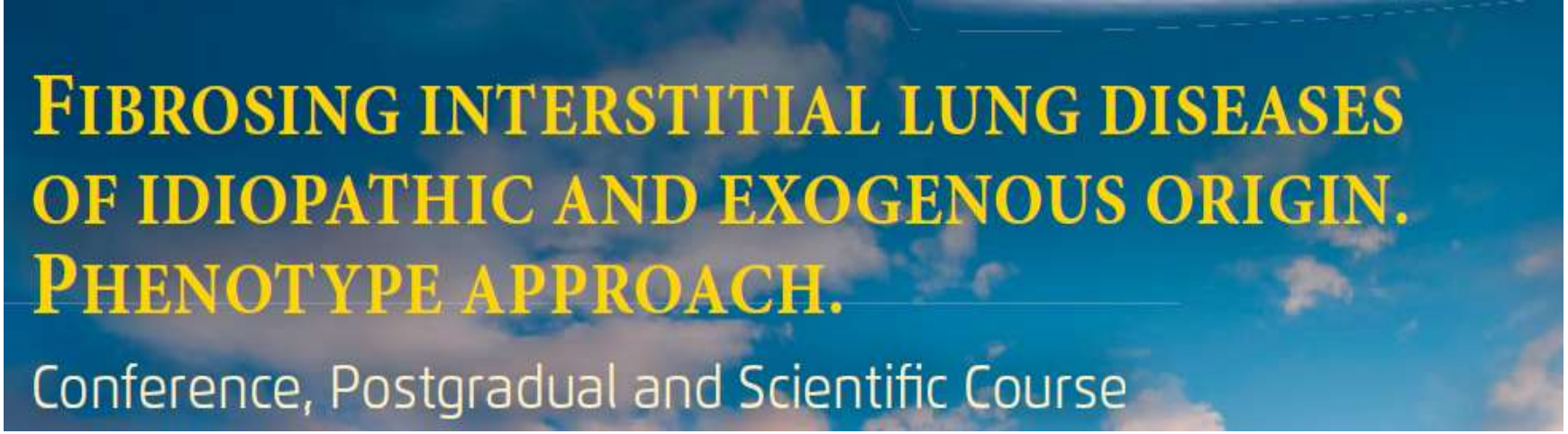


Pathogenesis of Hypersensitivity Pneumonitis

Katerina M. Antoniou, MD, PhD

As. Professor in Thoracic Medicine

ERS ILD Group Secretary

Medical School, University of Crete

Prague, June 2014

Definition

- Report of the NHLBI/ORD workshop:

- "Hypersensitivity pneumonitis, also known as extrinsic allergic alveolitis, is a *complex health syndrome* of varying intensity, clinical presentation, and natural history.

- HP is the result of an *immunologically induced inflammation of the lung parenchyma* in response to inhalation exposure to a large variety of antigens."

Definition

- The HP Study Group defined HP as:

- *“A pulmonary disease with symptoms of dyspnea and cough resulting from the inhalation of an antigen to which the patient has been previously sensitized.”*

General considerations

- HP is a pulmonary disease with or without systemic manifestations
- caused by the inhalation of an antigen to which the subject is sensitized and hyperresponsive;
- Sensitization and exposure alone in the absence of symptoms do not define the disease;

- ***An intriguing question is why only few of the exposed individuals develop the disease.***

Selman M, et al. AJRCCM 2012

Pathogenetic hypothesis

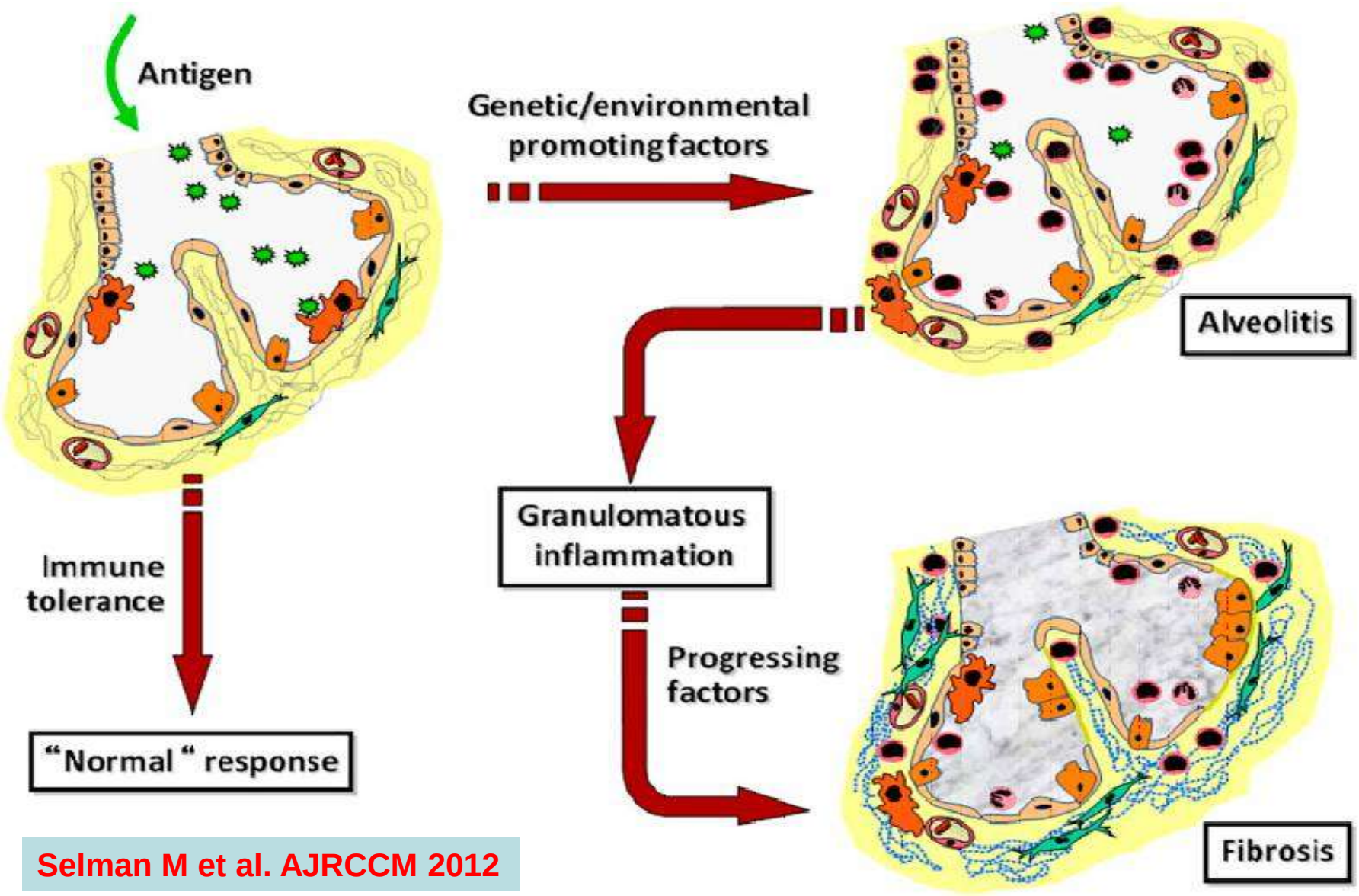
- **A two-hit hypothesis has been suggested,**

