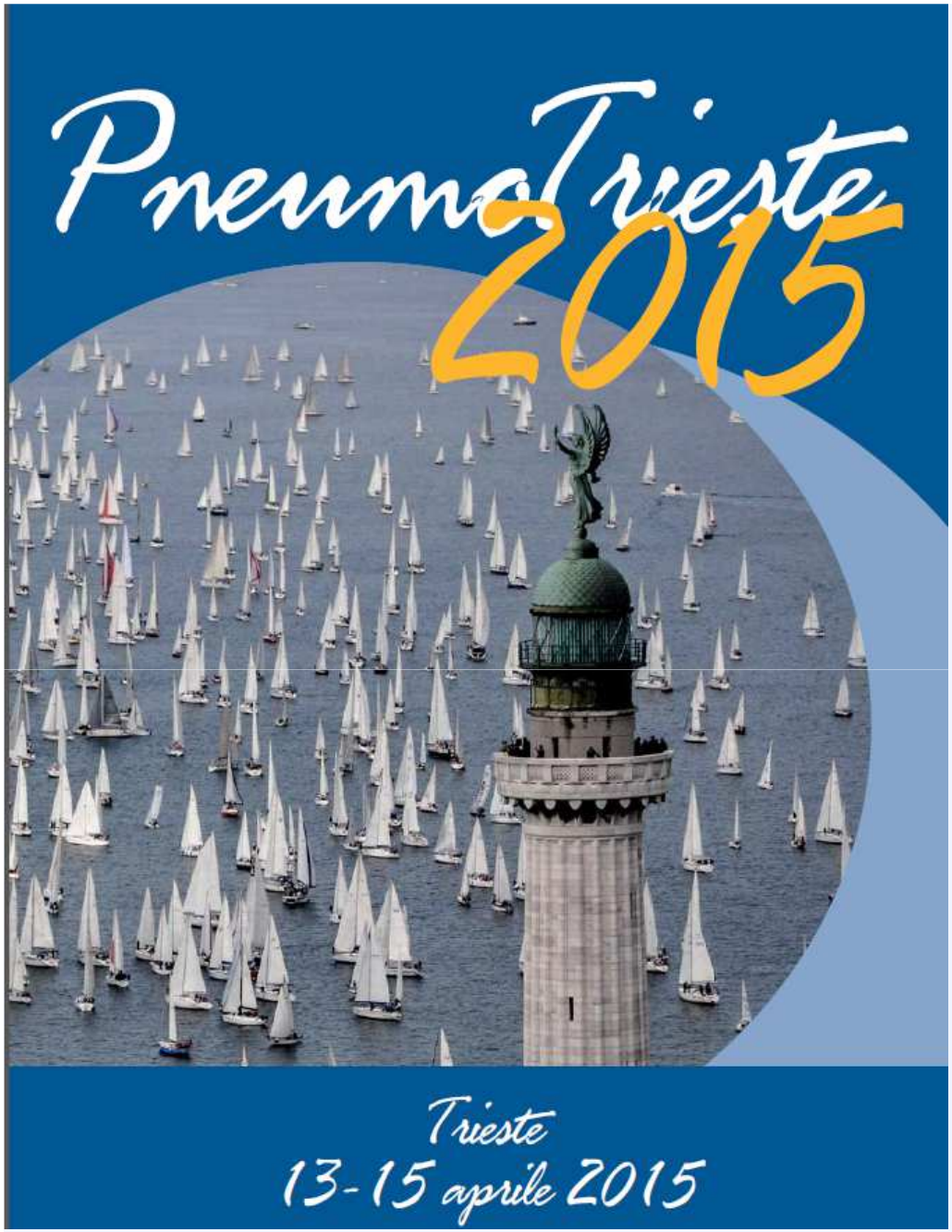


Understanding Acute Lung Injury/ARDS

Chairs: *K. Antoniou, V. Poletti*

A pathologist's view

Marco Chilosi
Anatomia Patologica
AOUI Verona

ONLINE FIRST

Acute Respiratory Distress Syndrome
The Berlin Definition

The ARDS Definition Task Force*

The acute respiratory distress syndrome (ARDS) was defined in 1994 by the American-European Consensus Conference (AECC); since then, issues regarding the reliability and validity of this definition have emerged. Using a consensus process, a panel of experts convened in 2011 (an initiative of the European Society of Intensive Care Medicine endorsed by the American Thoracic Society and the Society of Critical Care Medicine) developed the Berlin Definition, focusing on feasibility, reliability, validity, and objective evaluation of its performance. A draft definition proposed 3 mutually exclusive categories of ARDS based on degree of hypoxemia: mild (200 mm Hg $<$ $Pao_2/Fio_2 \leq$ 300 mm Hg), moderate (100 mm Hg $<$ $Pao_2/Fio_2 \leq$ 200 mm Hg), and severe ($Pao_2/Fio_2 \leq$ 100 mm Hg) and 4 ancillary variables for severe ARDS: radiographic severity, respiratory system compliance ($\leq$40 mL/cm H_2O), positive end-expiratory pressure ($\geq$10 cm H_2O), and corrected expired volume per minute ($\geq$10 L/min). The draft Berlin Definition was empirically evaluated using patient-level meta-analysis of 4188 patients with ARDS from 4 multicenter clinical data sets and 269 patients with ARDS from 3 single-center data sets containing physiologic information. The 4 ancillary variables did not contribute to the predictive validity of severe ARDS for mortality and were removed from the definition. Using the Berlin Definition, stages of mild, moderate, and severe ARDS were associated with increased mortality (27%; 95% CI, 24%-30%; 32%; 95% CI, 29%-34%; and 45%; 95% CI, 42%-48%, respectively; $P < .001$) and increased median duration of mechanical ventilation in survivors (5 days; interquartile [IQR], 2-11; 7 days; IQR, 