Appearance of remodelled and dendritic cell-rich alveolar-lymphoid interfaces provides a structural basis for increased alveolar antigen uptake in chronic obstructive pulmonary disease

Michiko Mori, Cecilia K Andersson, Kaj A Svedberg, Pernilla Glader, Anders Bergqvist, Medya Shikhagaie, Claes-Göran Löfdahl, Jonas S Erjefält

ABSTRACT

Rationale The alveolar pathology in chronic obstructive pulmonary disease (COPD) involves antigen-driven immune events. However, the induction sites of alveolar adaptive immune responses have remained poorly investigated.

Objectives To explore the hypothesis that interfaces between the alveolar lumen and lymphoid aggregates (LAs) provide a structural basis for increased alveolar antigen uptake in COPD lungs.

Methods Lung samples from patients with mild (Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I), moderate–severe (GOLD II–III), and very severe (GOLD IV) COPD were subjected to detailed histological assessments of adaptive immune system components. Never smokers and smokers without COPD served as controls.

Results Quantitative histology, involving computerised three-dimensional reconstructions, confirmed a rich occurrence of alveolar-restricted LAs and revealed, for the first time, that the vast majority of vascular or bronchiolar associated LAs had alveolar interfaces but also an intricate network of lymphatic vessels. Uniquely to COPD lungs, the interface epithelium had transformed into a columnar phenotype. Accumulation of langerin+ dendritic cells occurred in the interface epithelium, to COPD lungs but not controls. The antigen-capturing capacity of langerin+ dendritic cells was confirmed by increased alveolar protrusions and physical T cell contact. Several of these immune remodelling parameters correlated with lung function parameters.

Conclusions Severe stages of COPD are associated with an emergence of remodelled and dendritic cell-rich alveolar–lymphoid interfaces. This novel type of immune remodelling, which predicts an increased capacity to respond to alveolar antigens, is suggested to contribute to aggravated inflammation in COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide.1 Long-term exposure to inhaled chemical irritants in tobacco smoke results in a chronic and destructive inflammation in the lung.2–3 Apart from innate immune responses in COPD, it is becoming increasingly clear that antigen-driven adaptive immune responses are activated in COPD lungs.4

In conducting airways, epithelial dendritic cells (DCs) extend cytoplasmic protrusions to sample luminal antigens.5 When an antigen is encountered, DCs migrate to draining lymph nodes and present the antigen to naïve T cells, thus initiating adaptive immune responses.6 Importantly, adaptive immune responses in COPD also take place locally within the lungs as demonstrated by the presence of bronchiolar-associated lymphoid aggregates.7 Since only scarce numbers of such lymphoid aggregates (LAs) are present in healthy subjects, these structures are thought to develop as a result of extended periods of inflammation or infections (ie, lymphoid neogenesis).8–10 Consequently lung LAs are not regarded as an integral part of secondary lymphoid organs (eg, lymph nodes or Peyer’s patches in the
Lung LAs may acquire inhaled luminal antigens via migration of superficial airway DCs. However, LAs have also been observed at sites distant from the conducting airways. It remains unclear whether or not these structures acquire luminal antigens from other lung regions such as the alveolar parenchyma, a compartment that in COPD is frequently subjected to inflammation and infections. However, although alveolar adaptive immune responses are likely to occur extensively in the alveolar parenchyma, a compartment that in COPD is frequently subjected to inflammation and infections. The study was approved by the local ethics committee.

### Characteristics of peripheral lung LAs

For each subject multiple peripheral lung samples were subjected to standardised fixation and processing for generating paraffin sections. LAs, defined as more than 50 contiguous lymphoid cells, were quantified and subgrouped according to their anatomical location: small airway/bronchiolar-associated lymphoid tissue (abbreviated BRALT in order to make a distinction to bronchial-associated lymphoid tissue BALT), vascular-associated lymphoid tissue (VALT, defined as LAs in non-bronchiolar-associated solitary vessels with a diameter >140 μm) and alveolar-only lymphoid tissue (ALT, defined as LAs well separated from any bronchioles, pleural tissue or pulmonary vessels).

### Immunohistochemistry

Immunohistochemistry (IHC) was performed in an automated IHC robot (DakoCytomation, Glostrup, Denmark). All primary antibodies used (see table 2) and IHC protocols have been validated extensively for use on paraffin sections. The immunoreactivity was visualised using the Dako EnVision kit. For bright field double staining the first primary antibody was detected with horseradish peroxidase-conjugated antibodies and the second with alkaline phosphatase-conjugated antibodies and the second with alkaline phosphatase-conjugated antibodies.

