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Airway epithelium damage in acute respiratory distress syndrome

Ludovic Gerard[1,2*], Marylene Lecocq[2], Bruno Detry[2], Caroline Bouzin[5], Delphine Hoton[3], Joao Pinto Pereira[1], François Carlier[2,6], Thomas Plante-Bordeneuve[2,6], Sophie Gohy[2,4], Valérie Lacroix[7], Pierre-François Laterre[8] and Charles Pilette[2,4]

Abstract

Background The airway epithelium (AE) fulfils multiple functions to maintain pulmonary homeostasis, among which ensuring adequate barrier function, cell differentiation and polarization, and actively transporting immunoglobulin A (IgA), the predominant mucosal immunoglobulin in the airway lumen, via the polymeric immunoglobulin receptor (pIgR). Morphological changes of the airways have been reported in ARDS, while their detailed features, impact for mucosal immunity, and causative mechanisms remain unclear. Therefore, this study aimed to assess epithelial alterations in the distal airways of patients with ARDS.

Methods We retrospectively analyzed lung tissue samples from ARDS patients and controls to investigate and quantify structural and functional changes in the small airways, using multiplex fluorescence immunostaining and computer-assisted quantification on whole tissue sections. Additionally, we measured markers of mucosal immunity, IgA and pIgR, alongside with other epithelial markers, in the serum and the broncho-alveolar lavage fluid (BALF) prospectively collected from ARDS patients and controls.

Results Compared to controls, airways of ARDS were characterized by increased epithelial denudation ($p=0.0003$) and diffuse epithelial infiltration by neutrophils ($p=0.0005$). Quantitative evaluation of multiplex fluorescence immunostaining revealed a loss of ciliated cells ($p=0.0317$) a trend towards decreased goblet cells ($p=0.056$), and no change regarding cell progenitors (basal and club cells), indicating altered mucociliary differentiation. Increased epithelial permeability was also shown in ARDS with a significant decrease of tight ($p<0.0001$) and adherens ($p=0.025$) junctional proteins. Additionally, we observed a significant decrease of the expression of pIgR, ($p<0.0001$), indicating impaired mucosal IgA immunity. Serum concentrations of secretory component (SC) and S-IgA were increased in ARDS (both $p<0.0001$), along other lung-derived proteins (CC16, SP-D, sRAGE). However, their BALF concentrations remained unchanged, suggesting a spillover of airway and alveolar proteins through a damaged AE.

Conclusion The airway epithelium from patients with ARDS exhibits multifaceted alterations leading to altered mucociliary differentiation, compromised defense functions and increased permeability with pneumoproteinemia.

Keywords ARDS, Airway epithelium, Ciliated cells, Junctional proteins, Mucosal immunity, Immunoglobulin A, Pneumoproteinemia

*Correspondence:
Ludovic Gerard
ludovic.gerard@saintluc.uclouvain.be
Full list of author information is available at the end of the article

Introduction

Acute Respiratory Distress Syndrome (ARDS) is a common cause of respiratory failure in critically ill patients, and is associated with high morbidity and mortality [1]. ARDS is characterized by inflammation-mediated alveolar/capillary barrier dysfunction with interstitial and airspace edema, resulting in severe hypoxemia [2]. However, alongside with alveolar and vascular involvement, the role of a distal airway injury in the pathophysiology of ARDS is increasingly recognized [3–5]. Functional alterations related to increased airway resistance, measurable at the bedside in mechanically ventilated patients, have been found in up to 40% of ARDS patients, suggesting that small airway dysfunction is a common phenomenon [6–8]. Small airways are lined by a pseudostratified epithelium, which fulfils multiple functions to maintain pulmonary homeostasis, acting as a first line barrier against inhaled particles while participating in local innate and adaptative immune mechanisms and maintaining a tight control on inflammatory mechanisms [9]. Airway epithelial (AE) cells transport immunoglobulin A (IgA), the predominant mucosal immunoglobulin in the airway lumen. Mucosal IgA, produced by submucosal B-cells under the form of polymers (principally dimers), is actively transported from the basolateral side to the apical pole of epithelial cells by the polymeric immunoglobulin receptor (pIgR), where a cleavage releases the extracellular part of the pIgR called secretory component (SC), either alone or bound to IgA, resulting in the genesis of secretory IgA (S-IgA) [10]. In the airway lumen, S-IgA and SC exert anti-bacterial, anti-viral, pro-inflammatory as well as immunomodulating functions. Altered AE barrier and lung mucosal immunity are described in chronic respiratory diseases, such as chronic obstructive pulmonary disease (COPD) [9, 11], asthma [12] and cystic fibrosis (CF) [13, 14]. However, AE structure and functionality, and IgA mucosal immunity are poorly characterized in the acute diseased lung. Moreover, beyond its potential interest as a marker of epithelial dysfunction, the IgA/pIgR system, as one of the main frontline protection mechanisms of the lung, could be involved in the development of secondary pulmonary infections, which occur frequently in ARDS patients [15] and have been associated to deleterious outcomes [16]. Therefore, the aim of this study is to provide a comprehensive understanding of the structure and functionality of small airways in patients with ARDS, with a specific focus on IgA immunity. To address this question, we retrospectively assessed lung tissue samples of patients with ARDS and controls, to investigate and to quantify structural and functional changes in the small airways of ARDS patients. Additionally, we measured markers of pIgR/IgA immunity, alongside with other epithelial markers, in the serum and the broncho-alveolar lavage fluid (BALF) prospectively collected from ARDS patients and controls.

Material and methods

More details regarding study protocols, patient selection, data collection, sampling processing, immunostaining and imaging can be found in the online data supplement.

Retrospective analysis of lung tissue from ARDS and controls

Patients

Patients with ARDS were screened within a database of all patients who underwent open-lung biopsy for ARDS (according to Berlin definition criteria) between 2007 and 2020, either for non-resolving ARDS or for ARDS of unknown etiology (flow chart in online data supplement Fig. S1). The main characteristics and outcomes of most of these patients have been previously reported [17]. Among the 56 patients of the database for whom sufficient lung tissue could be retrieved, we excluded 31 patients for whom there were less than 3 full airway sections on the slides. The 25 remaining patients were included for morphological analysis. For quantitative evaluation of immunostaining on whole tissue sections, after a careful delineation of airways epithelial area, we selected the patients in whom there was sufficient material to perform a representative evaluation (AE area $> 10^5$ μm^2/slide, n = 15). The control group consisted of lung tissue from patients who had undergone lobectomy for a solitary lung tumor, with sections taken at maximal distance from the lesions. Patients and controls characteristics can be found in Table 1 with additional information in online data supplement (Tables E1–E3).