4-14; and 9 days; IQR, 5-17, respectively; $P < .001$). Compared with the AECC definition, the final Berlin Definition had better predictive validity for mortality, with an area under the receiver operating curve of 0.577 (95% CI, 0.561-0.593) vs 0.536 (95% CI, 0.520-0.553; $P < .001$). This updated and revised Berlin Definition for ARDS addresses a number of the limitations of the AECC definition. The approach of combining consensus discussions with empirical evaluation may serve as a model to create more accurate, evidence-based, critical illness syndrome definitions and to better inform clinical care, research, and health services planning.

JAMA. 2012;307(23):2526-2533
Published online May 21, 2012. doi:10.1001/jama.2012.5669 www.jama.com

VALID AND RELIABLE DEFINITIONS are essential to conduct epidemiological studies successfully and to facilitate enrollment of a consistent patient phenotype into clinical trials.[1] Clinicians also need such definitions to implement the results of clinical trials, discuss prognosis with families, and plan resource allocation.

Following the initial description of acute respiratory distress syndrome (ARDS) by Ashbaugh et al[2] in 1967, multiple definitions were proposed and used until the 1994 publication of the American-European Consensus Conference (AECC) definition.[3] The AECC defined ARDS as the acute onset of hypoxemia (arterial partial pressure of oxygen to fraction of inspired oxygen [Pao_2/Fio_2] $\leq$ 200 mm Hg) with bilateral infiltrates on frontal chest radiograph, with no evidence of left atrial hypertension. A new overarching entity—acute lung injury (ALI)—was also described, using similar criteria but with less severe hypoxemia ($Pao_2/Fio_2 \leq$ 300 mm Hg).[3]

The AECC definition was widely adopted by clinical researchers and clinicians and has advanced the knowledge of ARDS by allowing the acquisition of clinical and epidemiological data, which in turn have led to improvements in the ability to care for patients with ARDS. However,