### Table 1 Subject characteristics

<table>
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<tr>
<th>Characteristic</th>
<th>Never smokers</th>
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<th>GOLD II–III* COPD</th>
<th>GOLD IV COPD</th>
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<td>85.9 (80–95)</td>
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Values are median (range) or n.  
*Two patients with GOLD stage III COPD. 
†The mean value of the study group is 63 years. 
‡One patient with unknown medical history. 
COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; (F)VC, (forced) vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease.
antibodies. Immunofluorescence double staining was performed to evaluate colocalisation.18

Serial section-based three-dimensional reconstruction of LAs
A series of serial sections (>30) was used to generate image segmentation data of LAs, parenchymal tissue and immunostained lymphatic vessels. Structure-specific segmentation data were loaded into a software platform (Amira 5.4.2 Visage Imaging by manual cursor tracing. Langerin + DCs were manually counted along alveolar–LA interfaces and in distinct regions within the LAs. Quantiﬁcations involving manual counting or cursor tracing were performed on blinded sections.

Quantifications
For each subject multiple large sections (from two to three separated peripheral lung regions) were analysed. High-resolution digital images, representing the entire section area, were generated from all bright field sections using a slide-scanning robot (ScanScope, Aperio Technologies, Vista, California, USA). Morphometric analyses and quantiﬁcation of immunoreactivity were performed using Aperio ImageScope V10.0 software (Aperio Technologies). Apart from multiple alveolar regions, a total of around 1000 individual bronchioles and pulmonary vessels were evaluated. Alveolar–LA interfaces were measured by manual cursor tracing. Langerin+ DCs were manually counted along alveolar–LA interfaces and in distinct regions within the LAs. Quantiﬁcations involving manual counting or cursor tracing were performed on blinded sections.

Statistics
Values are given as median (range), unless otherwise stated. The Kruskal–Wallis non-parametric test followed by Dunn’s multiple comparisons post test was used for comparison between study groups and the Mann–Whitney test was used for comparisons between two groups (Prism V5.0, GraphPad Software, San Diego, California, USA). Spearman’s rank method was used to calculate correlation coefficients.

RESULTS
LAs are present in all major peripheral lung compartments
Two-dimensional approach to assess the distribution of LAs
Prompted by initial observations of a widespread anatomical distribution of LAs in COPD lungs (see online supplementary ﬁgure S1 and table S1), all LAs were quantiﬁed and divided into BRALTs, VALTs and ALTs (ﬁgure 1A, see online supplementary ﬁgure S1 and table S1). The BRALT structures were mainly observed in the adventitial layer of the bronchioles. Across all study groups an average of 17.7% of the BRALTs reached the bronchiolar epithelium. Although the percentage of bronchioles containing BRALTs was increased in GOLD stage IV COPD compared with never smokers (p<0.05), the number of BRALTs was not changed among the study groups. The number of VALTs, which had an almost exclusive adventitial distribution, increased in patients with GOLD stage IV COPD compared with never-smoking control subjects (p<0.01). In the alveolar parenchyma, patients with COPD displayed a tendency to increased number of ALTs (see online supplementary table S1). In GOLD stage IV, most LAs were well developed as indicated by Ki-67+ proliferating cells and increased CD21+ follicular DCs (see online supplementary data). Follicular CD21 immunoreactivity was observed equally frequently among BRALTs, VALTs and ALTs.

Three-dimensional conﬁrmation of true alveolar-only LAs
A serial section-based three-dimensional analysis was performed to reveal the true spatial distribution of the lung LAs. This analysis conﬁrmed the presence of true ALT structures; that is, alveolar-only LAs without physical contact with bronchioles or pulmonary vessels (ﬁgure 1B–D). In COPD, an average of 25.4% of all LAs were true ALTs and 31.2% of all LAs were VALTs. Consequently, the remaining 43.4% of the LAs were BRALTs. This serial section analysis also revealed that 79.8% of all identiﬁed ALTs in two-dimensional sections were in fact true ALTs. Similarly, 89.3% of all VALTs were true VALTs.

