The IgA-pIgR System Is Dysregulated in IPF

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TPB, CP and AF designed the study. TPB, YB, ML, DH, CF, VL, BR, WW, CB collected data. TPB, ML, YB, CF performed experiments. TPB, ML, CP, AF analyzed the data. TPB drafted the manuscript under the supervision of CP and AF. All authors revised the manuscript and accepted its final version.

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To the Editor:

Introduction

Idiopathic pulmonary fibrosis (IPF) lungs display aberrant epithelial structures, namely honeycomb cysts, lined by a bronchiolized epithelium and filled with mucus-like secretions, and hyperplasic type-2 alveolar epithelial cells (AEC2) cysts (1). The recent discovery of pathological bronchial epithelial cell subpopulations in IPF along with mucosal immunity modifications further questions the role of these structures (2).

Immunoglobulin A (IgA) is the predominant mucosal immunoglobulin and a key player of mucosal immunity. IgA subclasses, IgA1 and IgA2, differ by the length of their hinge region and glycosylation profile and by their effector activities. Airway cells express the polymeric immunoglobulin receptor (pIgR) at their basolateral side, allowing IgA binding and transcytosis. After transcytosis, the secretory component (SC), a part of the pIgR molecule, is cleaved and shed alone or complexed with IgA, generating free SC or secretory IgA (S-IgA) at the luminal level (3). Recent studies have linked IgA and pIgR to IPF severity (4, 5) and increased levels of IgA+ memory B-cells and autoreactive IgA are observed in IPF (6). However, a comprehensive overview of the IgA-pIgR system in IPF is lacking. Therefore, we studied in-depth the IgA-pIgR system IPF.

Methods

Following informed consent ((review board approval numbers PNEU-ILD-01 2017/JAN/010, 2007/19MARS/58, S52174 and S55877), serum was prospectively collected from IPF patients (n=45) and from age- and sex-matched controls without evidence of respiratory disease (n=22). Broncho-alveolar lavage (BAL) was performed according to standard operating procedures in IPF patients (n=39) and controls (n=6). We obtained lung tissue from IPF explants (Universitair Ziekenhuis Leuven (Leuven, Belgium) and Centre Hospitalier Universitaire UCL Namur-Godinne (Yvoir, Belgium)) or upon surgical lung resections at the Cliniques universitaires Saint-Luc (Brussels, Belgium)(n=11). Donor samples (n=10) originated from rejected donors without evidence of pulmonary disease or from

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surgical resection for solitary lung tumor, at maximum distance of the lesion. Patients' characteristics are summarized in Table 1. Total serum IgA, IgG and IgM were determined using a Cobas 8000 (Roche, Switzerland). ELISA for human IgA, S-IgA and SC was performed as previously described (7, 8). Tyramide signal amplification (TSA) staining was performed (7) using HPA006154 (pIgR, Sigma-Aldrich, Saint-Louis, MO), PA5-82342 (MUC5B, Thermo Fisher Scientific, UK), ab239472 (SCGB1A1, Abcam, UK), T7941 (β-tubulin, Sigma-Aldrich), SA-1-19258 (IgA, Thermo Fisher Scientific), and ab90716 (proSP-C, Abcam) antibodies. A single cell RNA sequencing database of IPF and control lungs was queried through the IPF cell atlas interface (https://p2med.shinyapps.io/IPFCellAtlas/) to evaluate potential *PIGR* expressing cell types (9). A549 cells (ATCC) were cultured at 37°C and 5% CO2 in DMEM (Capricorn Scientific, Germany) with 10% fetal bovine serum (Thermo Fisher Scientific), L-glutamine (Capricorn Scientific) and penicillin/streptavidine (Capricorn Scientific) . Cells were stimulated for 48 hours with either control medium, dimeric IgA or S-IgA (the latter obtained as previously described (10)). We compared groups with Mann-Whitney U test (2 groups) or Kruskal-Wallis test (>2 groups). We used χ^2 or Fisher's exact test for categorial variables. Correlations were assessed using Pearson's correlation coefficient followed by linear regression analysis. We analyzed survival with Kaplan-Meier approach with a log-rank test and a Cox proportional hazards model, considering age, smoking status, sex and IgA levels. Data are presented as median and interquartile range. A p-value <0.05 was deemed significant.

