Autoimmunity in IPF Pathogenesis: Novel insights

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IPF DEFINITION

- IPF is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults, *limited to the lungs*, and associated with the histopathologic and/or radiologic pattern of UIP.
(A) HRCT: peripheral and basal predominant reticular abnormalities, honeycombing, traction bronchiectasis, and bronchiolectasis.

(B) VATS biopsy shows UIP: heterogeneous appearance with alternating areas of normal lung, interstitial inflammation, fibrosis, and honeycomb change.

BOUROS D. Lancet 2009;374:180-182
Original and new hypotheses for the pathogenesis of IPF

**Original hypothesis**
- **Stimulus**
  - Chronic inflammation
  - Injury
  - Fibrosis

**New hypothesis**
- **Repeated stimulus**
  - Sequential lung injury
  - Aberrant wound healing
  - Fibrosis

**Genetic factors**

Inflammation

Th1 – Th2 balance

Gross, Hunninghake. *NEJM* 2001;345:517-525
Multiple pathologies and cascades in IPF

The same injury may result in one or a combination of histological lesions

Idiopathic pulmonary fibrosis: pathogenesis

**Injury**
- Epithelial damage
- Endothelial damage
- Destruction of alveolar capillary basement membrane
- Vascular leak
- Platelet activation
- Fibrin clot activation

**Epithelial-fibroblastic interaction**
- Release of profibrotic cytokines
- (myo)fibroblast recruitment, proliferation and differentiation
- Provisional matrix formation
- Angiogenesis
- Defective reepithelialisation

**Aberrant Repair and fibrosis**
- Exaggerated ECM accumulation
- Lack of matrix degradation
- Progressive lung remodelling
- Honeycomb changes

**Additional Notes**
Figure 1. The idiopathic pulmonary fibrosis transcriptome is influenced by both environmental and genetic factors. The epigenome links environmental exposures to gene-expression changes that lead to disease development. A number of genome-wide miRNA studies in IPF have been published, while DNA methylation and histone modification studies on the genomic scale are just emerging in IPF.
Autoimmunity in IPF

- Circulating antibodies to self antigens have been reported by several groups, without any definitive evidence of causality.

To examine the possibility that adaptive immunity may participate in IPF pathogenesis.
Comparison of phenotypes and effector functions of CD4 T cells, autoantibody production, and proliferative responses of pulmonary hilar lymphnodes CD4 T cells to autologous lung extracts from IPF patients and controls.

Feghali-Bostwick, J Immunol 2007
Main results

- CD4 Cells more frequently activated
- More frequently elaborate mediators $TGF-b1$, $IL-10$, and $TNF\alpha$
- Provide help to B cells to produce autoantibodies
- IPF lung extracts stimulated proliferations of autologous CD4 T cells

Feghali-Bostwick, J Immunol 2007
AUTOIMMUNITY IN IDIOPATHIC PULMONARY FIBROSIS
The presence of autoantibodies to self-antigen, perplakin, in 40% of IPF patients.

Anti-PPL antibodies have the potential to interfere with alveolar repair and are associated with a more severe disease.

Periplakin is localized in bronchial and alveolar epithelial cells.

Since T cells provide help for B cells to produce antibodies, these data are consistent with autoantigen-induced T cell proliferation in IPF.

Under normal conditions, regulatory T cells (Tregs) have key roles in suppressing T cell–mediated autoreactivity.
Global Impairment of CD4\(^+\)CD25\(^+\)FOXP3\(^+\) Regulatory T Cells in Idiopathic Pulmonary Fibrosis

Ioannis Kotsianidis\(^1\*\), Evangelia Nakou\(^2\*\), Irene Bouchliou\(^1\), Argyrios Tzouvelekis\(^2\), Emmanouil Spanoudakis\(^1\), Paschalis Steiropoulos\(^2\), Ioannis Sotiriou\(^2\), Vassilis Aidinis\(^3\), Dimitrios Margaritis\(^1\), Costas Tsatalas\(^1\), and Demosthenes Bouros\(^2\)

\(^1\)Department of Hematology and \(^2\)Department of Pneumonology, Democritus University of Thrace Medical School, Alexandroupolis; and \(^3\)Institute of Immunology, Biomedical Sciences Research Center Alexander Fleming, Athens, Greece