Lung tissue immunohistochemistry and imaging

Hematoxylin–eosin (HE) HE was performed according to conventional protocol. Semi-quantitative morphological evaluation of the small airways was performed by an experienced pathologist, blinded to the study groups, as described in the online date supplement.

Multiplex fluorescence immunostaining (mIF) in lung tissue Formalin-fixed, paraffin-embedded (FFPE) tissue processing and tyramide signal amplification (TSA)-based multiplex fluorescence immunostaining were performed as previously described [18]. Details regarding the antibodies used and their concentration can be found in online data supplement, Table E4.

Imaging and quantitative evaluation of immunostaining in whole tissue sections Digitalized stainings were quantified on entire tissue sections with software applications ("APP"s) using the image analysis tool Author version

Table 1 Baseline characteristics, adjunctive treatments and outcomes for ARDS (n = 25) and controls (n = 15), for morphological and semi-quantitative evaluation in Hematoxylin–Eosin staining

Variables	Controls (n = 15)	ARDS(n = 25)	*p*-value
Age, years	55 (47–66)	67 (52–76)	0.03
Male, n (%)	7 (46.7)	12 (48)	0.59
Diabetes, n (%)	1 (6.7)	7 (28)	0.11
Active smoking, n (%)	5 (33)	5 (20)	0.28
Chronic obstructive pulmonary disease, n (%)	0	2 (8)	0.385
Other chronic respiratory disease, n (%)	0	3 (12)	0.283
Active neoplasia, n (%)	14 (93.3)	7 (28)	< 0.0001
Type of tissue sampling, n (%)			< 0.0001
Open-lung biopsy	0	25 (100)	
Surgical lobectomy	15 (100)	0	
APACHE II, median (IQR)		18 (14–20)	
SOFA, median (IQR)		5 (4–7)	
Cause of ARDS, n (%)			
COVID-19		0	
Pneumonia		11 (44)	
Sepsis (of extra-pulmonary origin)		3 (12)	
Other		11 (44)	
Adjunctive treatments before tissue sample, n (%)			
Invasive mechanical ventilation		25 (100)	
Prone positioning		8 (32)	
Inhaled Nitric oxide		7 (28)	
NMBA		2 (8)	
ECMO		0	
Vasopressors		12 (48)	
Renal replacement therapy		7 (28)	
Diffuse alveolar damage on pathological examination, n (%)		18 (72)	
PaO2/FIO2 ratio (day of sample), mmHg		127 (98–170)	
PEEP (day of sample), cmH2O		8 (6–10)	
Tidal volume (ml/kg PBW)		6.7 (5.8–7–9)	
Duration between MV initiation and tissue sampling, days		5 (2–13)	
ICU mortality, n (%)		18 (72)	

Data are presented as count (%), or median (IQR)

ARDS acute respiratory distress syndrome, *APACHE II* acute physiology and chronic health evaluation II, *SOFA* sequential organ failure assessment, *NMBA* neuromuscular blocking agents, *ECMO* extra-corporeal membrane oxygenation, *PEEP* positive end-expiratory pressure, *PBW* predicted body weight, *ICU* intensive care unit, *IQR* interquartile range

2023.01 (Visiopharm, Hørsholm, Denmark). Results were expressed as the staining index, namely the proportion of stained pixels within the region of interest (ROI) (area of stained pixels within ROI/area of ROI, Fig. S2, online data supplement). No background subtraction was applied. For each slide series, acquisition parameters, including laser power, were kept constant across all slides. On one slide in each series, thresholds for each fluorescent channel were manually adjusted at high magnification (×20) on representative stained regions, compared to negatively stained areas. These thresholds were then uniformly applied to all slides within the series. All analyses were performed by an experienced observer, who was blinded to the clinical categories of the tissue samples.

Prospective analysis of BALF and serum from ARDS and controls

Patients

Consecutive adult patients undergoing invasive mechanical ventilation (IMV) with a diagnosis of moderate to severe ARDS (n = 24, full list of exclusion criteria and patient's characteristics in Table 2) were prospectively enrolled in an observational study between 2016 and 2020. They were compared with control subjects with no

Table 2 Baseline characteristics, adjunctive treatments and outcomes for ARDS (n = 24) and controls (n = 7, for serum and bronchoalveolar lavage fluid (BALF) assays

Variables	Controls (n = 7)	ARDS (n = 24)	*p*
Age, years	53 (39–87)	59.5 (48–74.5)	0.81
Male sex	4 (57)	18 (75)	0.32
Body mass index (kg/m^2)	22.2 (19.3–25.3)	27.7 (23.9–31)	0.027
Diabetes	0	3 (12)	0.45
Active smoking	2 (28.6)	4 (16)	0.43
Asthma	0	0	1
Chronic obstructive pulmonary disease (Gold 0/1)	0	2 (8)	0.59
Cirrhosis	0	5 (21)	0.25
Active cancer	2 (28.6)	3 (12)	0.25
Corticosteroids	0	0	1
APACHE II score	14 (5–15)	20 (15–26)	0.015
SOFA score	1 (0–4)	7 (4–10)	0.0005
Cause of ARDS			
COVID-19		0	
Pneumonia		11 (46)	
Sepsis, extra-pulmonary origin		8 (33)	
Other		5 (20)	
Delay ICU admission—sampling, days	0 (0–1)	2 (1–4)	0.002
Delay IMV initiation—sampling, hours	10 (6–18)	24 (12–25)	0.09
PaO2/FiO2 ratio (day of sampling), mmHg	312 (285–371)	130 (104–170)	< 0.0001
ARDS Severity (day of sampling)			< 0.0001
No	7 (100)	0	
Mild	0	0	
Moderate	0	19 (79)	
Severe	0	5 (21)	
CRP (day of sampling), mg/dl	3 (1–22)	226 (108–330)	< 0.0001
Reason for IMV			< 0.0001
Acute respiratory failure	0	24 (100)	
Elective surgery	4 (57)	0	
Airway protection	3 (3)	0	
Adjunctive treatments			
IMV	7 (100)	24 (100)	1
Prone positioning	0	5 (20.8)	0.25
NMBA	3 (43)	2 (8)	0.062
ECMO	0	0	1
Vasopressors	0	13 (54)	0.012
RRT	0	7 (29)	0.13
VFDays D28	27 (22–27)	5.5 (0–20)	0.0002
ICU LOS	1 (1–2)	12.5 (8–22)	0.0003
ICU mortality	0	8 (33)	0.17
30-day mortality	1 (14)	8 (33)	0.21
Hospital mortality	1 (14)	11 (46)	0.29