  wherein preexisting genetic susceptibility or environmental factors (*the first hit)* increases the risk for the development of HP after antigen exposure *(the second hit).*

- **Antigen exposure acts as the inducing factor, and genetic or environmental factors act as promoting risk factors.**

Selman M et al. AJRCCM 2012

PATHOGENESIS OF HP



Selman M et al. AJRCCM 2012

Environmental promoting factors

- Farmer's lung: high pesticide exposure, use of organochlorine & carbamate pesticides
- Respiratory viral infection: proteins from the influenza A virus have been revealed in BAL macrophages from most patients with acute HP.
- ***Viral infection induces the overexpression of B7 costimulatory molecules (CD80, CD86), increasing the antigen-presenting capacity by alveolar macrophages***

Hoppin JA, et al. Occup Environ Med 2007
Dakhama A, et al. AJRCCM 1999
Israël-Assayag E, et al. AJRCCM 1999

Smoking paradox in HP

- Activation of the nicotinic acetylcholine receptor a7 **reduces**
- the secretion of proinflammatory cytokines by macrophages
- it *decreases the reactivity of the Th1 and Th17 lineages, increasing the Th2 response,* on lymphocytes

- HP develops more frequently in *nonsmokers*

- *when HP occurs in smokers, they may develop a chronic clinical course*

Mechanisms of disease: Acute form

- Lung inflammation appears to be mediated by immune complexes :

- *presence of high titers of antigen-specific precipitating serum IgG, and*
- *increase of lung neutrophils*

Mechanisms of disease

- ***Cell-mediated immune reaction (type IV hypersensitivity):***
- Histology of lymphocytic interstitial infiltrates with granuloma formation
- BAL findings with a significant lymphocytosis & macrophage and lymphocyte activation
- can be passively transferred with sensitized lymphocytes of the Th1- type followed by inhalational challenge

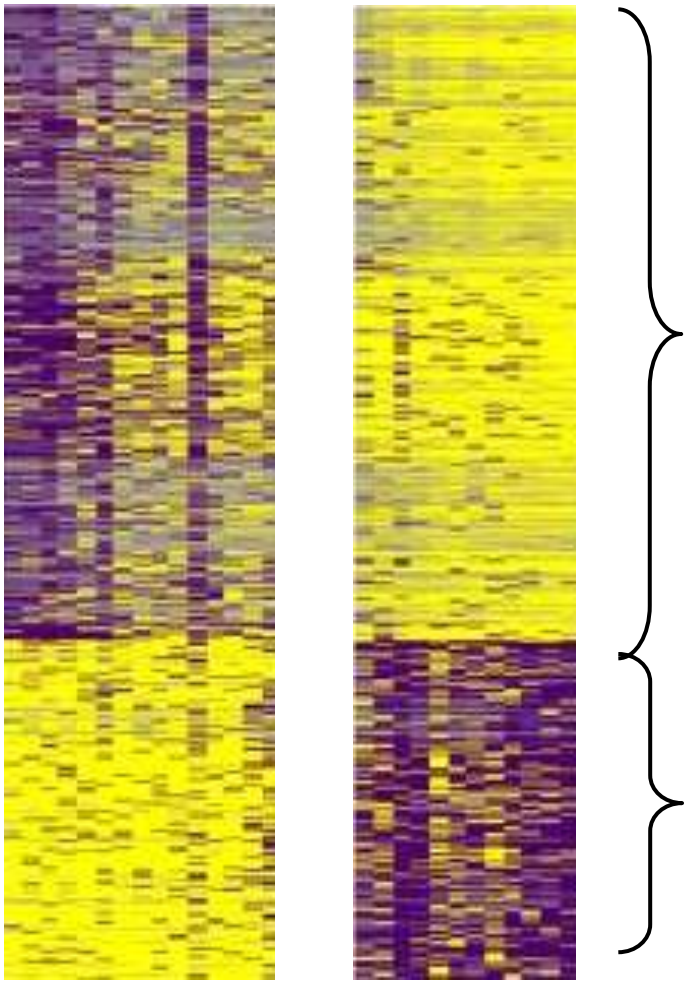
Genetic Susceptibility

- There is no evidence of a clear genetic susceptibility to develop HP.
- However, recent studies have described cytokine gene polymorphisms in patients with HP.
- ***Class II MHC molecules appear to be the primary susceptibility locus in HP.***
- Polymorphisms associated with HLA-DR and DQ have been associated with increased risk for HP in populations with different genetic backgrounds

Genetic Susceptibility

- Different genetic signature between HP & IPF.
- Increased expression of CCL24 and genes encoding for IL-1 receptor antagonist, TNF, and complement receptor 1, in IPF.
- In contrast, the expression of genes associated with inflammatory cytokines and chemokines is observed in HP.

Prominent Inflammatory Gene Expression in HP vs. IPF



Analysis of lung gene expression in IPF and Hypersensitivity Pneumonitis

- High gene expression
- Low gene expression

Genes expressed in HP > IPF

- Enriched for genes associated with
  - Inflammation
  - T cell activation
- Immune responses

Genes expressed in IPF > HP

- Enriched for genes associated with
  - Tissue remodeling
  - Epithelial cells
  - Fibroblasts

Selman et al. Am J Respir Crit Care Med Vol 173. pp 188–198, 2006

Genetic Susceptibility

- Increased frequencies of the alleles Gly-637 and the genotypes Asp-637/Gly-637 and Pro661/Pro661 on the *TAP1 (transporters associated with antigen processing 1)* gene.
- Others also found a polymorphism in the *PSMB8* gene among Mexican patients with HP.