Types, patterns, mechanisms, molecular pathways

Lung
Damage and Repair

Acute
Sub-acute
Chronic

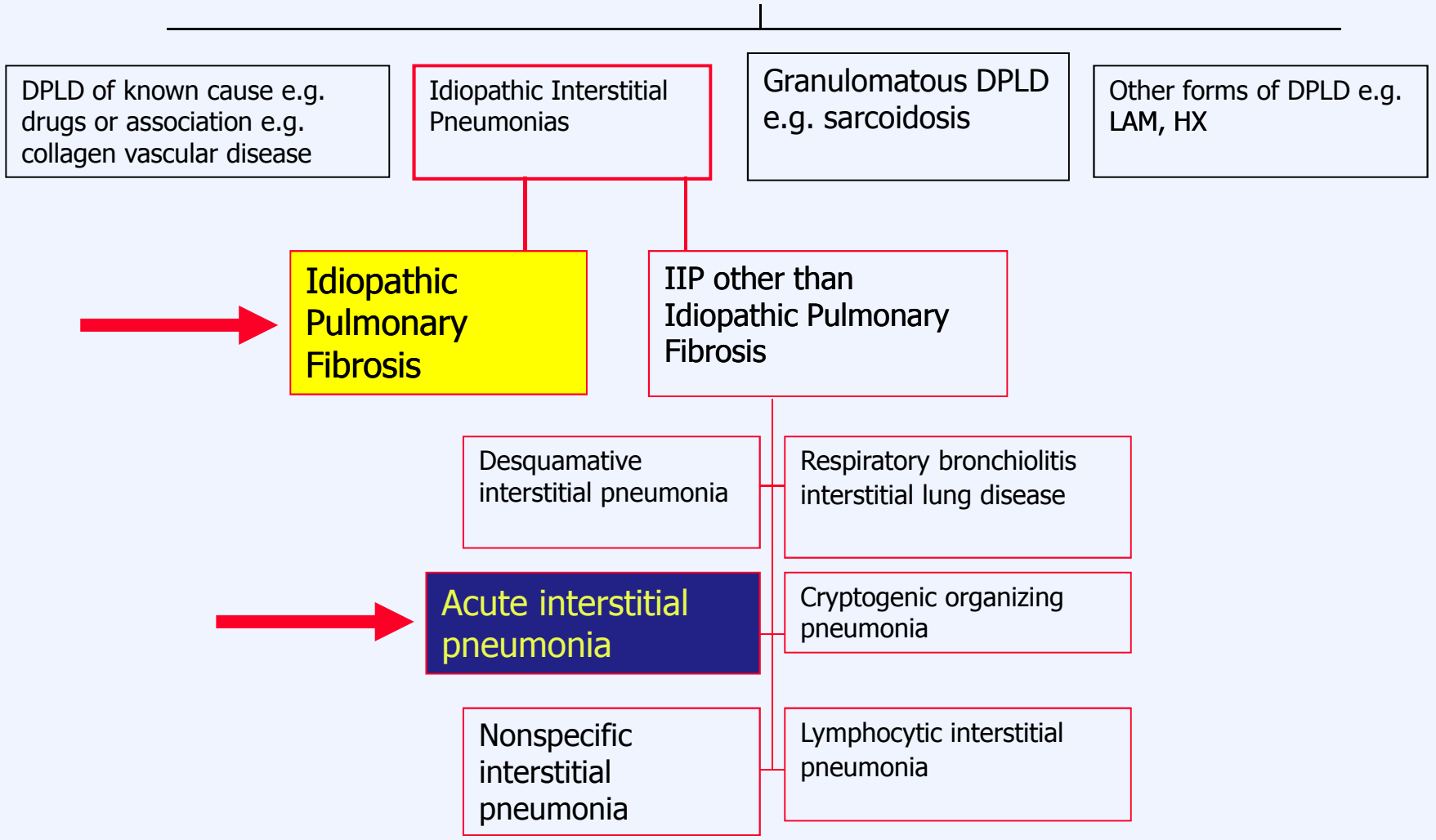
Direct
Indirect

TABLE 2. CLINICAL DISORDERS ASSOCIATED WITH THE DEVELOPMENT OF THE ACUTE RESPIRATORY DISTRESS SYNDROME.

DIRECT LUNG INJURY	INDIRECT LUNG INJURY
Common causes	**Common causes**
Pneumonia	Sepsis
Aspiration of gastric contents	Severe trauma with shock and multiple transfusions
Less common causes	**Less common causes**
Pulmonary contusion	Cardiopulmonary bypass
Fat emboli	Drug overdose
Near-drowning	Acute pancreatitis
Inhalational injury	Transfusions of blood products
Reperfusion pulmonary edema after lung transplantation or pulmonary embolectomy	

Ware LB, Matthay MA. *The acute respiratory distress syndrome.*
N Engl J Med. 2000 May 4;342(18):1334-49.

Diffuse Parenchymal Lung Disease



Am J Respir Crit Care Med 2002 Jan 15;165(2):277-304

American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias.

AIP

Biopsy shows an acute and/or organizing form of diffuse alveolar damage (DAD) that is indistinguishable from the histologic pattern found in ARDS. In the organizing phase, when most patients undergo biopsy, hyaline membranes may be inconspicuous or absent and the key findings include diffuse distribution, loose organizing connective tissue causing alveolar wall thickening and prominent pneumocyte hyperplasia.