Data are presented as count (%), or median (IQR)

ARDS acute respiratory distress syndrome, *APACHE II* acute physiology and chronic health evaluation II, *SOFA* sequential organ failure assessment, *IMV* invasive mechanical ventilation, *CRP* C-reactive protein, *NMBA* neuromuscular blocking agents, *ECMO* extra-corporeal membrane oxygenation, *RRT* renal replacement therapy, *VFDays D28* ventilatory-free days at day 28, *ICU* intensive care unit, *LOS* length of stay

evidence of respiratory disease and who were under IMV for reasons other than acute respiratory failure (n = 7). Controls should have a Chest X-Ray performed in the 12 h before inclusion with no pulmonary infiltrates, have a paO2/FiO2 ratio > 300 mmHg with FiO2 < 30%.

Serum and BALF sampling

Serum and BALF were sampled within the 24 h of the diagnosis of ARDS or within 24 h of the initiation of IMV for controls. Serum was sampled through a central venous line or through a dedicated intravenous access. Serum samples were immediately centrifuged (1500 RPM) and stored at − 80 °C. BALF was always obtained from the territory corresponding to the most infiltrated segment on chest X-rays. In case of bilateral diffuse opacities and in controls, BALF was collected from right middle lobe.

Enzyme-linked immunosorbent assay (ELISA)

Serum and BALF IgA and SC, as well as SC concentrations in the apical washings of ALI AE were determined by sandwich ELISA as previously described [19, 20]. Other ELISA assays were performed according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using SPSS 21 (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY) and figures were created using Graphpad Prism 10 (GraphPad Software, LaJolla, CA). Values were expressed as median (interquartile range [IQR]) unless otherwise stated. Categorical and continuous variables were analysed using the chi-square test and Mann–Whitney test, respectively. All tests were two-sided, with a significance level set at 0.05.

Results

Structural changes of the AE and altered cell differentiation in patients with ARDS

Small airways from ARDS patients and controls (baseline and detailed characteristics are available in Table 1 and in Table E1 (online data supplement)), were stained by HE, revealing the presence of basal membrane denudation (white arrow, Fig. 1a), which was quantified at 20% [IQR 5–40] of the epithelium surface in ARDS patients, while this feature was absent in controls (0% [0–0], $p = 0.0003$, Fig. 1c). In addition, airway walls of ARDS patients were diffusely infiltrated by neutrophils (Fig. 1b, semi-quantitative evaluation in Fig. 1d, $p = 0.0005$).

To further assess the different cell types and their abundance, as well as the integrity of the AE, we performed a quantitative evaluation of fluorescence immunostaining of whole tissue sections from a subset of ARDS patients and controls (data in Tables E2 and E3, and Fig. S1, online data supplement). First, we performed costainings for β-tubulin and Forkhead box protein J1 (Fox-J1,

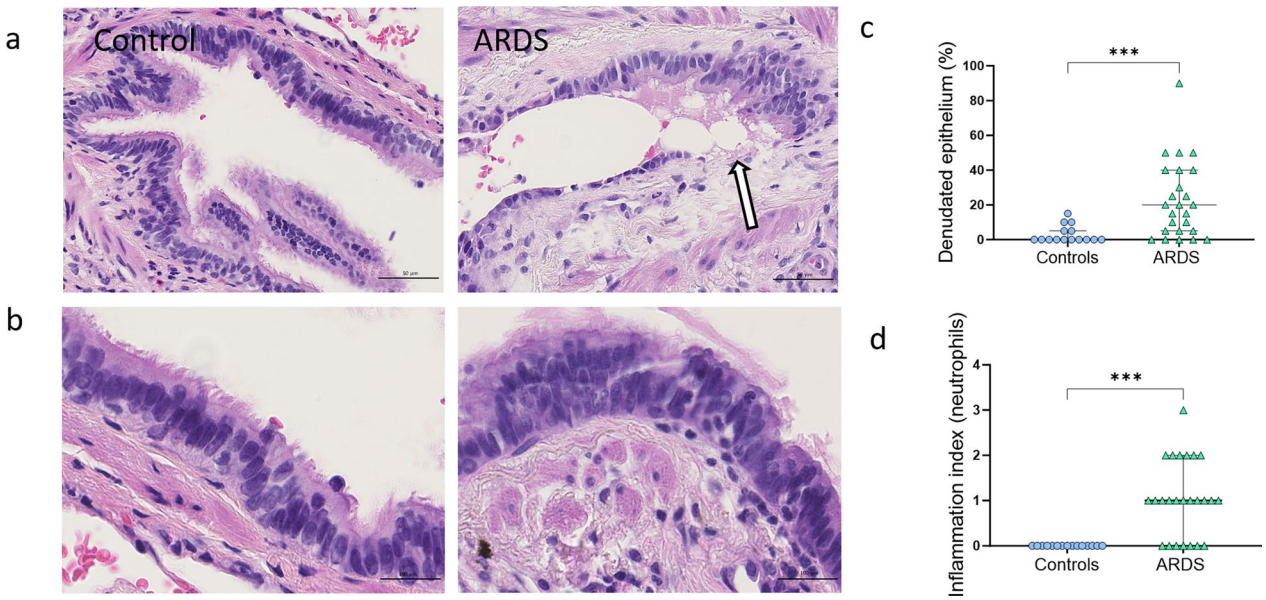


Fig. 1 Structural changes in the airways of ARDS patients. **a**, **b** Representative HE staining of samples from a control (left) and a patient with ARDS (right) showing epithelial denudation (**a**, white arrow) and neutrophilic infiltration (**b**). **c**, **d** Quantitative analysis comparing the extent of epithelial denudation (**c**), and the presence of inflammatory cells infiltrating airway epithelia, using a four-grade scale (**d**) in controls (n = 15) and ARDS (n = 25). Between-group difference was evaluated using the non-parametric Mann–Whitney U test. *** indicates $p < 0.0005$