MHC and HP

- Polymorphisms in TAP gene may lead to exacerbated immune response and interruption of antigen tolerance, *which may explain susceptibility of patients with HP to the disease.*

- *PSMB8* is involved in the antigenic presentation by the degradation of proteins and the generation of antigenic peptides.

TNFa & IL-6 in HP

- patients with farmer's lung display high frequency of TNFA2 (-308) allele, *which increases its biological activity*
- patients with BFL this allele is similar to control subjects.

- Correlation of IL-6 gene polymorphisms with BAL fluid cytokine and chemokine levels in BAL from patients with HP.

Immune Tolerance as Protective Factor

- Many exposed individuals develop a mild lymphocytic alveolitis *but remain asymptomatic, suggesting the development of a tolerant response to HP antigens*.

- Although the mechanisms are unclear, tolerance may be mediated by regulatory T cells (Treg).

Eur Respir J 2011; 37: 632–639
DOI: 10.1183/09031936.00055210

Impaired function of regulatory T-cells in hypersensitivity pneumonitis

M. Girard, E. Israël-Assayag and Y. Cormier

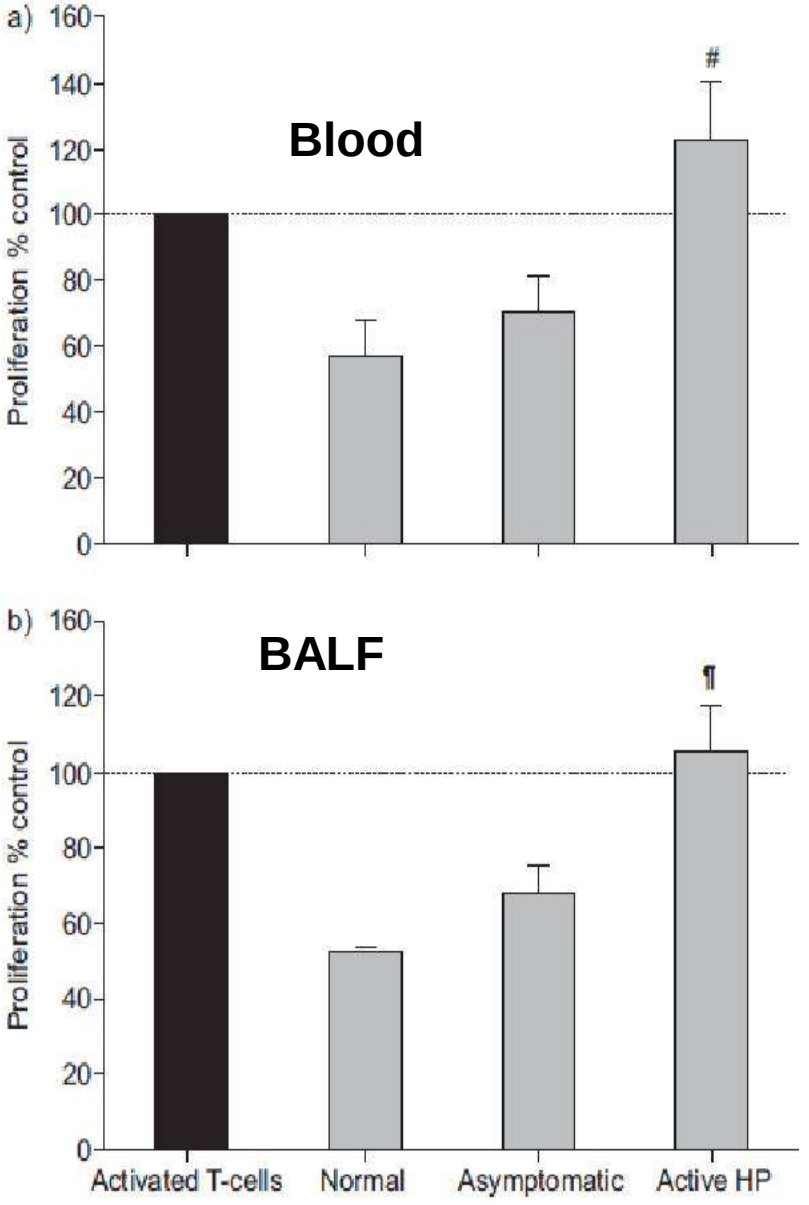
- **BALF and blood Treg-cells were totally nonfunctional and unable to suppress proliferation.**
- **Low levels of IL-17 were detected in sera and BALF from both normal and asymptomatic individuals, whereas measurable levels were found in patients.**
- **Defective Treg-cell function, potentially caused by increased IL-17 production, could account for the exacerbated immune response of HP.**

BRONCHOALVEOLAR LAVAGE IN HP

TABLE 1 Cells recovered from bronchoalveolar lavage (BAL) of normal individuals, asymptomatic subjects and hypersensitivity pneumonitis (HP) patients

	Normal	Asymptomatic	HP patients
Subjects n	4	4	6
Total inflammatory cells $\times 10^6$	29.82	35.63	134.57
Macrophages $\times 10^6$	24.72	28.58	72.13
Lymphocytes $\times 10^6$	3.97	7.20	51.00
Treg cells $\times 10^6$	0.12	0.17	2.81
Neutrophils $\times 10^6$	0.37	0.24	11.84
Eosinophils $\times 10^6$	0	0	0

Girard et al. ERJ 2011



JLB

$CD4^+CD25^+$ regulatory T cells attenuate hypersensitivity pneumonitis by suppressing IFN-γ production by $CD4^+$ and $CD8^+$ T cells

Yuna Park,[1] Sae Jin Oh,[1] and Doo Hyun Chung[2]

- IL-10 production of CD4+CD25+ Treg cells and direct contact between CD4+CD25+ Treg cells and CD4 or CD8 T cells in BALF resulted in reduced IFN- production.

- Taken together, CD4+CD25+ Treg cells play a protective role in SR-induced HP by suppressing IFN- production by T cells.