TABLE 1. DEMOGRAPHIC AND SPIROMETRIC CHARACTERISTICS OF PATIENTS ENROLLED

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IPF</th>
<th>nIPF</th>
<th>CVD-IP</th>
<th>Healthy Volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>19</td>
<td>35</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Sex, no. of males/no. of females</td>
<td>19/0</td>
<td>24/11</td>
<td>6/12</td>
<td>16/12</td>
</tr>
<tr>
<td>Age, years</td>
<td>66 (44–79)</td>
<td>50 (38–62)</td>
<td>64 (41–72)</td>
<td>48 (23–82)</td>
</tr>
<tr>
<td>Smokers/nonsmokers</td>
<td>15/4</td>
<td>28/7</td>
<td>8/12</td>
<td>14/14</td>
</tr>
<tr>
<td>6MWT distance, m</td>
<td>355 ± 34</td>
<td>534 ± 45</td>
<td>485 ± 27</td>
<td>525 ± 32</td>
</tr>
<tr>
<td>6MWT ΔSaO(_2), %, points</td>
<td>7.2 ± 3.4</td>
<td>4.6 ± 2.9</td>
<td>4.5 ± 2.2</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>FVC, % pred</td>
<td>68 ± 5</td>
<td>78 ± 4</td>
<td>71 ± 3</td>
<td>104 ± 10</td>
</tr>
<tr>
<td>TLC, % pred</td>
<td>67 ± 2</td>
<td>77 ± 3</td>
<td>66 ± 5</td>
<td>89 ± 13</td>
</tr>
<tr>
<td>Kco, % pred</td>
<td>51 ± 5</td>
<td>68 ± 5</td>
<td>67 ± 4</td>
<td>90 ± 4</td>
</tr>
</tbody>
</table>
Decreased Tregs in the peripheral blood

A

<table>
<thead>
<tr>
<th></th>
<th>HV</th>
<th>nIPF</th>
<th>IPF</th>
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</thead>
<tbody>
<tr>
<td>CD25</td>
<td>1.2%</td>
<td>3.1%</td>
<td>0.24%</td>
</tr>
</tbody>
</table>

B

\[
P = 0.002 \qquad P = 0.01 \qquad P = 0.026
\]

C

\[
P = 0.022
\]

Decreased Tregs in the BALF
Marked reduction in suppressor function of PB and BALF Tregs on Teff cells compared to HV and nIPF.

Impaired control of BALF and PB Treg cells of Th1 cytokine release.

Defective inhibition of Th2 cytokine production by Treg cells.

‘The low numbers and the systemic and local Treg dysfunction may either result in inefficient control of the pre-existing overexuberant Th2 response or contribute to a Th2 skew’.

Investigation of the citrullination pathway in the pathogenesis of Fibrotic Lung Disorders

Antoniou KM, Samara K, Lasithiotaki I, Pantelidis P, Siafakas NM and Wells AU

ERS 2012
RA-ILD: prognosis

- In RA, clinically significant ILD
  - affects 7-10% patients
  - reduces life expectancy by 2.6 years (men) to 3.5 years (women)

- Bongartz, Arthritis Rheum 2010; Olson, AJRCCM 2011
Rheumatoid Lung Disease

Kevin K. Brown

Lung disease in RA

- Usual interstitial pneumonia is the pattern in IPF and is also the most frequent RA histology

- Logical, therefore, to compare and contrast IPF and rheumatoid lung
Smoking also increases the incidence and severity of rheumatoid arthritis (RA), possibly by means of epitope citrullination in a predisposing genetic background, increasing the risk of severe extra-articular RA manifestations.
Review

Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis

Lars Klareskog\textsuperscript{a,•}, Vivianne Malmström\textsuperscript{a}, Karin Lundberg\textsuperscript{a}, Leonid Padyukov\textsuperscript{a}, Lars Alfredsson\textsuperscript{b}

\textbf{Interplay between two genes (HLA-DR-SE, PTPN22) and one environmental factor (smoking) in two subsets of RA}

\textbf{ACPA-positive RA}

\begin{itemize}
\item No SE
\item Single SE
\item Double SE
\end{itemize}

\textbf{ACPA-negative RA}

\begin{itemize}
\item No SE
\item Single SE
\item Double SE
\end{itemize}

It is not clear if autoimmunity is involved in IPF pathogenesis.