ciliated cells), Clara Cell 16 (CC16, also called CC10 or SCGB1A1, club cells), Muc5AC and Muc5B (goblet cells) as well as p63 and CK5 (basal cells) (Fig. 2a). A selective loss of ciliated cells in ARDS patients (Fig. 2b, β-tubulin stained area of 0.61% [0.25–1.0] of the total epithelial area versus 3.67% [1.09–5.76], $p=0.0317$ and Fig. 2c, Fox-J1 stained area of 20.4% [5.1–30.8] versus 32.2 [27.1–43.5], $p=0.034$) was shown, as well as a trend for decreased goblet cells (Fig. 2e; Muc5AC 1.09% [0.02–2.9] versus 7.4% [0.88–12.88], $p=0.055$). Conversely, there were no significant changes in other cells such as club (Fig. 2d) or basal cells (Fig. 2f).

Loss of integrity of the AE

Moreover, additional costainings (Fig. 3a) for adherens (E-Cadherin) and tight junction proteins (Zonula Occludens 1 [ZO-1], Claudin-1 and Occludin), showed a loss of both adherens ($p=0.025$) and tight junctions ($p<0.0001$, $p=0.025$ and $p=0.0014$ respectively) in the airways of ARDS patients compared to controls (Fig. 3b–e). However, there was significant heterogeneity in the expression of junctional proteins between ARDS patients, as exemplified in Fig. S3.

Downregulation of mucosal pIgR expression in the airways of ARDS patients

Multiplex immunofluorescence quantitative analysis revealed that the tissue expression of the pIgR (Fig. 4a) was significantly lower in patients with ARDS in comparison with controls (Fig. 4b, 14.42% of AE area [7.82–22.87] versus 41.57% [38.49–49.21], $p<0.0001$), whereas no significant difference was shown regarding the abundance of IgA (Fig. 4c) and of S-IgA (area of IgA and pIgR co-staining, Fig. 4d) within the AE.

Increased secretory IgA and epithelial proteins in the serum of ARDS patients

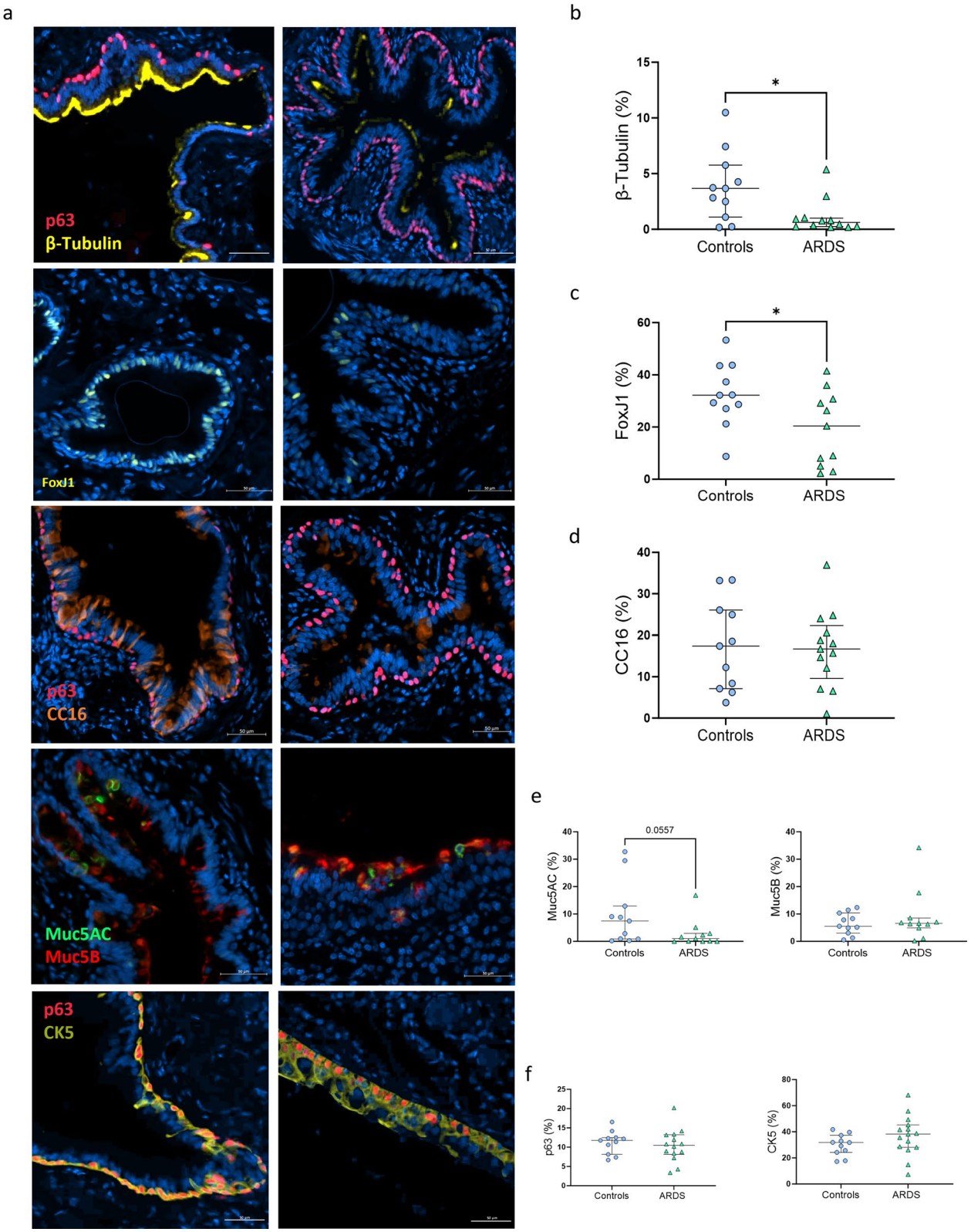
To confirm alterations of the IgA-pIgR system in ARDS, we measured the levels of IgA, S-IgA and SC, along other airway/alveolar epithelial injury markers, in the serum and broncho-alveolar lavage fluid (BALF) of ARDS patients (n = 24) and control subjects (n = 7, Table 2). A large increase of SC (Fig. 5a, 599 pg/ml [413–952] versus 133 pg/ml [103–137], $p<0.0001$), and of S-IgA1 (Fig. 5b, 71.4 µg/ml [49.9–117.4] versus 10.8 µg/ml [4.08–13.6], $p<0.0001$) but not of total IgA1 (Fig. 5c, $p=0.368$), total IgA2 or S-IgA2 (Fig. S4a–b, online data supplement) was observed in the serum of ARDS patients compared to controls. Conversely, BALF SC (Fig. 5d, 218 ng/ml [117–667] versus 341 ng/ml [267–498], p = 0.508), S-IgA1 (Fig. 5e, 8.4 mcg/ml [3.6–15.8] versus 10.8 mcg/ml [3.7–14.5], $p=0.99$) or S-IgA2 (Fig. S4d, online data supplement) were unaltered, whereas total IgA1 (Fig. 5f, 286 µg/ml [23.5–534.5] versus 7.8 µg/ml 3.6–45.6], $p=0.002$) and IgA2 (Fig. S4c, online data supplement) were increased. Altogether, this data suggests a local dysfunction of IgA-pIgR system in the airways of ARDS patients, with a leakage of SC and S-IgA1 from the airway lumen towards the blood and a spillover of IgA1 and IgA2 from the blood to the airway lumen, probably through the damaged AE. Interestingly, IgA and pIgR/SC merged staining revealed an overlap of these proteins immediately beneath the AE, further indicating spilling of S-IgA from the airway lumen (Fig. S5, online data supplement). Furthermore, serum concentrations of CC16/SCGB1A1 (Fig. S6a, online data supplement), Surfactant Protein-D (SP-D, alveolar type 2 cells, Fig. S6b, online data supplement) and soluble Receptor for Advanced Glycation End-products (s-RAGE, alveolar type 1 cells, Fig. S6c, online data supplement) were also increased in the serum of ARDS patients compared to controls, associated with a BALF concentration increase of the latter (Fig. S6d–f, online data supplement), suggesting that the epithelial injury observed in ARDS patients is not restricted to airways ciliated cells, but extends to club cells, alveolar epithelial cells type 2 and type 1, respectively.