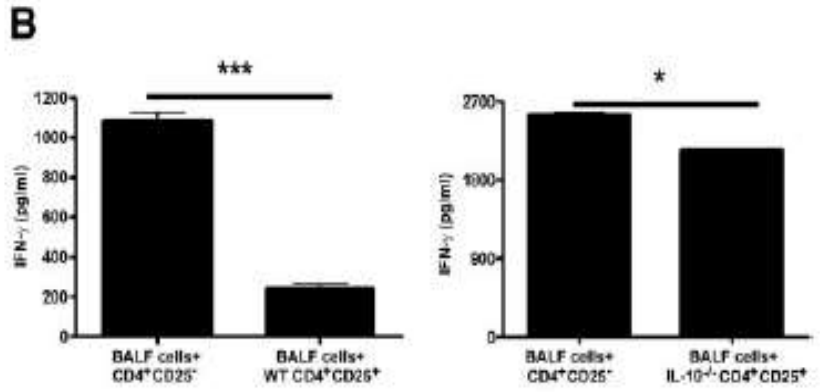


Figure 7. $CD4^+CD25^+$ T_{reg} cells suppress $CD4^+$ and $CD8^+$ T cells in the lungs during SR-induced HP via IL-10 production.

Immunopathogenesis

- Subacute and chronic forms of HP are provoked by T lymphocytes through a Th1 immune response *under the specific "master regulator" transcription factor, T-bet.*

Aune TM, et al. Immunology 2009

Immunopathogenesis

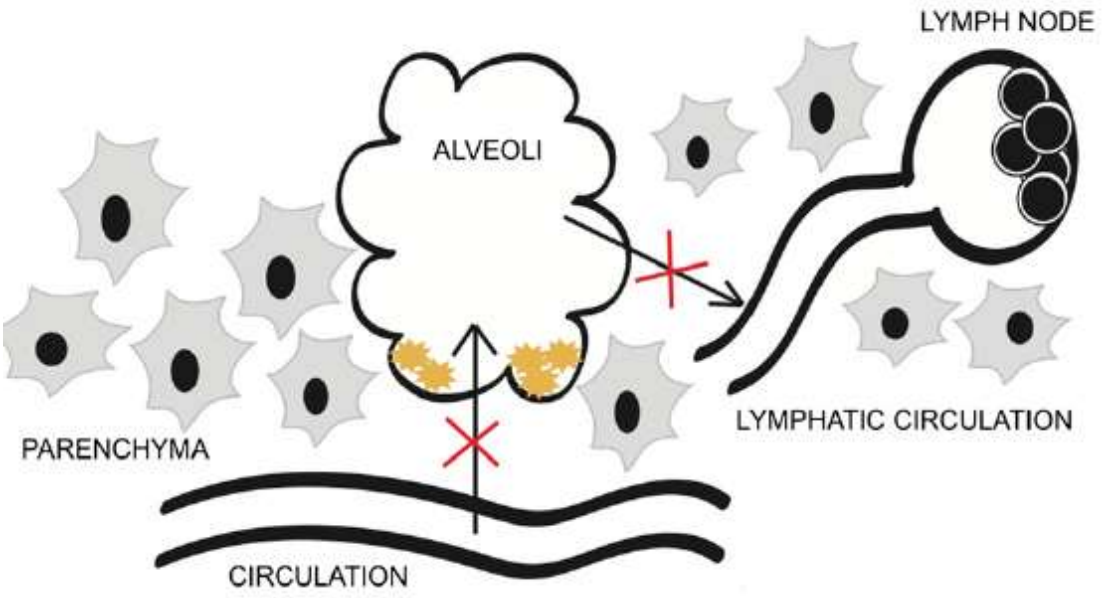
- *Data in experimental models of HP suggest that the expression of CD34+ and TLR-9 are critical for efficient trafficking of DC through the lungs and for development of a Th1 granulomatous inflammatory response.*

Bhan U, et al. Am J Pathol 2011;
Blanchet MR,et al. Am J Respir Crit Care Med 2011

CD34 Is Required for Dendritic Cell Trafficking and Pathology in Murine Hypersensitivity Pneumonitis

Blanchet MR,et al.

Am J Respir Crit Care Med Vol 184. pp 687-698, 2011



•Cd34-/- DCs fail to traffic from the alveolar space and migrate to the draining node and instead accumulate in the parenchyma.
• recruited DCs from the circulation accumulate in the parenchyma and fail to enter the alveolar space in response to inflammation.

Immunopathogenesis

- Microarray analysis in human HP revealed that in addition to Th1 factors, ***IL-17 and IL-17–associated transcripts were also up-regulated***.

- In chronic exposure to S. rectivirgula, *CD4+ T cells were not polarized to Th1 but rather to Th17 with differential expression of IL-17A and IL-22* .

Selman M, et al. AJRCCM 2006
Simonian PL, et al. J Immunol 2009

TLR9-Dependent IL-23/IL-17 is Required for the Generation of *Stachybotrys chartarum*-induced Hypersensitivity Pneumonitis

Urvashi Bhan, Michael J. Newstead, Xianying Zeng, Amy Podsaid, Moloy Goswami, Megan N. Ballinger, Steven L. Kunkel, and Theodore J. Standiford

- A novel role of TLR9 in driving IL-17 responses in experimental HP.
- Murine model: repeated intraperitoneal sensitization and i.t challenge of mice with the S.chartarum.
- ***IL-17 is an important cytokine mediator of SC-induced HP.***
- ***Production of IL-17 in this model requires TLR9-dependent IL-23 expression from DC.***

J Immunol. 2013

γδ T cells protect against lung fibrosis via IL-22

Philip L. Simonian,[1] Fabian Wehrmann,[1] Christina L. Roark,[2]
Willi K. Born,[2] Rebecca L. O'Brien,[2] and Andrew P. Fontenot[1,2]

- **In a mouse model of HP that progresses to lung fibrosis, *γd T cells expand in the lung and inhibit collagen deposition*.**
- **We show that a subset of these cells represents the predominant source of the Th17 cytokine IL-22.**
- ***Presence of protective γd T cells and IL-22 diminished recruitment of CD4+ T cells to lung.***
- ***These data reveal a protective pathway that involves the inhibition of γd T cells by regulatory IL-22–secreting ab T cells***