Results

IPF patients had a higher serum concentration of both IgA1 (median: 3.29 (IQR: 2.70-4.02) vs 2.51 (1.77-3.16)mg/mL, p=0.005) and IgA2 (0.14 (0.10-0.23) vs 0.033 (0.02-0.09)mg/mL, p<0.0001), (**figure 1A**) while serum IgM or IgG did not differ (0.83 (0.61-1.13) vs 0.79 (0.42-1.24), p=0.54 and 12.00 (10.15-14.50) vs 11.30 (9.35-13.70) mg/mL, p=0.32 respectively, data not shown). Higher S-IgA (**figure 1B**), S-IgA1 (17.48 (12.47-26.85) vs 12.08 (7.86-14.80) µg/mL, p=0.0005) and SC (98.74 (86.37-126.10) vs 81.24 (73.22-91.54) ng/mL, p=0.0004), but not S-IgA2 levels (0.61 (0.34-0.88) vs 0.54 (0.33-0.78)

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µg/mL, data not shown) were detected in IPF patients compared to controls. In BAL, IPF patients displayed higher levels of IgA (figure 1A), including IgA1 (8.68 (5.72-14.24) vs 4.33 (3.14-5.82)µg/mL, p=0.01), and S-IgA (figure 1B) but not SC (523.20 (335.20-826.40) vs 662.90 (295.80-842.70)ng/mL, p=0.78, data not shown) or S-IgA2 (0.45 (0.33-0.60) vs 0.32 (0.24-0.43) µg/mL, p=0.20, data not shown). We detected a trend towards increased S-IgA1 levels (4.36 (2.75-6.32) vs 3.00 (2.11-4.13), p=0.08). Furthermore, serum SC negatively correlated with baseline diffusion capacity of the lung for carbon monoxide (DLCO) (r=0.42, p=0.0032, **figure 1C**) and patients displaying more than 5% FVC loss had a higher serum SC at baseline than patients who remained stable (124.70 (97.48-138.90) vs 93.44 (79.57-116.20) ng/mL, p=0.02). Using the previously reported cut-off for serum IgA of 2.85 g/L (4), a trend was observed towards increased mortality of IPF patients with higher serum IgA concentration at diagnosis (p=0.06). A Cox model with age, smoking status, sex and IgA levels as variables showed a trend between survival and age (p=0.05, HR: 1.10 (1.00-1.20)) and IgA status (IgA>2.85g/L, p=0.09, HR: 3.07 (0.33-11.35)).

We detected pIgR expression in the epithelium of bronchiolized area and S-IgA in the luminal mucus (figure 1D). Query of a single cell RNAseq dataset (9) revealed that ciliated, goblet and club cells were the airway cells expressing *PIGR.* Co-stainings for β-tubulin (ciliated cells), MUC5B (goblet cells) and SCGB1A1 (club cells) confirmed this, showing the presence of pIgR+β-tubulin+, pIgR+MUC5B+ and pIgR+SCGB1A1+ cells (**figure 1D**). Further co-staining for SC and IgA showed overlapping mucus marking within these structures, confirming the presence of S-IgA (**figure 1E**) and suggesting a functional transcytosis by the HC epithelium. Furthermore, the intraluminal presence of S-IgA was observed within cystic AEC2 structures, while such structures and potential contact were absent from control lungs (**figure 1F**). Rare zones of continuity between pIgR+ bronchiolized areas and S-IgA filled proSP-C+ cysts were present, suggesting a passage from the former to the latter. Finally, stimulating A549 baso-alveolar cells with dimeric IgA or S-IgA, induced an upregulation of *VIM* (coding for vimentin) and *SLUG*, implicated in epithelial-mesenchymal transition (EMT), after S-IgA treatment (**figure 1G**).

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Discussion

In this study, we show an activation of the IgA-pIgR system in IPF, with several components correlating with severity. Additionally, S-IgA and pIgR expression in HC cysts suggests a role in the disease. Indeed, overexpression of the pIgR has been shown to promote EMT in cancer cells (11), while S-IgA is profibrotic *in vitro* (12, 13), strengthening this link between IgA-pIgR components and (epithelial) fibrotic features. In addition, we observe here a potential role in the EMT of alveolar cells, a process implicated in IPF (14) . All in all, our results pave the way for further research on the implication of mucosal immunity in pulmonary fibrosis.

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Table 1. Demographic characteristics of patients. Data are presented as median and interquartile range. Data analysis was performed using a Mann-Whitney U test, χ^2 or Fisher's exact test when appropriate. FVC: forced vital capacity; DLCO: diffusion of the lung for carbon monoxide; %PV: percent predicted value; ns: not significant; NA: not available. *: data available for 19/21 patients. †: data available for 20/21 patients. ‡: data available for 11/21 patients.

Serum	**IPF (n=45)**	**Controls (n=22)**	**p-value**
Age (years)	73 (66-78)	71 (59-82)	ns
Sex (M/F (n))	37/8	15/7	ns
Smoking status (never/ever (n))	8/37	16/6	<0.001
Origin (Europe/North Africa/Central Africa (n))	40/4/1	22/0/0	ns
FVC at diagnosis (%PV)	79 (67-93)	NA	
DLCO at diagnosis (%PV)	45 (34-56)	NA	
BAL	**IPF (n=39)**	**Controls (n=6)**	**p-value**
Age (years)	73 (66-76)	60 (50-78)	ns
Sex (M/F (n))	32/7	6/0	ns
Smoking status (never/ever (n))	5/34	1/5	ns
Origin (Europe/North Africa/Central Africa (n))	34/4/1	5/1/0	ns
FVC at diagnosis (%PV)	78 (69-88)	NA	
DLCO at diagnosis (%PV)	45 (33-59)	NA	
IHC	**IPF (n=11)**	**Controls (n=10)**	**p-value**
Age (years)	61 (59-63)	64 (61-71)	ns
Sex (M/F (n))	8/3	8/2	ns
Smoking status (never/ever (n))*	3/6	4/6	ns
Origin (Europe/North Africa/Central Africa (n))†	9/0/1	10/0/0	ns
FVC at inclusion (%PV)‡	57 (42-73)	101 (86-104)	0.01
DLCO at inclusion (%PV)‡	28 (23-31)	71 (60-94)	0.003