Occult background fibrosis may be present and if this shows features of UIP, acute exacerbation of underlying IPF should be considered . AIP can progress to a pattern similar to fibrotic NSIP or to severe fibrosis resembling honeycombing .

Am J Respir Crit Care Med Vol 188, 6, 733–748, 2013

Eur Respir J 2003; 22: 821–826
DOI: 10.1183/09031936.03.00022703
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European Respiratory Journal
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Acute exacerbation of idiopathic pulmonary fibrosis: report of a series

V. Ambrosini*, A. Cancellieri#, M. Chilosi¶, M. Zompatori+, R. Trisolini§, L. Saragoni*f*, V. Poletti*

Acute exacerbation of idiopathic pulmonary fibrosis: report of a series. V. Ambrosini, A. Cancellieri, M. Chilosi, M. Zompatori, R. Trisolini, L. Saragoni, V. Poletti. ©ERS Journals Ltd 2003.

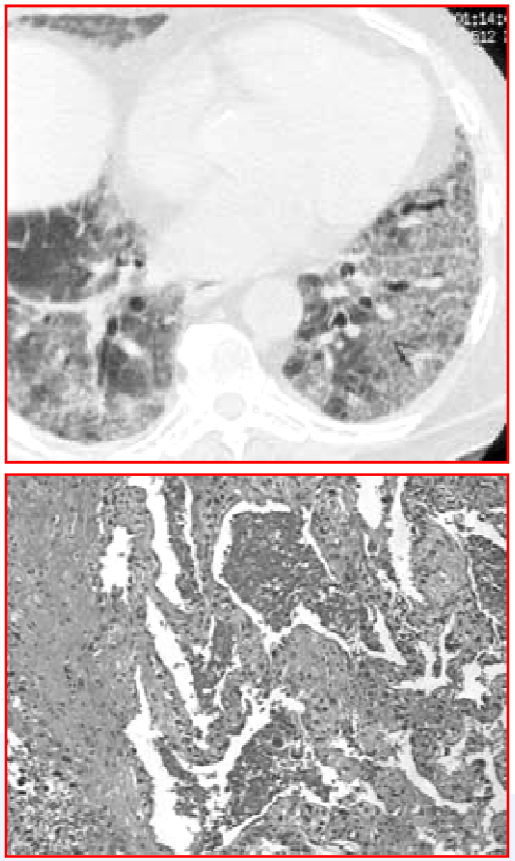
ABSTRACT: This study describes five cases presenting an acute clinical course of pulmonary fibrosis, in the absence of specific precipitating factors.

A retrospective chart review of five patients with histologically proved usual interstitial pneumonia was carried out in 2001–2002. Clinical data, bronchoalveolar lavage (BAL) findings, high resolution computed tomography and histological features were reported.

On admission all cases presented hypoxemia and dyspnoea, while some showed an increase of carbohydrate antigen 19.9 or laboratory tests typical of infection, although appropriate cultures were all negative. Altogether, four subjects died and only one is on follow-up. A pattern of diffuse ground-glass or alveolar opacification superimposed on reticular and linear findings was evident on lung imaging in all cases. Marked neutrophilia, together with type II reactive cells hyperplasia, was detected on BAL.

Histological findings, from open lung biopsy or autopsy, showed all the aspects of usual interstitial pnemonia with superimposed features of acute lung injury, such as diffuse alveolar damage, with or without hyaline membranes, type II reactive cells hyperplasia and numerous fibroblastic foci. This study also underlines the diagnostic value of bronchoalveolar lavage *versus* open lung biopsy.

Eur Respir J 2003; 22: 821–826.



ALI/ARDS:
a pathologist's view

- Histological Patterns
- Pathogenesis
- Diagnosis

ALI/ARDS: a pathologist's view

- Diagnosis
  - Autopsy
  - Diagnosis *(rarely)*
  - Evaluation in *borderline cases*
  - *DAD in IPF and other IIP*

Hum Pathol. 2003 Aug;34(8):743-8. # Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore

Franks TJ, Chong PY, Chui P, Galvin JR, Lourens RM, Reid AH, Selbs E, McEvoy CP, Hayden CD, Fukuoka J, Taubenberger JK, Travis WD. Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology, Wshington, DC 20306, USA.