JEM 2010

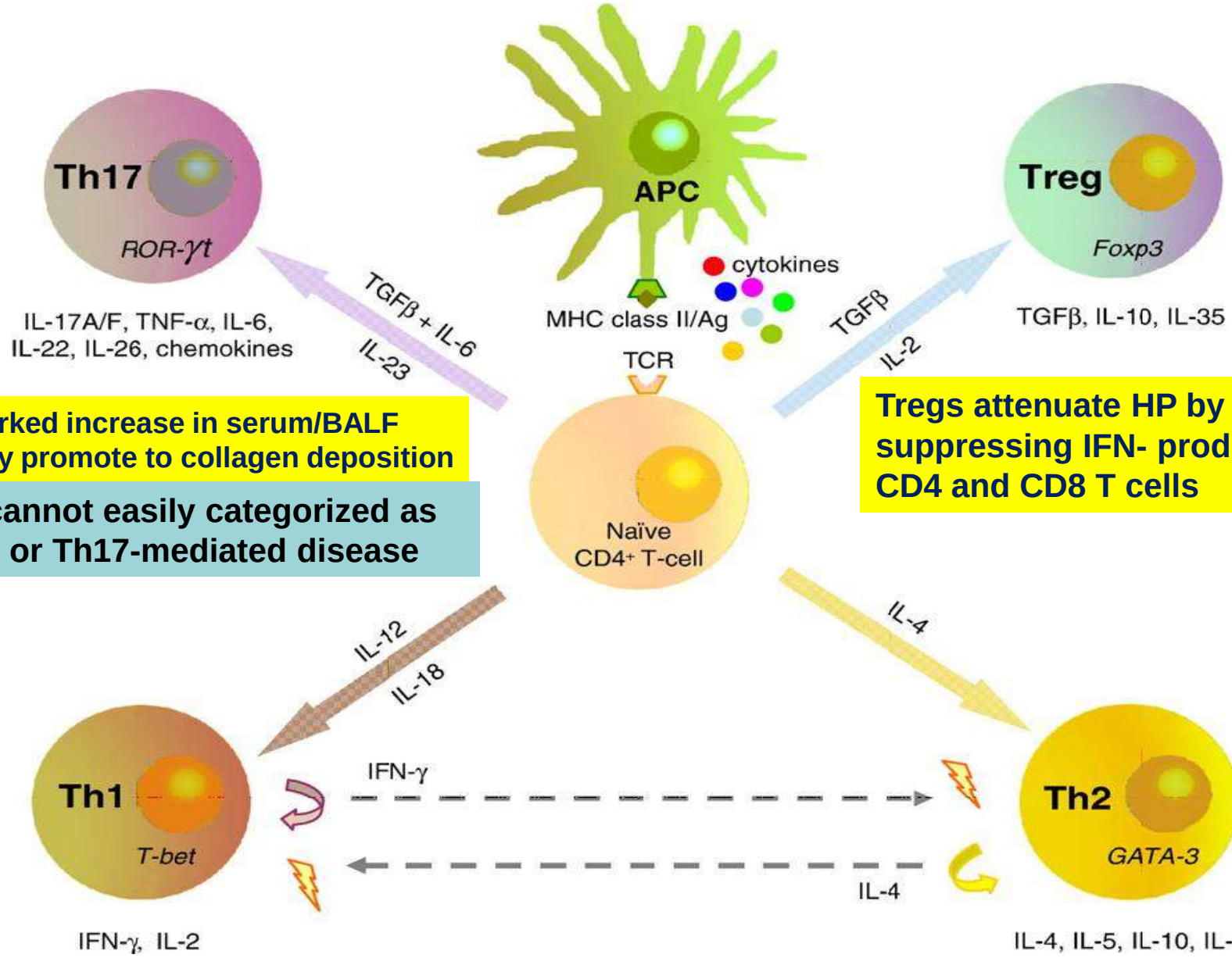
Immunopathogenesis: chronic disease -fibrosis

- increase in CD4+:CD8+ratio
- decrease of γδ T cells,
- skewing toward Th2 as opposed to Th1
- exhaustion of effector CD8+ T cells

- ***increase of Th17 cells may promote collagen deposition in the lung in response to chronic exposure of HP antigens.***

EFFECTOR

REGULATORY



Marked increase in serum/BALF
May promote to collagen deposition

HP cannot easily categorized as Th1- or Th17-mediated disease

Tregs attenuate HP by suppressing IFN- production by CD4 and CD8 T cells

Immunoglobulin Free Light Chains Are Increased in Hypersensitivity Pneumonitis and Idiopathic Pulmonary Fibrosis

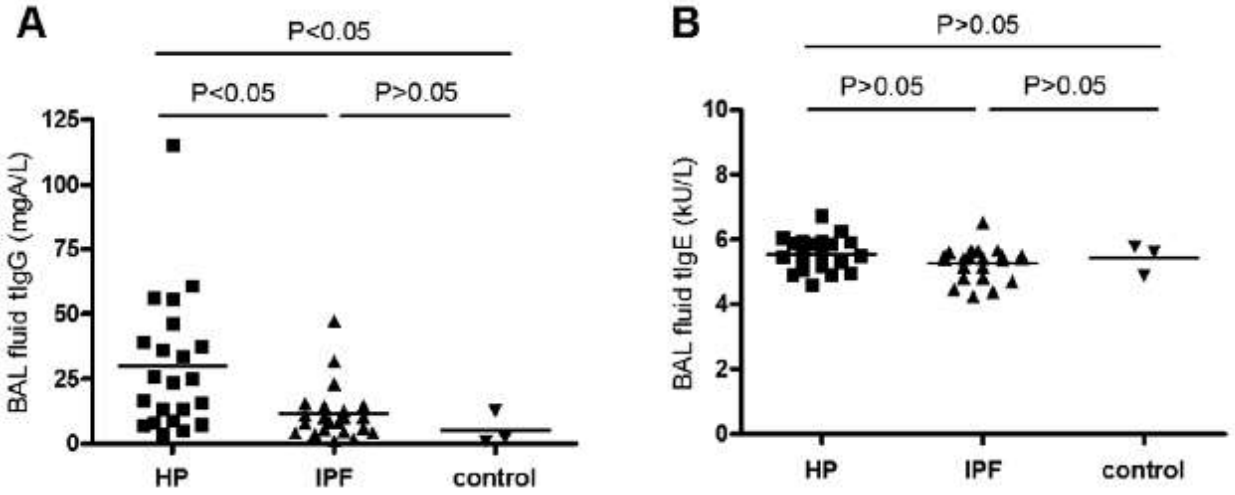
Tom Groot Kormelink[1], Annie Pardo[2], Karen Knipping[1,3], Ivette Buendía-Roldán[4], Carolina García-de-Alba[4], Bart R. Blokhuis[1], Moises Selman[4], Frank A. Redegeld[1]*