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Figure Legend:

Figure 1. The IgA-pIgR system is altered in IPF patients. A. Total serum and broncho-alveolar lavage (BAL) IgA is elevated in IPF patients compared to controls. B. Serum and BAL secretory (S-)IgA is increased in IPF patients compared to controls. C. Serum secretory component (SC) negatively correlates with DLCO at inclusion (r=0.42, p=0.0032). PV: predicted value. D. pIgR expression is largely absent from the distal control lungs, outside of bronchial structures (upper left panel). pIgR is present within club (SCGB1A1, purple), ciliated (beta-tubulin, yellow) and mucus (MUC5B, red) cells. The distal IPF lung presents a profound architectural remodeling with the presence of cystic spaces filled with pIgR/SC (green). HC pIgR positive cells express several bronchial markers including SCGB1A1 (purple), MUC5B (red) and beta-tubulin (yellow). Scale bar overview 200µm, zoom 20µm. E. HC are filled with secretory IgA, as shown by the overlap of IgA (red) and pIgR/SC staining (green). Scale bar: 100µm. F. upper left panel: S-IgA is absent from the distal control lung. Other panels: Cystic AEC2 structures (expressing proSP-C, yellow) are filled with S-IgA, as shown by the overlap of IgA (red) and pIgR/SC staining (green). Scale bar 100µm. Blue staining: Hoechst. G. A549 cells treated with control medium (ctrl), dimeric IgA (dIgA) or S-IgA show a an upregulation of *VIM* (coding for vimentin) and *SLUG*, two features of EMT. HG: Housekeeping genes. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. (A-B) Between group difference was evaluated using a non-parametric Mann-Whitney U test. (C) Correlation was assessed using Pearson's correlation coefficient followed by linear regression analysis. G. Between group difference was evaluated using a Kruskal-Wallis test

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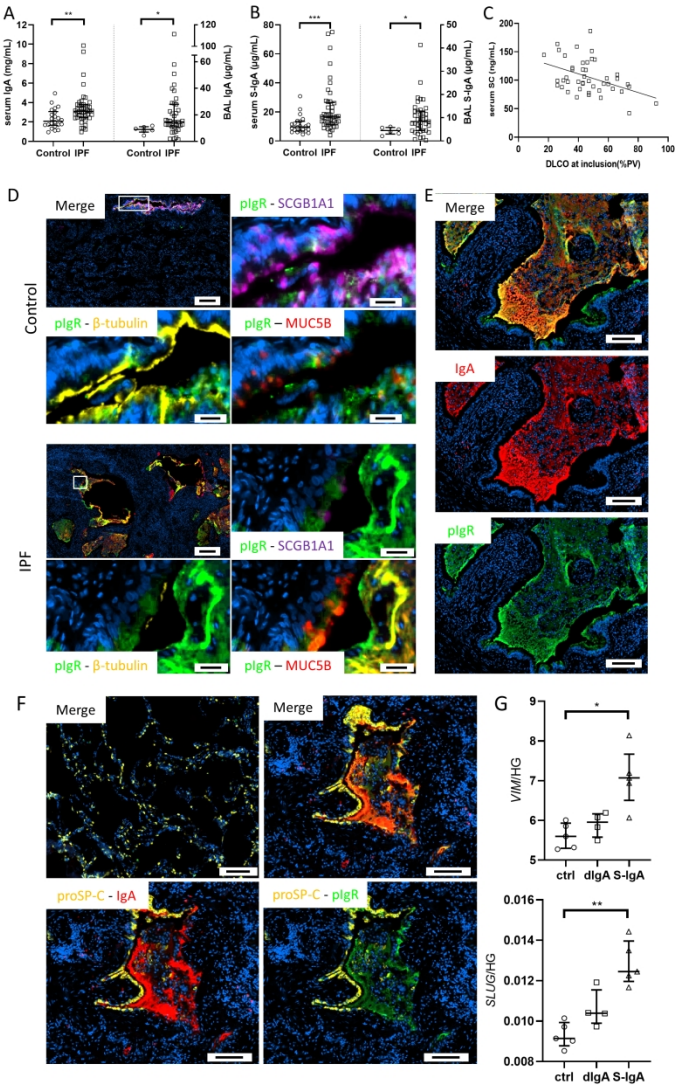

Figure 1

190x338mm (300 x 300 DPI)

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