We explored whether an autoimmune process implicating anticitrullinated peptide immunity may take place in patients with IPF.
Hypothesis

The citrullination pathway is implicated in the pathogenesis of pulmonary fibrosis, both idiopathic and autoimmune.
Citrullination is a posttranslational modification of arginine by peptidylarginine deiminase (PADI) enzymes. Citrullinated residues may be neoepitopes that break immunologic tolerance and lead to Rheumatoid Arthritis.
Aim of the study

- a) activation of the citrullination pathway
- b) linkage between citrullination and outcome in both diseases.
Patients and Methods

- We have evaluated the expression of PADI-2 and PADI-4 in BALF of:
  - 53 patients with IPF
  - 37 patients with rheumatoid lung (RA-ILD)
  - 10 healthy controls
Western blot analysis of peptidylarginine deiminase type II (PADI2) in BALF

- Protein levels of PADI2 were determined via western blotting, using actin as internal control.
- PADI2 levels were higher in the RA-ILD group compared to the control group (p<0.005), as well as compared to the IPF group (p<0.05). There was no statistically significant result in the IPF vs control group analysis (p>0.05).
Protein levels of PADI4

Control group
RA-ILD
IPF

0.0
0.5
1.0
1.5
2.0

p<0.05
p<0.005
p<0.005

Relative protein expression
Western blot analysis of peptidylarginine deiminase type IV (PADI4) in BALF

- Protein levels of PADI4 were determined via western blotting, using actin as internal control.
- PADI4 levels were higher in the RA-ILD group compared to the control group (p<0.005), as well as compared to the IPF group (p<0.05).
- PADI4 levels were higher in the IPF group compared to the control group analysis (p<0.005).
Survival analysis

- Twenty-nine of 53 IPF patients died during a (mean ± SD) follow-up time of 35 ± 28 months.

- Sixteen of 37 RA-ILD patients died during a (mean ± SD) follow-up time of 42 ± 26 months.
PADIs and IPF

- PADI4 levels were predictive of late mortality in IPF, *before* ($p<0.005$) and *after* ($p=0.03$) adjustment for CPI, age, gender and smoking.

- PADI2 levels were not predictive of mortality in IPF ($p = 0.935$).
PADI4 levels were predictive of late mortality in RA-ILD, before (p=0.04) and after (p=0.03) adjustment for CPI, age, gender and smoking.

PADI2 levels were predictive of late mortality in RA-ILD, before (p=0.04) and after (p = 0.05) adjustment with CPI, age, gender and smoking.
When both PADI2 and PADI4 were included in the same multivariate model, *both were independently predictive of late mortality after adjustment with CPI, age, gender and smoking status.*
Citrullination in extra-articular manifestations of rheumatoid arthritis

T. Bongartz¹, T. Cantaert², S. R. Atkins¹, P. Harle³, J. L. Myers¹, C. Turesson¹,⁴, J. H. Ryu¹, D. Baeten² and E. L. Matteson¹

Results. Presence of citrulline could be detected in eight lung specimens of patients with RA-associated IP (44%) and nine patients with idiopathic IP (46%). Conversely, lung tissue from control patients showed weak extracellular citrullination in only two cases (20%). Citrullination did not show any significant associations with demographic or clinical characteristics such as age, gender, smoking habits, disease severity, histological subtype, degree of inflammation or steroid use. Rheumatoid nodules were citrulline positive in a majority of cases (70%).
High Levels of Anti-Cyclic Citrullinated Peptide Autoantibodies Are Associated with Co-occurrence of Pulmonary Diseases with Rheumatoid Arthritis

Fleur Aubart, Bruno Crestani, Pascale Nicaise-Roland, Florence Tubach, Caroline Bollet, Karen Dawidowicz, Emilie Quintin, Gilles Hayem, Elisabeth Palazzo, Olivier Meyer, Sylvie Chollet-Martin and Philippe Dieudé

**Objective.** To investigate whether levels of anti-cyclic citrullinated peptide antibodies (anti-CCP2) in patients with rheumatoid arthritis (RA) are associated with the co-occurrence of lung diseases.

**Methods.** A total of 252 RA patients were included in a cross-sectional study. Pulmonary disease was confirmed by high-resolution chest computed tomography scan. Circulating anti-CCP2 were quantified using ELISA. Multivariate logistic regression was conducted to identify independent risk factors for lung disease.

**Results.** Male sex (OR 3.29, 95% CI 1.59–6.80) and high anti-CCP2 levels (OR 1.49, 95% CI 1.25–1.78) were identified as independent risk factors for lung disease in the RA population.