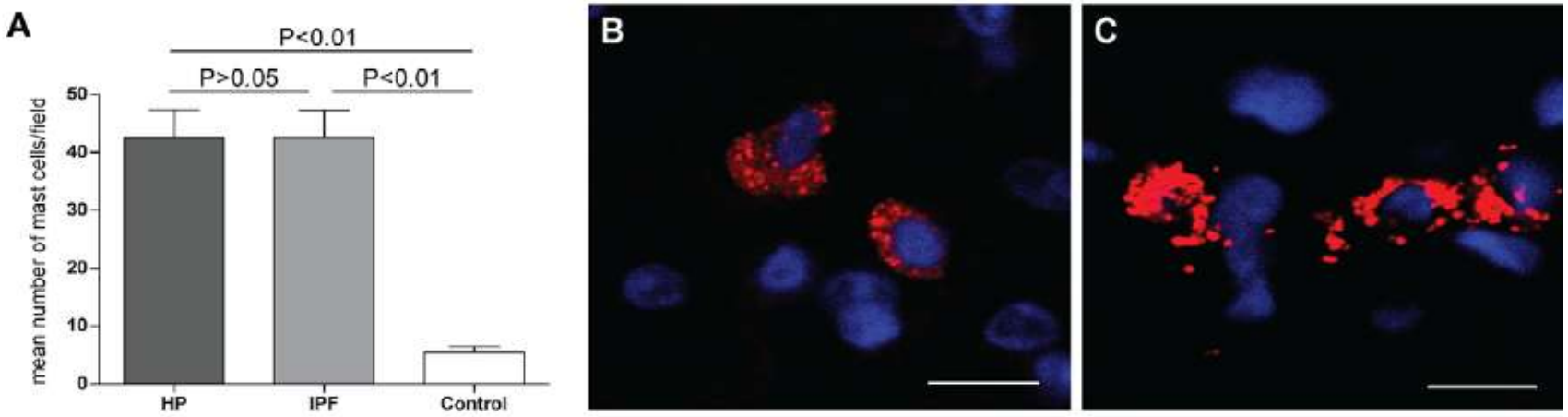
- ***Little is known about B lymphocyte involvement.***

- *Antibody response to inhaled antigens resulting in high titers of circulating specific antibodies*
- *presence of plasma cells in the BAL mainly in sub-acute cases*

Plos one 2011

Free Light Chains Are Increased in HP and IPF





Plos one 2011

“Regulation of microRNAs in Idiopathic Pulmonary Fibrosis & non-IPF in Bronchoalveolar Lavage Fluid (BALF) cells”

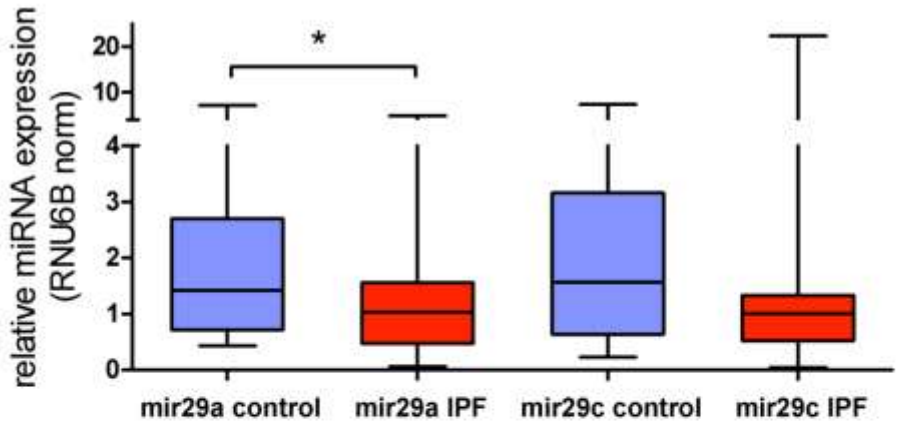
K.M. Antoniou, E. Tsitoura, S. Sarantoulaki, A. Psaraki, K. Karagiannis, H. Sato, A.U. Wells, G. Sourvinos, N.M. Siafakas

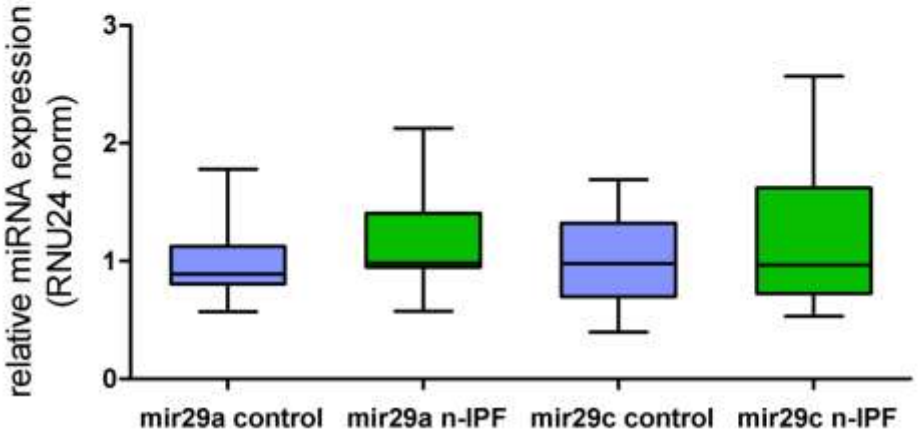
ΕΛΛΗΝΙΚΗ ΠΝΕΥΜΟΝΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ
HELLENIC THORACIC SOCIETY

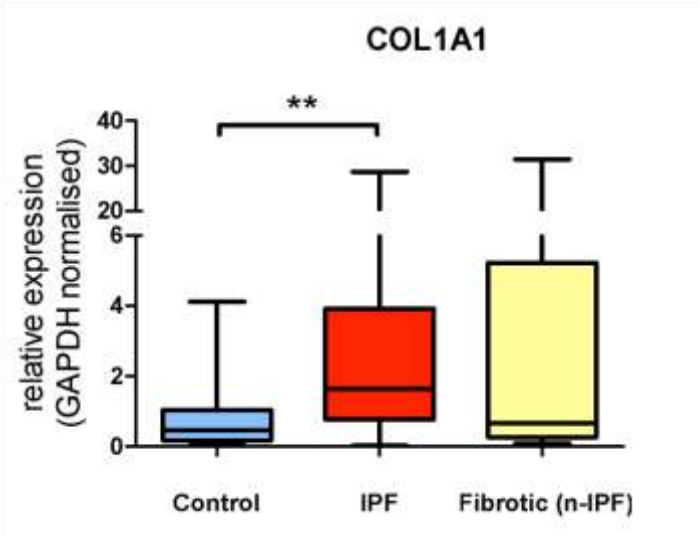
Ιατρική Σχολή
TransPOT

ATS 2014

mir29a and mir29c expression in IPF cells is down regulated inversely correlates with COL1A1 expression







