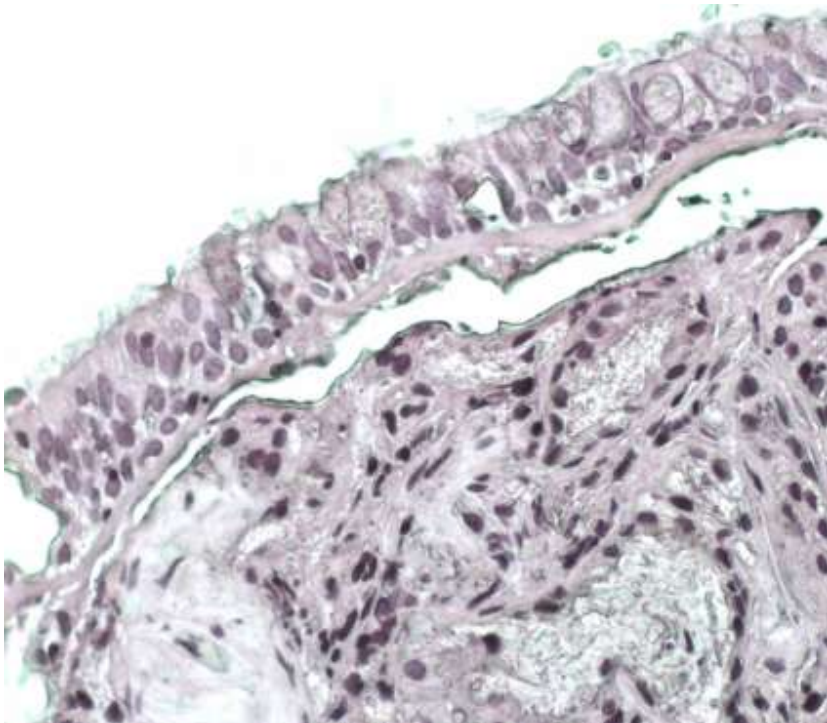


Expression of Opn in subepithelial inflammatory cells

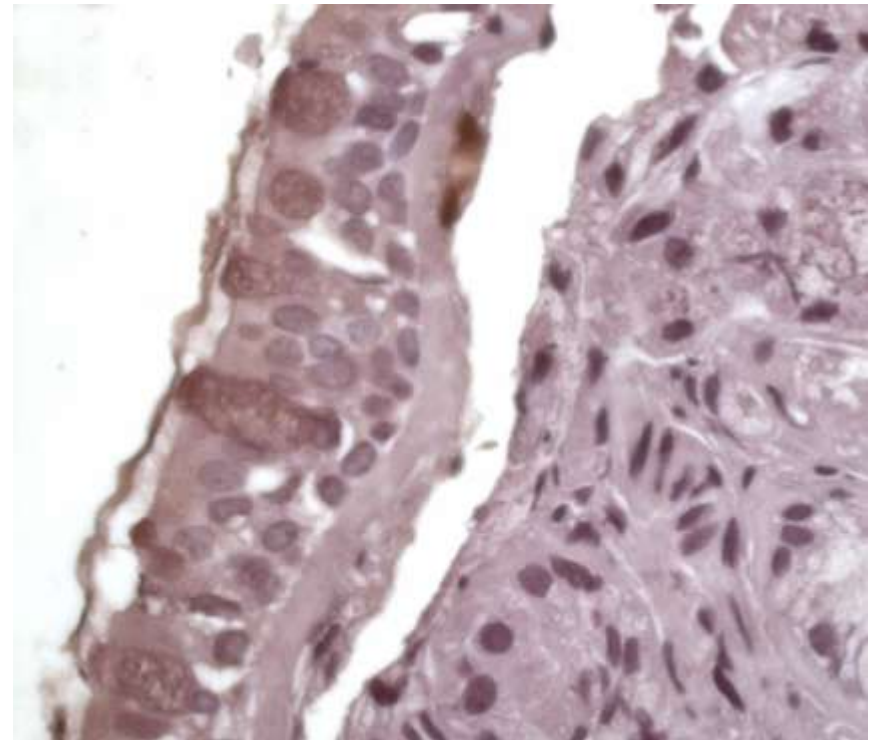


OPN Expression in Lung Biopsies of Severe Asthmatics

Isotype control



Osteopontin mab

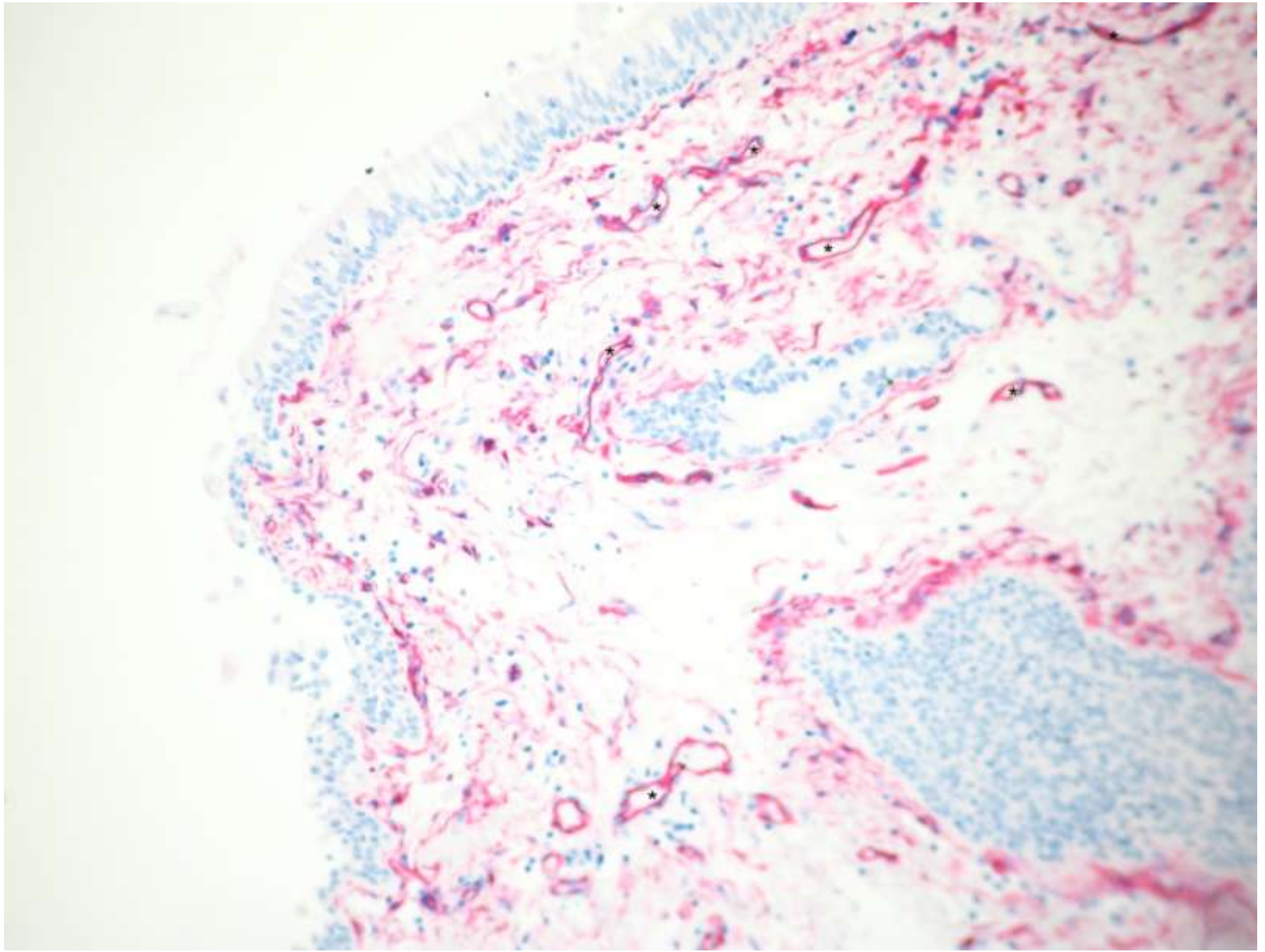


Conclusions

- Opi levels are significantly increased in the serum, BAL and bronchial tissue in stable asthma, while serum Opi levels are decreased during exacerbations.
- Opi expression is higher in the subepithelium in SA compared to MMA.
- Taken together with the previous work on mice, the data support the hypothesis of a protective effect of Opi.
- Overall, our data suggest that Opi plays a significant role in the pathogenesis of human asthma and that its expression might be dependent on asthma severity and level of control.

Ongoing research

- Differential counts from BALF/sputum cytopins and Opn cellular expression patterns (ICH)
- Opn may be involved in remodeling (Simoes et al, 2009)
 - Evaluation of “classic” remodeling markers (basement membrane thickness, goblet cells etc)
 - IHC for markers remodeling such as collagen type IV, hCD34
 - Investigation of possible correlation of Opn expression and remodeling



Future work

- Culture human epithelial cell lines (induced allergic model)
 - Expression of intracellular or secreted Opm (rtPCR)
 - Evaluation of steroids effect on epithelial Opm expression
- PBMC cultures (controls – asthmatics) to investigate steroids effect and correlate the expression of Opm (EIA, rtPCR) with that of other cell adhesion molecules (FACS)

Some Sources

- The role of Opn in lung disease