**Conclusion.** High anti-CCP2 levels are associated with lung disease in the RA population. (J Rheumatol First Release March 1 2011; doi:10.3899jrheum.101261)
Lung disease and ACPA

<table>
<thead>
<tr>
<th>ACPA (median)</th>
<th>Lung (+)</th>
<th>Lung (−)</th>
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<tbody>
<tr>
<td></td>
<td>N = 59</td>
<td>N = 168</td>
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</table>

P < 0.0001

2157

184

N=227

(Aubart, Crestani et al, J Rheumatol 2011)
Lung disease with anti-CCP antibodies but not rheumatoid arthritis or connective tissue disease

Objective: We sought to characterize a novel cohort of patients with lung disease, anti-cyclic citrullinated peptide (CCP) antibody positivity, without rheumatoid arthritis (RA) or other connective tissue disease (CTD).

Methods: The study sample included 74 subjects with respiratory symptoms, evaluated January 2008–January 2010 and found to have a positive anti-CCP antibody but no evidence for RA or other CTD. Each underwent serologic testing, pulmonary physiology testing, and thoracic high-resolution computed tomography (HRCT) scan as part of routine clinical evaluation.

Results: The majority of subjects were women, and most were former cigarette smokers. Four distinct radiographic phenotypes were identified: isolated airways disease (54%), isolated interstitial lung disease (ILD) (14%), mixed airways disease and ILD (26%), and combined pulmonary fibrosis with emphysema (7%). This cohort had a predominance of airways disease, either in isolation or along with a usual interstitial pneumonia-pattern of ILD. Among subjects with high-titer anti-CCP positivity (n = 33), three developed the articular manifestations of RA during a median follow-up of 449 days.

Conclusion

- Our results suggest that citrullination is an active process in both autoimmune and idiopathic lung fibrosis.

- The role of citrullination enzymes as biomarkers predicting late mortality merits further evaluation.
ΜΕΛΕΤΗ ΤΗΣ ΕΝΕΡΓΟΠΟΙΗΣΗΣ ΤΟΥ ΦΛΕΓΜΟΝΟΣΩΜΑΤΟΣ ΣΤΟ ΒΡΟΓΧΟΚΥΨΕΛΙΔΙΚΟ ΕΚΠΛΥΜΑ (BALF) ΑΣΘΕΝΩΝ ΜΕ ΠΝΕΥΜΟΝΙΚΗ ΙΝΩΣΗ

Γιανναράκης Ι., Σαμαρά Κ., Λασηθιωτάκη Ι., Μαργαριτόπουλος Γ., Χουλάκη Χ., Σιδηρόπουλος Π., Σιαφάκας Ν., Αντωνίου Κ.

Πνευμονολογική Κλινική, Εργαστήριο Μοριακής και Κυτταρικής Πνευμονολογίας, Ιατρική Σχολή Πανεπιστημίου Κρήτης, Ηράκλειο Κρήτης
Ρευματολογική Κλινική, Ερευνητικό Εργαστήριο Ρευματολογίας, Ιατρική Σχολή Πανεπιστημίου Κρήτης, Ηράκλειο Κρήτης.
Inflammasome Pathway Activation In Fibrotic Lung Diseases In Human Bronchoalveolar Lavage Fluid

Preliminary results

NLRP3-inflammasome activation products -TNFa and IL1β- exhibited higher secretion levels, both at baseline and after treatment with infectious stimuli (LPS), in the disease-specific samples (BALF) than in peripheral blood leukocytes in all groups studied.

The RA-ILD group showed a tendency to up-regulate this pathway compared to controls and IPF.

The IPF group seems to lose the ability to activate the NLRP3 pathway in the presence of infectious stimuli, thus suggesting a compromise of the innate immunity regulation in IPF.
Whether autoantibodies in patients with ILD are disease triggers, contribute to disease pathogenesis, or are simply epiphenomena and disease or prognostic biomarkers remains to be determined.
PATHOGENETIC MECHANISMS IN IPF

- Inflammation oxidants
- Epithelial apoptosis
- Epithelial-mesenchymal transformation
- Coagulation
- Fibroblast growth factors
- Angiogenesis
- Bone marrow-derived fibroblasts
- Myofibroblasts
- IMMUNODEREGULATION Th1/Th2/TH17/Tregs
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Dept of Clinical Virology
Prof DA Spandidos
As. Prof, G. Sourvinos
Soufia Giannoula, PhD
Thank you for your attention