*: $p<0.05$
**: $p<0.005$

Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization

Alicia Arranz[a,b], Christina Doxaki[a], Eleni Vergadi[a], Yeny Martinez de la Torre[a], Katerina Vaporidi[c], Eleni D. Lagoudaki[d], Eleftheria Ieronymaki[a], Ariadne Androulidaki[a], Maria Venihaki[a], Andrew N. Margioris[a], Efstathios N. Stathopoulos[d], Philip N. Tsichlis[b], and Christos Tsatsanis[a,1]

[a]Department of Clinical Chemistry, [c]Department of Intensive Care Medicine, and [d]Department of Pathology, School of Medicine, University of Crete, Heraklion 71003, Crete, Greece; and [b]Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA 02111

Akt1-/- macrophages give rise to M1 phenotype in response to LPS

increased sensitivity to LPS

Increased mir155 pro-inflammatory and decreased let7e (anti-inflammatory)
(Androulidaki et al 2009)

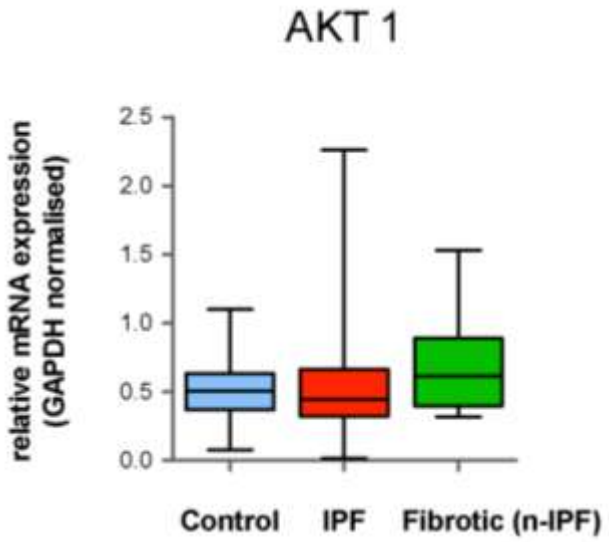
Akt2-/- macrophages give rise to M2 in response to LPS (hypo-responsive)

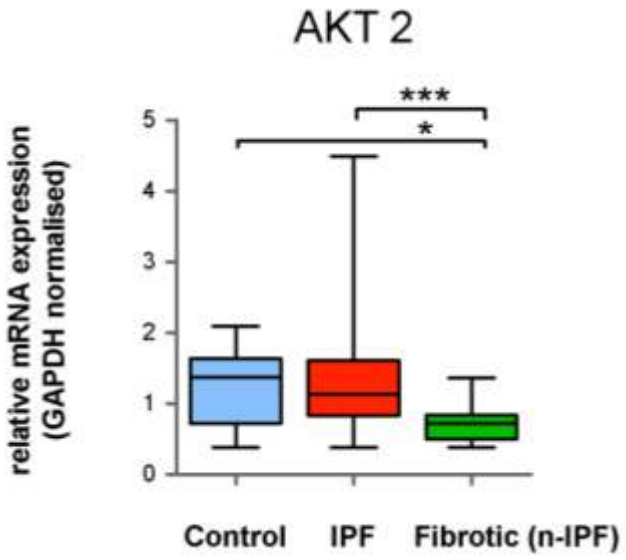
protection from acute lung injury

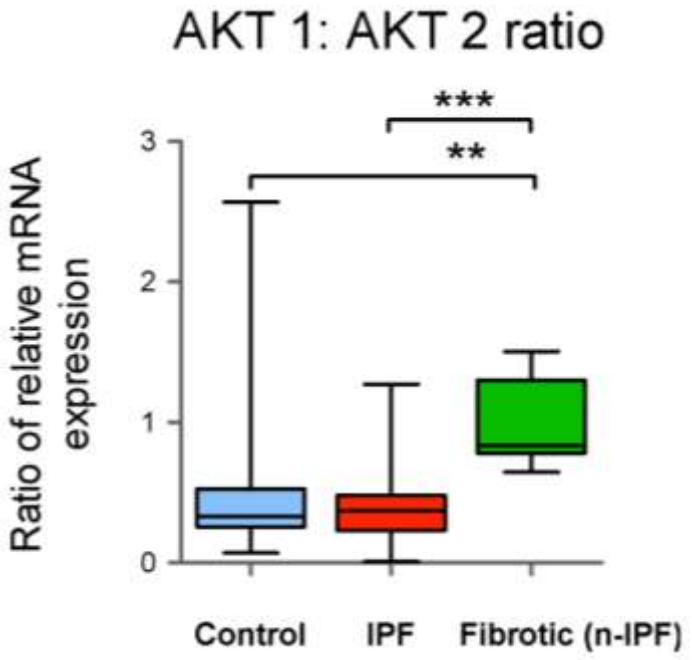
mir146a up-regulation (anti-inflammatory)

(Vergadi et al 2013)

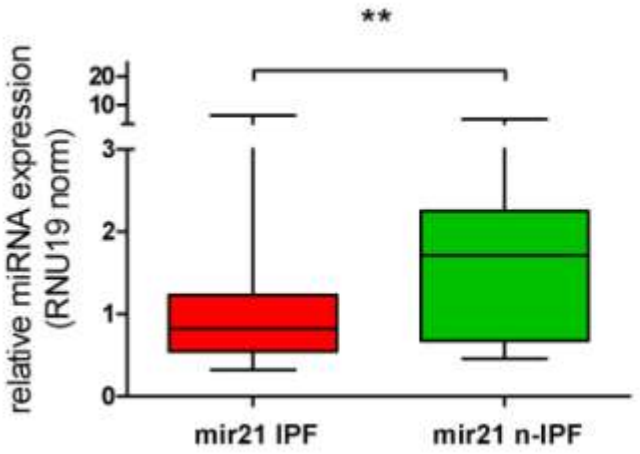
AKT2 expression is lower in the Non-IPF fibrotic group