Severe acute respiratory syndrome (SARS) is an infectious condition caused by the SARS-associated coronavirus (SARS-CoV), a new member in the family Coronaviridae. To evaluate the lung pathology in this life-threatening respiratory illness, we studied postmortem lung sections from 8 patients who died from SARS during the spring 2003 Singapore outbreak. The predominant pattern of lung injury in all 8 cases was diffuse alveolar damage. The histology varied according to the duration of illness. Cases of 10 or fewer days' duration demonstrated acute-phase diffuse alveolar damage (DAD), airspace edema, and bronchiolar fibrin. Cases of more than 10 days' duration exhibited organizing-phase DAD, type II pneumocyte hyperplasia, squamous metaplasia, multinucleated giant cells, and acute bronchopneumonia. In acute-phase DAD, pancytokeratin staining was positive in hyaline membranes along alveolar walls and highlighted the absence of pneumocytes. Multinucleated cells were shown to be both type II pneumocytes and macrophages by pancytokeratin, thyroid transcription factor-1, and CD68 staining. SARS-CoV RNA was identified by reverse transcriptase-polymerase chain reaction in 7 of 8 cases in fresh autopsy tissue and in 8 of 8 cases in formalin-fixed, paraffin-embedded lung tissue, including the 1 negative case in fresh tissue. Understanding the pathology of DAD in SARS patients may provide the basis for therapeutic strategies. Further studies of the pathogenesis of SARS may reveal new insight into the mechanisms of DAD.

Histologic Patterns

- ALI/ARDS
- AIP
- Infective pneumonitis
- Autoimmune lung
- Drugs

- AFOP
- AEP
- COP

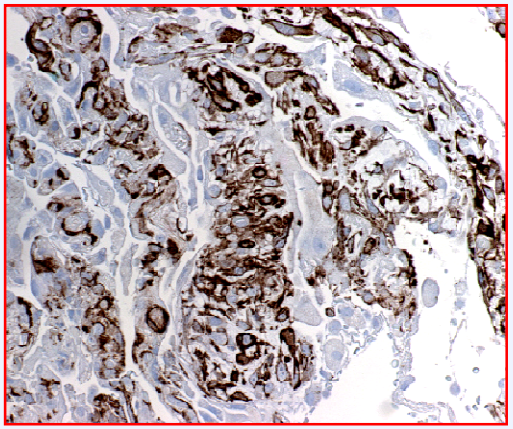
- DAD

- *Mixed* acute-subacute
- *Borderline* acute/subacute

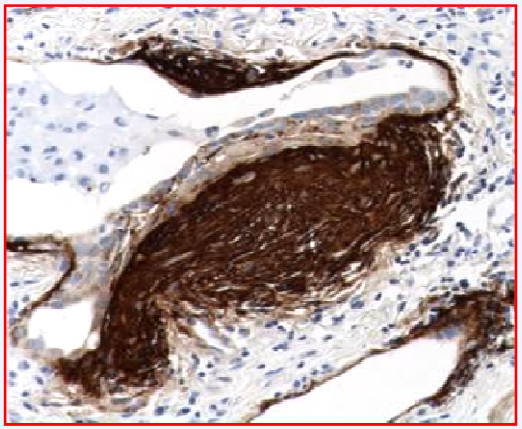
- AFOP
- AEP
- OP

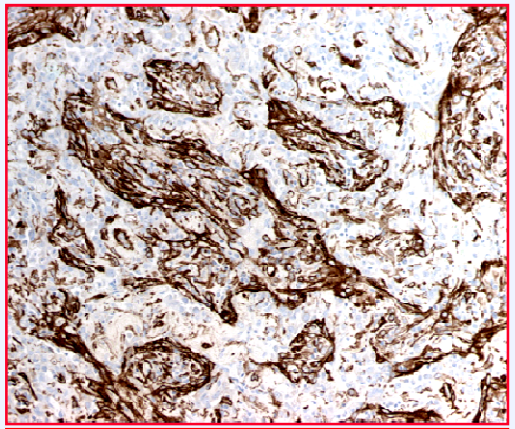
Patterns of Myofibroblast distribution in IIP