Discussion

This study demonstrates that multifaceted epithelial changes take place in the airways of patients with ARDS. First, structural abnormalities of ARDS airways consist of reduced mucociliary differentiation and loss of integrity in terms of adherens/tight junctional proteins. Second, functional alterations involve defective mucosal immunity, with a decreased expression of pIgR. Third, serum assays reveal increased systemic concentrations of secretory component and IgA, presumably resulting from a leakage (airway proteins such as SC and S-IgA and CC16) and spillover (alveolar proteins) of lung-derived proteins through the damaged epithelium.

(See figure on next page.)

Fig. 2 Changes in airway epithelial populations. **a** Representative immunofluorescence staining of sections from a control (left) and an ARDS patient (right) stained for β-tubulin and Fox-J1 (ciliated cells), CC16/SCGB1A1 (club cells), Muc5AC and Muc5B (goblet cells) and p63/CK5 (basal cells). Scale bars, 50 µm. **b**–**e** Quantification of the area expressing β-tubulin (in proportion of the total AE area, (**b**), Fox-J1 (**c**), CC16/SCGB1A1 (**d**), Muc5AC and Muc5B (**e**), p63 and CK5 (**f**) showing a loss of ciliated cells in patients with ARDS (n = 13), compared to controls (n = 11). Between-group difference was evaluated using the non-parametric Mann–Whitney U test. *indicates $p<0.05$



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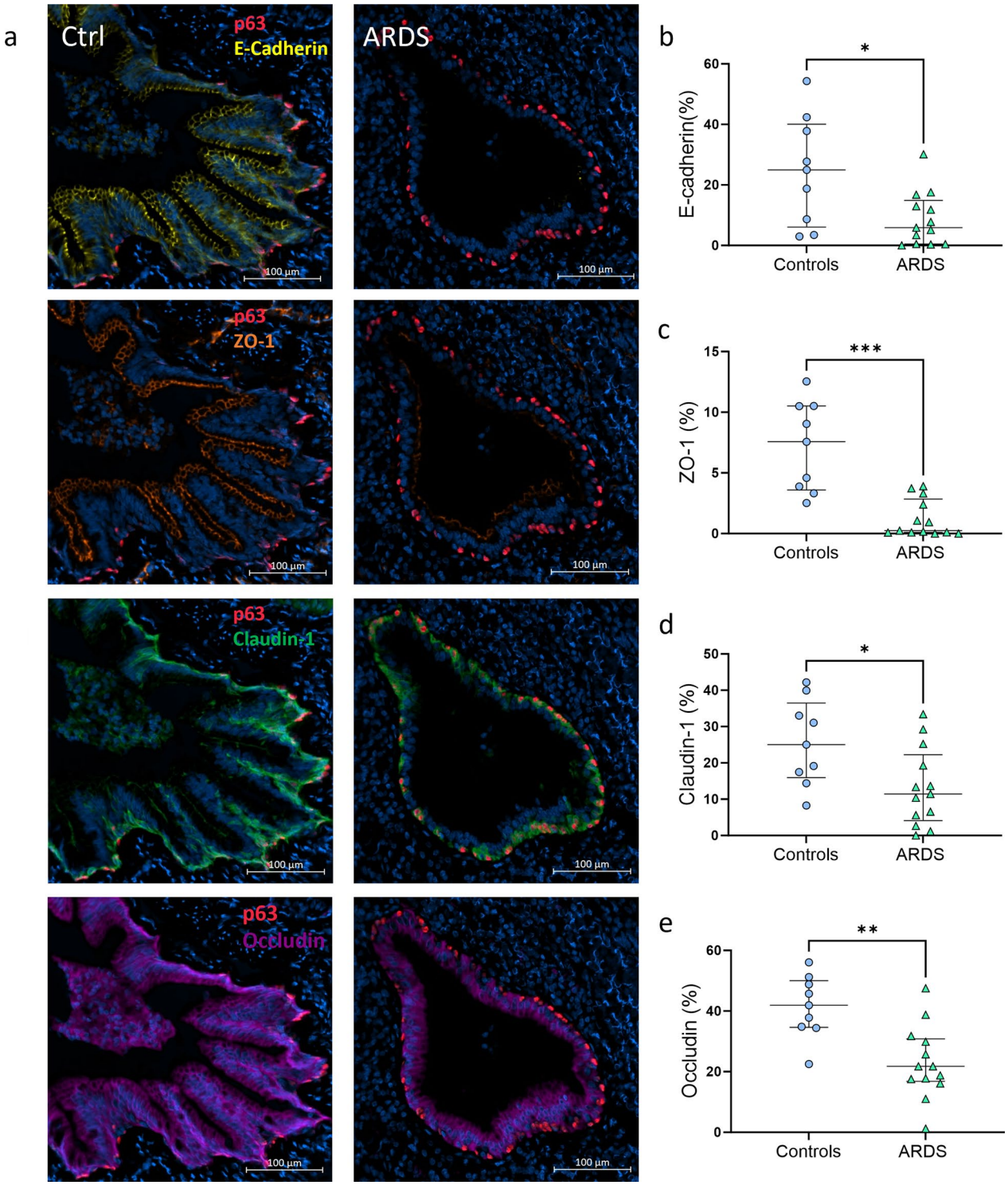