BRALT and VALT structures have extensive interfaces with the alveolar lumen and the lymphatics
The vast majority of the LAs, including BRALTs and VALTs, had a direct interface towards the alveolar lumen (ﬁgure 2A,C,D).

### Table 2 Primary antibodies used for immunohistochemistry

<table>
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<th>Antigen</th>
<th>Clone*</th>
<th>Supplier</th>
<th>Antigen retrieval</th>
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<th>Against</th>
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<td>F6</td>
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<td>CD11c</td>
<td>D11</td>
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<td>CD68</td>
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<td>Dako</td>
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<td>BDCA-2</td>
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<td>Multi-Cytokeratin</td>
<td>AE1/AE3</td>
<td>Novocastra§</td>
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<td>Langerin (CD207)</td>
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<td>Smooth muscle cells</td>
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*Mouse monoclonal antibodies unless otherwise stated.
†Working dilution for bright field staining, unless otherwise stated.
§EnVision FLEX Target Retrieval Solution, pH 6.1, K8005, Dako.
¶Distributed by Leica Biosystems Newcastle, UK.
‡DIVA Decloaker, pH 6, DV2004 MX, Biocare Medical.
IF, immunofluorescence; PC, pressure cook, 2100 Retriever, Prestige Medical Ltd., Blackburn, England; PT, pre-treatment module, Dako, Glostrup, Denmark.
Irrespective of anatomic location, no difference in the frequency of LAs with alveolar interfaces was found between the study groups (figure 2A and table 3).

The total length of alveolar interfaces, which is the combination of LA numbers and mean length of alveolar interfaces, was increased in advanced COPD compared with never-smoking controls (figure 2B). Among all LAs and study groups, an average of 33.4% of the LA perimeter was facing alveolar lumen (figure 2F).

Immunohistochemical visualisation of podoplanin+ lymphatic vessels demonstrated a close spatial relationship between LAs and lymphatics (figure 2C,D). Three-dimensional reconstruction of podoplanin-stained lymphatic vessels further revealed the intricate connection among BRAL T, VAL T, AL T and lymphatics (exemplified in figure 2E). Across all study groups lymphatic vessels comprised an average of 20.3% of the LA perimeter (figure 2F).

Remodelling of alveolar–lymphoid interface epithelium in COPD

In never-smoking controls the alveolar–lymphoid epithelium was, at all locations, primarily of a simple squamous type (figure 3A,E).

A similar epithelium, but with increasing stretches of cuboidal epithelium, was present in smokers and patients with COPD (figure 3B,F). At more advanced stages of COPD (GOLD II–IV) the occurrence of cuboidal epithelium was accompanied by a higher columnar, non-ciliated epithelium (figure 3G,H). The emergence of columnar epithelium occurred similarly among BRAL T, VAL T and AL T (table 3) and resulted in a significantly increased proportion and increased total columnar epithelial length in patients with GOLD IV COPD (figure 3C,D). Importantly, the columnar epithelium in patients with GOLD II–IV COPD was restricted to alveolar–LA interfaces (similar epithelium was not found in surrounding lung parenchyma or along other alveolar interfaces facing, for example, the adventitia of pulmonary vessels or conducting airways).

Selective accumulation of antigen-presenting DCs along alveolar–lymphoid interfaces in COPD lungs