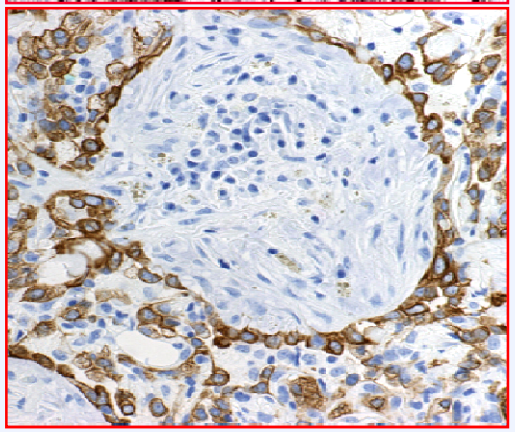
DAD



UIP







OP

Elementary patterns of lung damage and repair

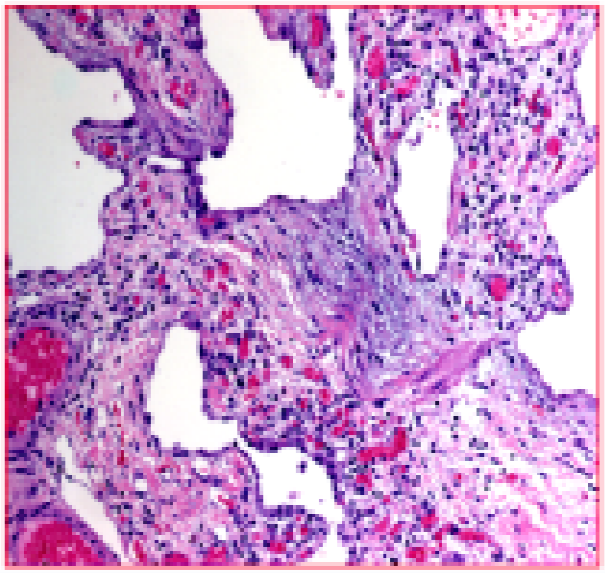
- *Chronic*
- *Subacute*
- *Mixed* acute-subacute
- *Borderline* acute/subacute
- Acute

- Alveolitis
- Interstitial thickening (NSIP)
- Organazing pneumonia
- Eosinophilic pneumonia
- Acut fibrinous organizing pneumonia (AFOP)
- Diffuse alveolar damage (DAD)