 - 2003, Cytokine & Growth Factor Reviews
- Opn – a molecule for all seasons
 - 2002, Q J Med
- Up-Regulation and Profibrotic Role of Opn in Human IPF
 - 2005, PLoS Medicine Vol2, Issue 9, e251
- Opn as a means to cope with environmental insults: regulation of inflammation, tissue remodeling and cell survival
 - 2001, The Journal of Clinical Investigation
- Eta-1 (Opn): an early component of type-1 (cell mediated) immunity
 - 2000, Science
- Opn (Eta-1) in cell-mediated immunity: teaching an old dog new tricks
 - 2000, Trends Immunology Today
- Opn-induced relapse and progression of autoimmune brain disease through enhanced survival of activated T-cells
 - Nature immunology, Advanced Online Publication

Article	Serum Opn				EIA Kit
	Ctrls		Pts		
	Range	Mean	Range	Mean/Median	
2007 - Clinical significance of elevated osteopontin levels in head and neck cancer patients	0-128 ng/ml	99±114 ng/ml	9-459 ng/ml	55±35 ng/ml	IBL Takasaki
2007 - Osteopontin expression in oral lichen planus		0.705 ± 0.173 U/ml		1.576 ± 0.511 U/ml	RnD
2007 - Osteopontin is elevated in Parkinson's disease and its absence leads to reduced neurodegeneration in the MPTP model	59–1295 ng/ml	468.2±282.9 ng/ml	88–3521 ng/ml	718.3±770.7 ng/ml	Assay Designs
2007 - Serum Markers to Detect Metastatic Uveal Melanoma		9 ng/ml		16 ng/ml	RnD
2008 - Elevated Osteopontin Levels in Patients with Peripheral Arterial Disease		6.54 ± 6.49 ng/ml		8.83 ± 9.90 ng/ml	RnD
2008 - The relationship between serum levels of vascular calcification inhibitors and carotid plaque vulnerability		43.57 ± 29.63 ng/ml		71.33 ± 45.35 ng/ml	RnD