Langerin (CD207)+ DCs represent a major antigen-presenting cell type in the conducting airways in COPD.20 21 Hence, we explored if langerin+ DCs were present at alveolar–LA interfaces. Irrespective of anatomic location, no or very few interface langerin+ DCs were present in never-smoking and smoking...
Lymphoid aggregates (LAs) have extensive interfaces with the alveolar lumen and peripheral lymphatic vessels. (A) Quantitative data showing that in all study groups the majority of LAs had an alveolar lumen–LA interface, irrespective of anatomical localisation. Values are given as mean±SEM. (B) Quantification of the total length of alveolar–LA interfaces. Statistical analysis was performed using the Kruskal–Wallis non-parametric test followed by Dunn’s multiple comparison post test. Horizontal lines indicate medians for each group. *p<0.05. (C) Photomicrograph of a typical bronchiolar-associated lymphoid tissue (BRALT) with its interface with the alveolar lumen (asterisk) and podoplanin+/α-smooth muscle actin+ lymphatic vessels (arrowheads; brown). α-Smooth muscle actin+ cells are shown in red. Arrow indicates alveolar macrophages and 'Ep' the bronchiolar epithelium. Black endogenous pigment depositions are also visible. Epithelial basal cells and pneumocytes showed a weak immunoreactivity for podoplanin. Cell nuclei were counterstained with Mayer’s haematoxylin (blue stain). (D) Photomicrograph of a similarly stained vascular-associated lymphoid tissue (VALT) with its direct interface with the alveolar lumen and extensive connection to podoplanin+/α-smooth muscle actin+ lymphatic vessel (arrowheads). (E) Three-dimensional rendering of an image stack with 40 (4 μm thick) serial sections revealed an intricate relationship between immunostained lymphatic vessels and peripheral lung LA. White: LA; blue: lymphatic vessels. (F) The relative proportion of anatomic structures along the LA perimeters, divided into alveolar interface, lymphatic vessel borders and remaining firm tissue (ie, airway wall tissue, adventitial tissue of pulmonary vessels, or parenchymal tissue). Values are given as mean±SEM. Scale bars: (C–E) 100 μm.
controls (figure 4D–F). In contrast, abundant and increased numbers of langerin+ DCs were observed in COPD (figure 4D–F and table 3). No, or few such DCs were present in non-LA alveolar interfaces.

A detailed analysis of the distribution of langerin+ DCs in LAs with an alveolar interface revealed a selective accumulation of DCs to the superficial region just beneath the alveolar interface epithelium (figure 4G). In all COPD groups the superficial LA regions had significantly higher DC densities compared with central or opposing regions (the overall p value was 0.034 for GOLD I and 0.002 for GOLD II–III and GOLD IV). The selective accumulation of interface DCs was equally present irrespective of the anatomical distribution of the LAs. In total, across the COPD groups more than 80% of the LA-associated langerin+ DCs were present within the interface epithelium or in the superficial LA region (exemplified in figures 4A and 5).

The langerin+ DCs within the alveolar–lymphoid epithelium frequently displayed apical protrusions reaching the alveolar border (figures 4A,B and 5A,C,D). This phenomenon was more frequent in the alveolar–LA epithelium compared with langerin+ DCs along the epithelium in the conducting airways. Due to the thin interface at alveolar–LA borders, several DCs were observed with simultaneous physical contact with the alveolar surface and underlying lymphocyte populations within the LAs, including CD20+ B cells and CD4+ T cells (figure 5B,C). Other DC populations, such as CD68+ CD11c+ myeloid-like DCs or BDCA-2+ plasmacytoid DCs did not have similar accumulation at the alveolar interfaces as the langerin+ DCs (see online supplementary figure S3).
Figure 3  Altered epithelial phenotype at alveolar–lymphoid aggregate (LA) interfaces in patients with chronic obstructive pulmonary disease. The proportion of (A) simple squamous, (B) simple cuboidal and (C) columnar interface epithelium was quantified on podoplanin (lymphatic vessels)/\(\alpha\)-smooth muscle actin stained-sections from peripheral lung samples. (D) Quantification of the total length of columnar interface epithelium. (A–D) Statistical analyses were performed using the Kruskal–Wallis non-parametric test followed by Dunn’s multiple comparison post test. Horizontal lines indicate medians for each group. *p<0.05; **p<0.01; ***p<0.001. (E–G) Micrographs where brown cytokeratin immunoreactivity visualise the epithelium at alveolar–LA interfaces. Representative images of (E) a simple squamous epithelium; (F) a simple cuboidal epithelium (arrowhead) surrounded by squamous epithelium; (G) a patchy stretch of non-ciliated columnar epithelium (arrowhead, note the sharp border to the flanking squamous epithelium). Cell nuclei were counterstained with Mayer’s haematoxylin (blue stain). (H) Computerised three-dimensional rendering of podoplanin-stained sections demonstrated the functional spatial arrangement between the alveolar lumen and interface epithelium (red), the LA (white) and the opposing intertwined network of lymphatic vessels (blue). The inset exemplifies an image from the series with 40 (4 \(\mu\)m thick) podoplanin-stained sections used for the three-dimensional reconstruction (see online supplementary data for methodological details). The asterisk indicates the interface epithelium. Scale bars: (E–G) 25 \(\mu\)m; (H, inset) 100 \(\mu\)m.
Correlation with lung function parameters

Several of the parameters associated with the present expansion and remodel of alveolar–LA interfaces, including the accumulation of interface DCs, correlated inversely with lung function (figure 6 and online supplementary table S2).