Chronic damage and repair

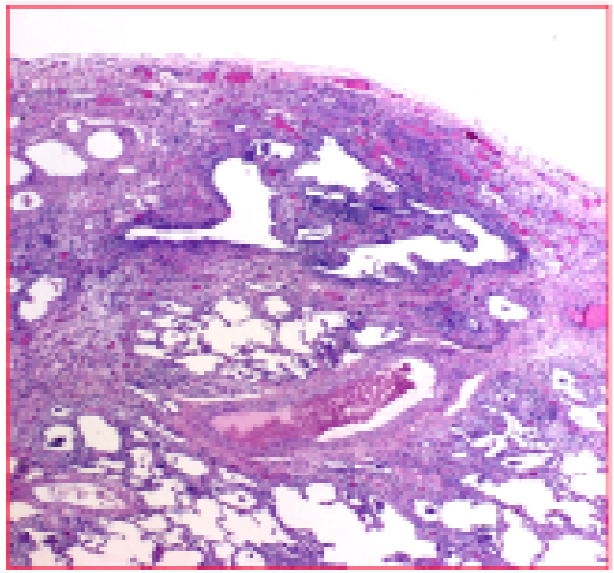
Inflammatory Pathway

NSIP

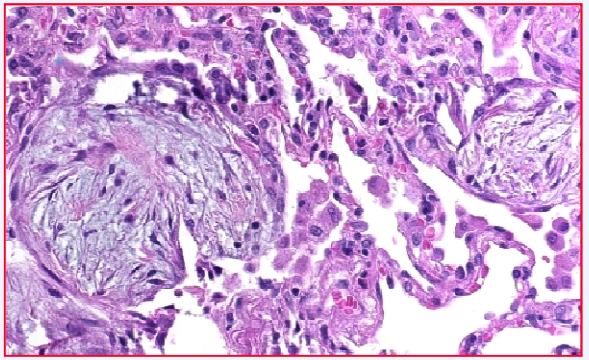


Epithelial-mesenchymal Deranged proliferation

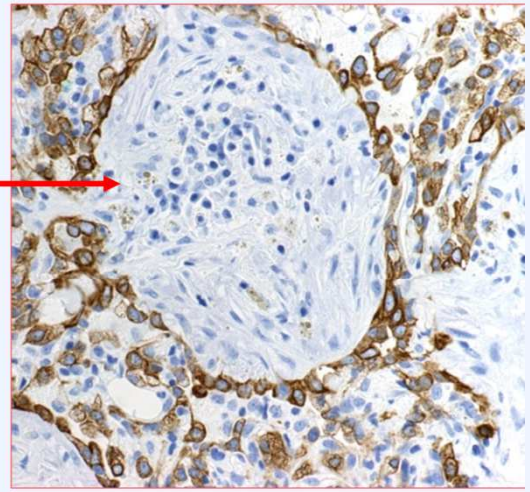
UIP



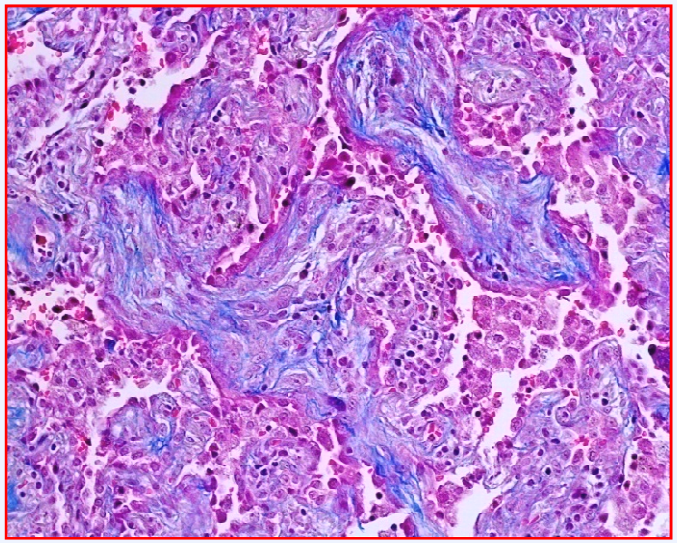
Organizing Pneumonia

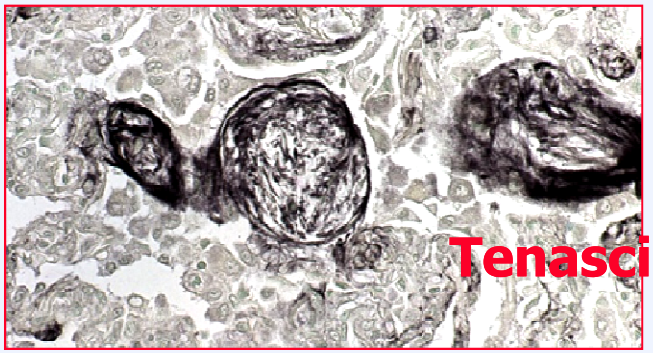


- intraluminal Masson's bodies
- Pneumocyte hyperplasia
- *Restitutio ad integrum*