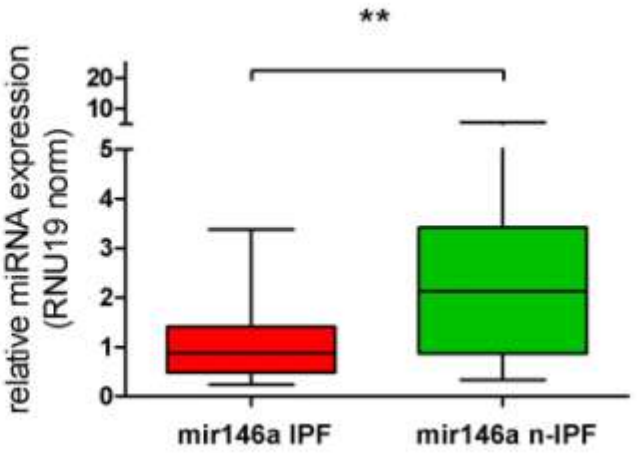


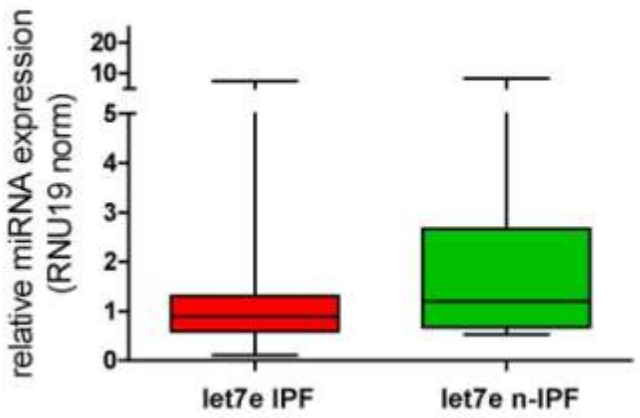


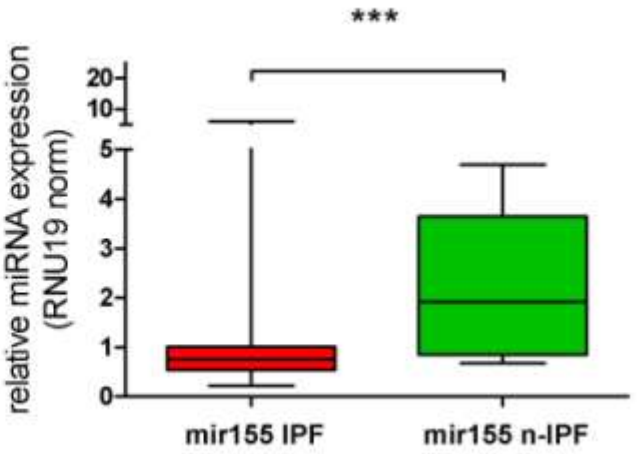


Immunoregulatory miRNA expression is up regulated in Non-IPF BALF cells

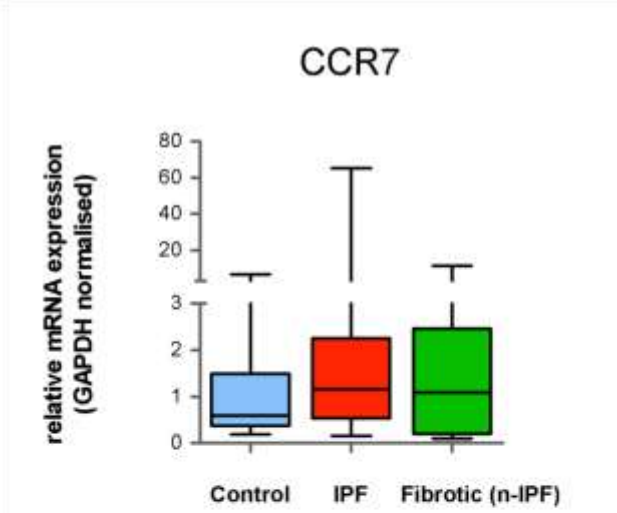


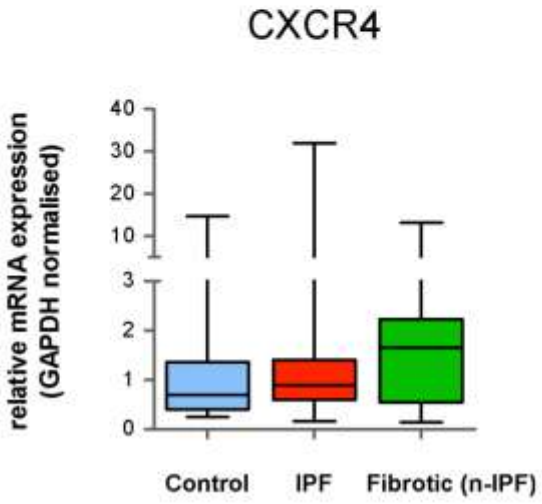


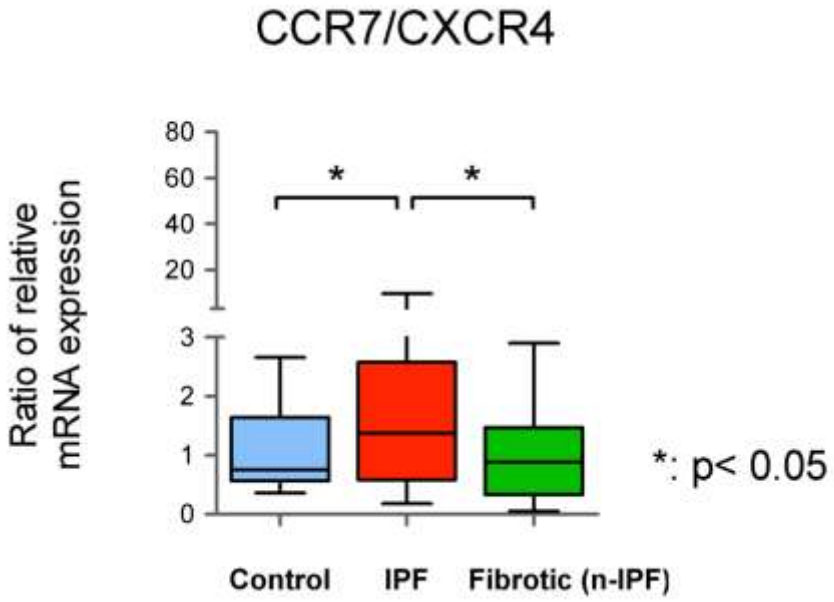




CCR7 (M1) vs CXCR4 (M2) expression in BALF cells







Conclusions

- *Genetics linked to MHC*
- *Immunopathogenesis unclear*
- *Progress regarding the role of Tregs & Th17*
- *Few insights regarding* B cells