DISCUSSION

The present study reveals new aspects of lung LAs in COPD and supports the emerging notion of the alveolar region as a critical site for induction of adaptive immune responses in inflammatory airway diseases. In light of this, the selective accumulation of langerin+ DCs at alveolar–LA interfaces in COPD, which represents a major novel finding in this study, is intriguing and suggests an adaptation for increased alveolar antigen uptake. This adaptation is likely to start early during COPD development because interface DCs were observed in relatively mild disease. Although, for obvious reasons, we could not perform functional studies, it seems evident that the present emergence

Figure 4  Accumulation of langerin+ dendritic cells (DCs) along alveolar–lymphoid aggregate (LA) interfaces. (A) Micrograph exemplifying homing of langerin+ DCs (brown) along the interface epithelium overlying a LA with a germinal centre (GC) (scale bar, 50 μm). Asterisks indicate alveolar lumen filled with macrophages. Arrowhead points to a langerin+ DC reaching the alveolar lumen. Black pigment deposition is visible. (B,C) Langerin+ DCs displayed a typical dendritic morphology with protrusions extending into the alveolar lumen (arrowhead in B) or a more basolateral position (scale bars in B,C 7 μm). Cell nuclei were counterstained with Mayer’s haematoxylin (blue stain). (D,E) Quantification of langerin+ DCs along peripheral lung LAs with squamous and cuboidal interface epithelium. (F) Quantification of the total number of interface langerin+ DCs, including DCs in the columnar interface epithelium. (D–F) Statistical analyses were performed using the Kruskal–Wallis non-parametric test followed by Dunn’s multiple comparison post test. Horizontal lines indicate medians for each group. *p<0.05; **p<0.001. (G) The densities of langerin+ DCs within distinct LA regions. Values are given as mean±SEM.
of DC-rich alveolar–LA interfaces in patients with COPD will result in an increased capacity for faster immune responses in the peripheral lung. Indeed, the intimate spatial relationship between the alveolar lymphoepithelial langerin+ DCs and the adjacent T cell areas, and the LAs and extensive network of lymphatic vessels, would create an ideal structural basis for a prompt and highly efficient induction of adaptive immune responses. In this sense the present remodelled LA interface resembles the highly specialised lymphoepithelium overlying the apical part of gut-associated lymphoid tissue (GALT) or classical BALT structures in bronchi. Unfortunately, due to a lack of cell-specific markers, we could not study whether M cells, similar to those present in the lymphoepithelium of GALTs, are also present in the alveolar lymphoepithelium. Theoretically, the occurrence of a higher epithelium at the interfaces could be due to lambertosis (ie, peribronchiolar metaplasia). However, the fact that alveolar columnar epithelium was in the parenchyma exclusively present along the alveolar–lymphoid interfaces suggests that traditional lambertosis is not the major cause of the columnar lymphoepithelium in our study. In any case, regardless of the aetiology of the epithelial changes, it seems clear that the present lymphoepithelium and its selective accumulation of langerin+ DCs represent a novel arena for alveolar antigen uptake. Apart from the release of DC-activating mediators by epithelial cells, physical DC–epithelial interactions are needed to maintain the epithelial barrier when DCs protrude across the tight junctions. Whether this types of interactions are increased in the columnar interface epithelium remains to be established.

Our study also provides the first quantitative data to show that the LAs in COPD are present in all major anatomic regions of the peripheral lung. In agreement, previous studies exploring lung LAs in COPD demonstrated that, apart from BRALTs, LAs were also increased in the alveolar parenchyma. However, it has previously been speculated that such seemingly alveolar LAs have physical contact with airways or pulmonary vessels in their three-dimensional in vivo context. Using three-dimensional reconstructions, this study thus proves for the first time that true alveolar-only LAs exist and shows that in COPD these represent around 25% of the total lung LAs. Moreover, this study provides novel quantitative data showing that irrespective of anatomical distribution, the vast majority of the total LAs in COPD lungs have direct interfaces towards the alveolar lumen. Thus, BRALTs and VALTs should conceptually also be viewed as alveolar associated (ie, ALTs). This new view of LAs agrees with previous findings in COPD lungs. For example, van der Strate and colleagues reported that BRALTs very rarely reached the bronchiolar epithelium. In agreement with our study, a similar adventitial distribution has also been noted for VALTs. Collectively, it thus seems that an extensive interface towards the alveolar lumen is a general feature of all types of lung LAs.