Fig. 3 Loss of tight and adherens junctions in the airways of patients with ARDS. **a** Representative immunofluorescence staining of sections from a control (left) and an ARDS patient (right) stained with E-cadherin, Zonula Occludens-1 (ZO-1), Claudin-1 and Occludin, costained with p63 and Hoechst. Scale bars, 100 µm. **b**–**e** Quantification of the area expressing E-Cadherin (in proportion of the total AE area, (**b**), ZO-1 (**c**), Claudin-1 (**d**), and Occludin (e) showing a loss of adherens (E-Cadherin) and tight (ZO-1, Claudin-1, Occludin) junctions in patients with ARDS (n = 13), compared to controls (n = 9). Between group difference was evaluated using the non-parametric Mann–Whitney U test. *indicates $p < 0.05$, ** indicates $p < 0.005$ and *** indicates $p < 0.0005$

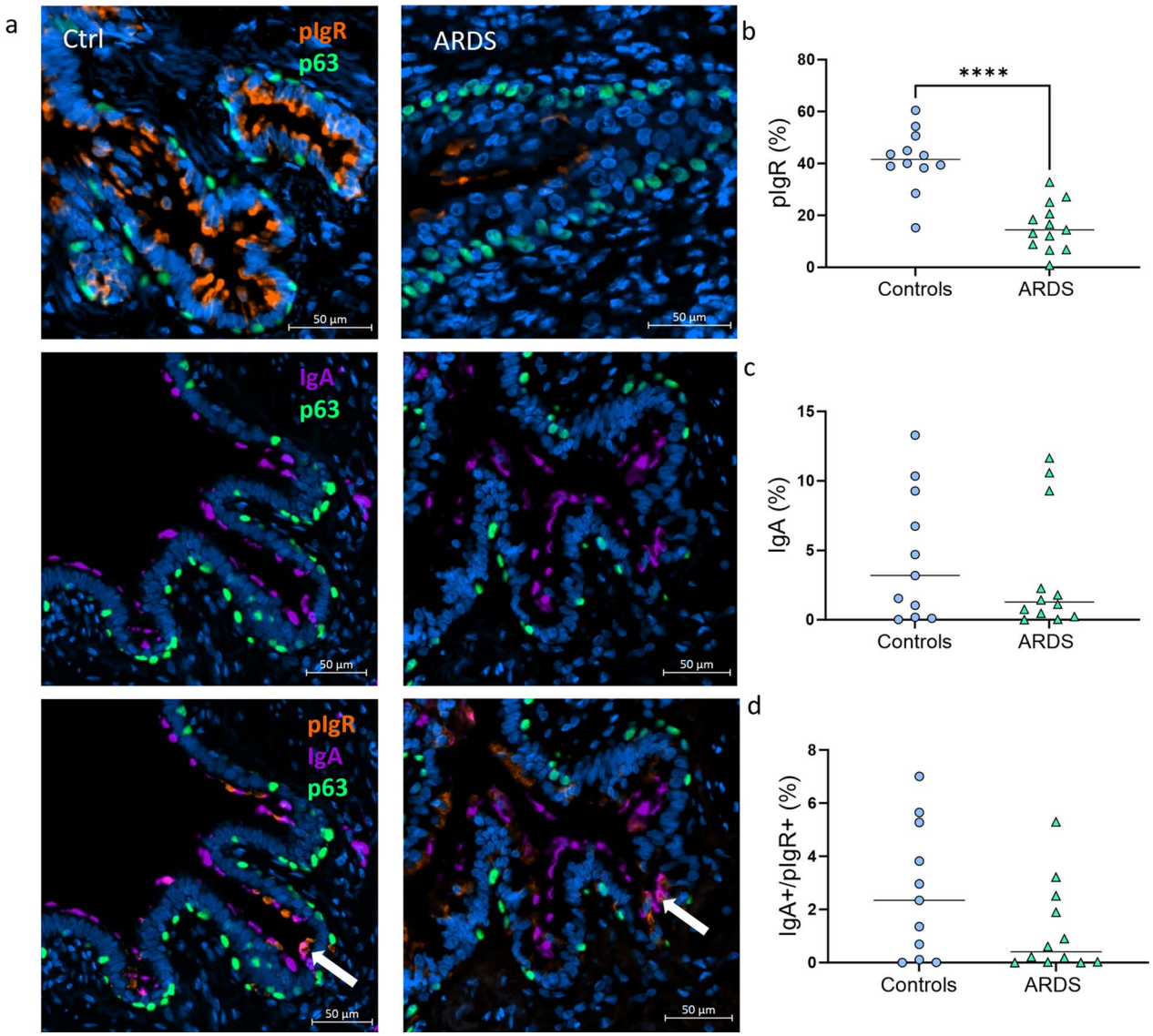

Fig. 4 Decreased pIgR expression in the airways of patients with ARDS. **a** Representative immunofluorescence staining of sections from a control (left) and an ARDS patient (right) stained for polymeric Immunoglobulin Receptor (pIgR, upper panel), Immunoglobulin A (IgA, middle panel) and both pIgR and IgA (white arrows, lower panel), costained for p63 and Hoechst. Scale bars, 50 µm. **b**–**d** Quantification of the area expressing pIgR (**b**), IgA (**c**), and of the area co-expressing IgA and pIgR (reflecting Secretory IgA [S-IgA], (**d**), all expressed in proportion of the total AE area, showing a significant decrease of pIgR expression within the AE of ARDS patients (n = 12) compared to controls (n = 13). Between-group difference was evaluated using the non-parametric Mann–Whitney U test. **** indicates $p < 0.0001$

Airway involvement in ARDS has been previously described [21, 22], and some studies have suggested that small airways alterations play a significant role in the pathophysiology of ARDS [4] and may be linked to deleterious outcomes [8, 23]. In retrospective autopsy studies, structural changes including epithelial denudation, loss of normal epithelium and inflammation were observed in small airways of patients with ARDS, paralleling our findings [3, 24]. However, beyond these structural alterations, we also provide robust evidence that changes regarding epithelial