The origin of the antigens driving the LA formation in COPD is still debated. One source of peripheral antigens comes from lower respiratory tract infections. Apart from the exacerbation-associated infections in COPD, recent microbiome studies reveal a chronic and low-grade presence of lung pathogens outside exacerbations. In addition, inhaled immunogenic microparticles may also induce alveolar adaptive immunity in COPD, as indicated by LA formation in pathogen-free mice exposed to cigarette smoke. Several studies have also demonstrated elevated levels of antibodies to self-antigens. Taken together, the antigens captured by the alveolar interface DCs revealed in this study should be expected to be multifaceted. Although the immune responses initiated at alveolar–LA interfaces are likely to have beneficial host defence purposes, it is also likely that in severe COPD these may turn into pathogenic events. It can thus be surmised that, apart from any autoimmune components in COPD, a non-proportional expansion of peripheral alveolar–LA interfaces at advanced stages of COPD results in an ‘immunological hyperreactivity’ that may evoke harmful and exaggerated immune responses to the frequent infections that hit this patient category.

As for most invasive patient studies, a potential weakness with our study was the limited number of subjects. In part this was compensated by the fact that several separated lung regions (each containing multiple anatomic compartments) were analysed from each patient. In any case, despite restricted patient numbers, robust changes could be observed for the main parameters. In the present study the excised lung samples were immersed into a fixative rather than using a perfusion fixation approach. Although this may result in tissue samples with different degrees of compression, the key parameters in the present study, such as the quantitative analysis of epithelial alterations and degree of DC infiltration along the alveolar–lymphoid boundaries, were determined in our study. Thus, our results can be compared with those from previous studies using perfusion fixation.
interface, are independent of the degree of compression. Also, it cannot be excluded that suspected cancer in several of our cohorts has influenced the immunological status of the lung. However, since we only included subjects with well delineated bronchial tumours and restricted the analyses to regions far away from the tumour, we believe that any such influence is minimal. Furthermore, the most striking findings were found in patients with GOLD stage IV disease who did not have cancer.

The present findings are expected to have a significant bearing on many other diseases characterised by peripheral lung LAs, for example, long-term distal infections, cystic fibrosis and idiopathic pulmonary fibrosis. Notably, patients with autoimmune diseases like idiopathic pulmonary arterial hypertension or rheumatoid arthritis with pulmonary complications develop ectopic lung LAs. Further work is needed to establish if emergence of the DC-rich alveolar–LA interfaces revealed in this study is a general feature of all diseases characterised by lung LAs.

This study may also have important implications for the growing field of pulmonary vaccinology. Respiratory pathogens enter the body at mucosal or alveolar surfaces, but systemically delivered vaccines fail to induce a therapeutic protective response at these sites. This has created a rational for local pulmo-

delivered vaccines fail to induce a therapeutic protective response at these sites. This has created a rational for local pulmonary delivery of vaccines to increase lung protection and systemic immunity. The novel type of DC-rich alveolar–LA interface shown in this study would likely contribute to an increased efficacy of new and emerging pulmonary vaccine strategies.

In summary, this study shows that severity of COPD is associated with a marked remodelling of alveolar–LA interfaces. These alterations, which involve a selective accumulation of interface DCs and correlate with lung function, predict an increased capacity to respond to alveolar antigens and may thus contribute to aggravated inflammation in COPD.

Acknowledgements We thank Karin Jansner and Britt-Marie Nilsson for skilful technical assistance with tissue processing and serial sectioning.

Contributors MM collected tissue samples, performed laboratory work, analysed the data and wrote the manuscript. CKA contributed to tissue handling and sample characterisation. KAS performed three-dimensional reconstructions. PG participated in early preliminary experiments. CGL contributed to the clinical characterisation. JSE designed and supervised the study and wrote the manuscript. All authors participated in manuscript editing and approved the final version of the manuscript.

Funding This work was supported by the Swedish Heart and Lung Foundation and the Swedish Medical Research Council.

Competing interests None.

Ethics approval Ethics approval was provided by the Swedish Research Ethics Committee in Lund.

Provenance and peer review Not commissioned; externally peer reviewed.

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Thorax 2013 68: 521-531 originally published online February 14, 2013
doi: 10.1136/thoraxjnl-2012-202879

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