populations are observed in the airways of ARDS patients, with a selective loss of ciliated cells, contrasted results regarding goblet cells (trend towards lower expression of MUC5AC and no change in the expression of MUC5B) whereas club cell and basal cell populations appear unaffected. To the best of our knowledge, changes in airway epithelial subpopulations during ARDS have never been fully characterized in vivo. Evidence is scarce and most studies rely on animal/cell line models of acute injury [25–27] or on sampling of

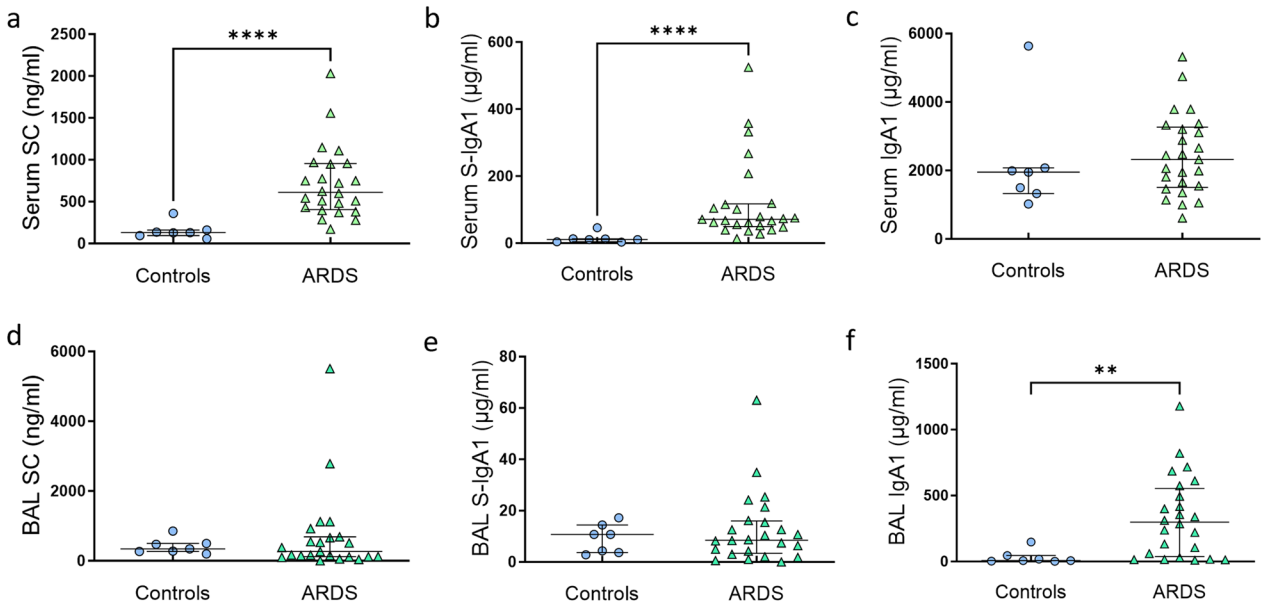

Fig. 5 Dysregulated IgA-pIgR/SC system in ARDS patients. **a**–**d** Serum secretory component (SC, **a**), and S-IgA1 (**b**) concentrations are increased in the serum of patients with ARDS (n = 24) compared to controls (n = 7), whereas no difference was found regarding total IgA1 (**c**). **e**–**f** Conversely, no difference was found in the BALF concentrations of SC (**d**), S-IgA (**e**) and S-IgA1 (**f**) comparing the same ARDS patients to controls, whereas total IgA1 is increased. Between-group difference was evaluated using the non-parametric Mann–Whitney U test. ** indicates $p < 0.005$, **** $p < 0.0001$

target proteins in BAL fluid/serum [27], often with contradictory results [28–30]. Interestingly, the pattern of reprogramming of AE lineage differentiation most often observed during chronic lung diseases such as COPD or asthma consist of hypoplasia of ciliated cells and hyperplasia of goblet cells [30–33]. Therefore, the observed loss of ciliated cells observed during ARDS might reflect that this altered programming is an early event, occurring only days after lung injury. Conversely, our results do not support early goblet cells hyperplasia and even suggest a decrease in MUC5AC production in the airways of ARDS compared to controls. The combined inhibition of both ciliary and secretory lineages could represent a specific phenotype of AE damage during acute lung injury. Alternatively, this discrepancy could be linked to a potentially more distal sampling in ARDS patients compared to controls, as the distribution of MUC5AC and MUC5B in the airways has been shown to be region-specific, the latter being predominant in distal airways [31].

Furthermore, we show that the barrier function of the airway epithelium is impeded by the loss of tight and adherens junctions during ARDS, with potential important effects on paracellular permeability and on the exposure of subepithelial tissues to luminal pathogens. Dissociation of intercellular junctions has been described between epithelial alveolar [32–34] and endothelial [35, 36] cells during acute lung injury, as well as in culture models of viral/bacterial infection of human airway epithelial cells [37]. However, it has never been shown in vivo, in the context of ARDS of various origins.

The second major finding of this study relates to mucosal immunity in ARDS, with reduced airway expression of pIgR. Impaired mucosal IgA-pIgR system was reported in several chronic disorders including COPD [11], asthma [12], and cystic fibrosis [13], whilst this study argues for an early dysregulation of mucosal IgA immunity in the pathogenesis of acute lung injury. Furthermore, reduced airway expression of pIgR in ARDS was combined with an increase of SC and S-IgA concentrations in the blood of ARDS patients compared to controls, whereas BALF concentrations were similar (although non-secretory IgAs were increased), probably reflecting a leakage of SC and S-IgA from the airway lumen to the blood across the damaged epithelium and endothelium [38]. The fact that marker proteins for club cells, alveolar epithelial cells type I and II were also elevated in the serum of ARDS patients, in line with recent literature [39], further suggests that pneumoproteinemia [40] may reflect epithelial damage during ARDS, which extends from conducting airways to alveoli. Whether this airway epithelial dysfunction is a mere bystander during ARDS, or whether it plays an active role in the pathophysiology remains elusive. However, it could be hypothesized that alterations in airway permeability and mucosal immunity play an important role in the development of secondary lung infections, which

occur frequently during the course of ARDS and impact patient's prognosis [16]. Therefore, pharmacologically targeting AE damage might prove clinically relevant. However, the mechanisms of these AE alterations during ARDS remain elusive. They could involve local inflammation and/or hypoxia, both of which have demonstrated effects on epithelial cellular permeability [41–43] or differentiation [9, 44–46] in vitro. Nonetheless, the presence of such airway epithelial alterations in ARDS patients has another important implication for experimental models of ARDS. Since many species, particularly rodents, differ from humans in the structure of their conducting airways [47], it is essential that animal models of ARDS utilize species with small airway structures that closely mimic those of humans.

Our study has several limitations. First, lung tissue analysis was performed in patients who underwent lung biopsy, who constitute a highly selected subgroup of ARDS patients, which may not be representative of a general, unselected ARDS population. However, increases in serum concentrations of S-IgA and SC were also observed in a prospective cohort of consecutive, unselected ARDS patients, suggesting that increase in airway epithelial permeability is present during ARDS, irrespectively of its etiology. Second, the study's limited sample size may have affected the ability to detect small but relevant differences in lung tissue quantifications between ARDS and controls, or between different ARDS etiologies. Third, ARDS and control tissues were taken using open-lung biopsy and surgical lobectomy respectively, and the different nature of the specimens may have influenced some findings such as cellular population distribution. Fourth, a more complete view of the causative mechanisms and of downstream mechanism pathways engaged in AE alterations during ARDS should be obtained.

Overall, this study demonstrates that the AE undergoes multifaceted alterations during ARDS, involving defects in IgA mucosal immunity, impaired cell differentiation, and loss of integrity leading to a leakage of airway and alveolar proteins into the bloodstream. It also provides a rationale to further study the AE and its causal implication in ARDS as well as future novel therapeutic targets.

Supplementary Information

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Supplementary Material 1

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Author contributions
LG drafted the work, collected the data and was a major contributor in writing the manuscript. ML contributed to data collection, performed technical analysis, and extensively revised the manuscript. BD contributed to data collection, performed technical analysis, and extensively revised the manuscript. CB contributed to data collection, performed image analysis, and extensively revised the manuscript. DH contributed to the acquisition of tissue material and to technical analysis and extensively revised the manuscript. JPP contributed to image analysis, and extensively revised the manuscript. FC contributed to data collection and extensively revised the manuscript. TPB contributed to data collection and extensively revised the manuscript. SG extensively revised the manuscript. VL contributed to the acquisition of tissue material and extensively revised the manuscript. PFL contributed to the acquisition of the data and extensively revised the manuscript. CP contributed to drafting of the work, was a major contributor in writing the manuscript and extensively revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Data is provided within the manuscript or supplementary information files. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
For the retrospective analysis of lung tissue, the study was conducted following the ethics committee approval (Comité d'Ethique Hospitalo-Facultaire, Cliniques universitaires Saint-Luc, UCLouvain N°2019/22jan/026). For the prospective evaluation of BAL fluid and serum from ARDS and controls, the study was conducted following the ethics committee approval (Comité d'Ethique Hospitalo-Facultaire, Cliniques universitaires Saint-Luc, UCLouvain N°2016/25avr/182), in accordance with the World Medical Association's Declaration of Helsinki, the Belgian law related to experiments in humans dated May 7, 2004, the General Data Protection Regulation 2016/679 and the Belgian law of July 30, 2018, regarding the protection of personal data. A written consent was received prior to participation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Critical Care Medicine, Cliniques universitaires Saint Luc, Université catholique de Louvain (UCLouvain), Avenue Hippocrate 10, 1200 Brussels, Belgium. [2]Pôle de Pneumologie, O.R.L. et Dermatologie (LuNS, Lung-Nose-Skin), Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain (UCLouvain), Brussels, Belgium. [3]Department of Pathology, Cliniques universitaires Saint Luc, Université catholique de Louvain (UCLouvain), Brussels, Belgium. [4]Department of Pulmonology, Cliniques universitaires Saint Luc, Université catholique de Louvain (UCLouvain), Brussels, Belgium. [5]IREC Imaging Platform (2IP, RRID:SCR_023378), Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain (UCLouvain), Brussels, Belgium. [6]Department of Pulmonology, CHU-UCL Namur, Yvoir, Belgium. [7]Department of Cardiovascular and Thoracic Surgery, Cliniques universitaires Saint Luc, Université catholique de Louvain (UCLouvain), Brussels, Belgium.

[8]Department of Intensive Care Medicine, Centre Hospitalier Régional Mons-Hainaut, Mons, Belgium.

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