- Plasma cells
- Lymphocytes
- neoangiogenesis



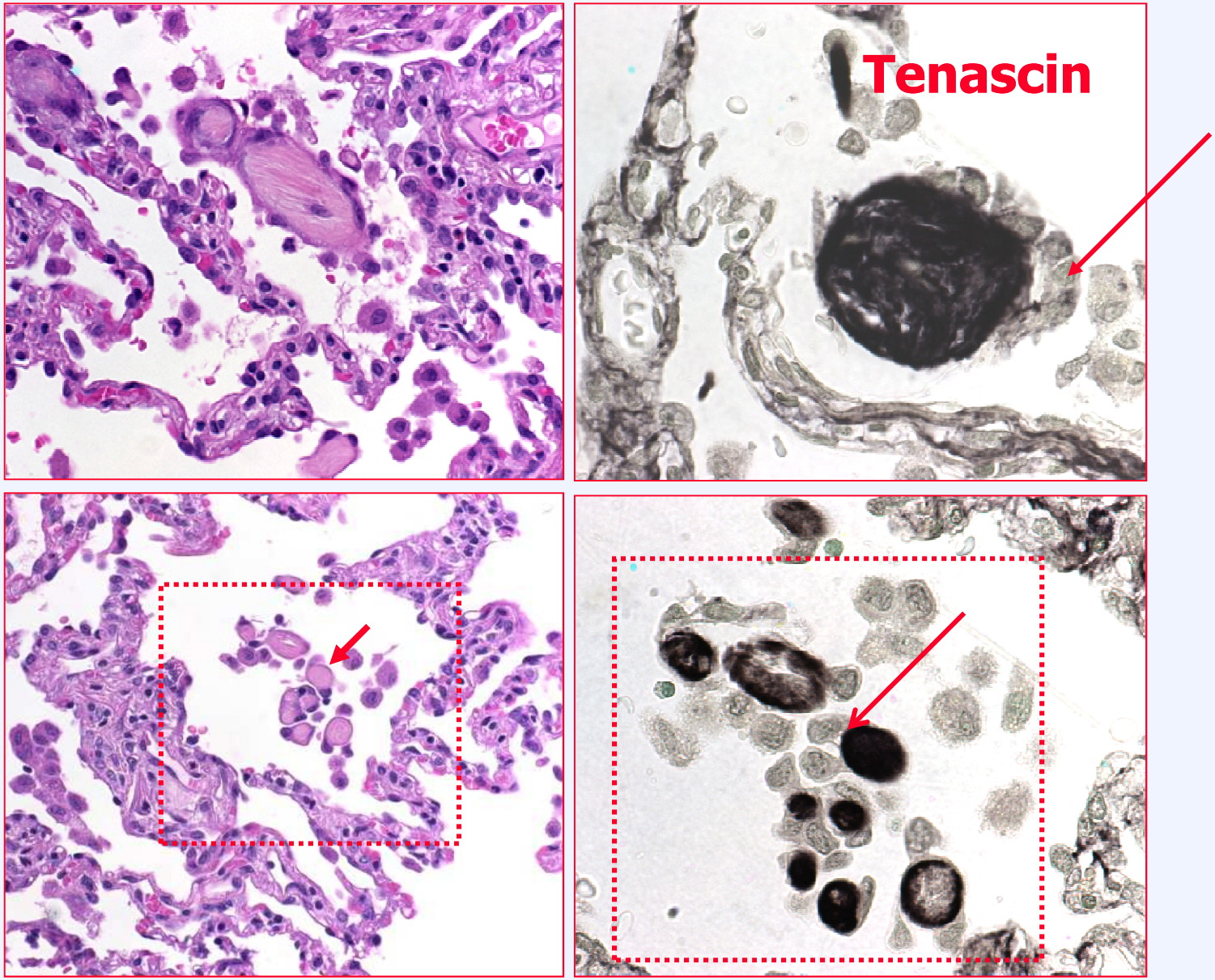


Tenascin

OP, COP (ex-BOOP)

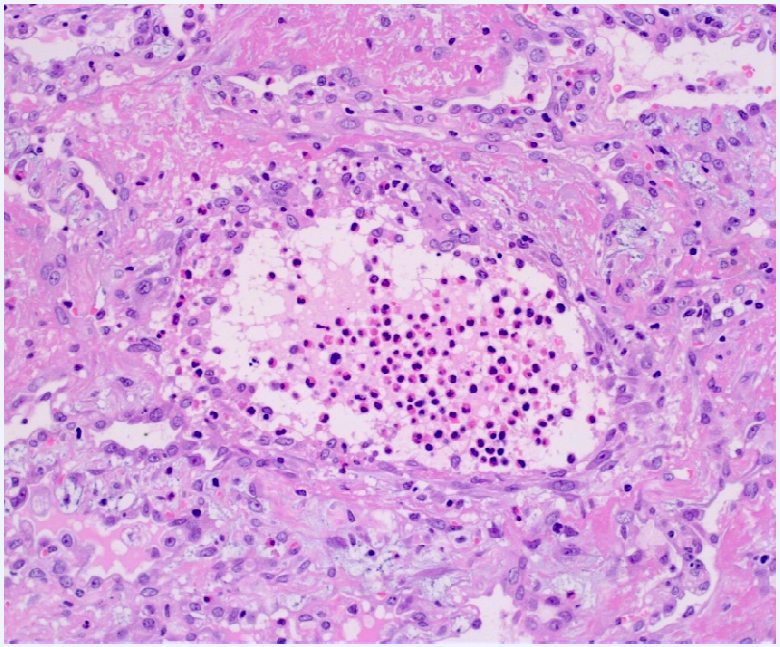
Organizing Pneumonia OP

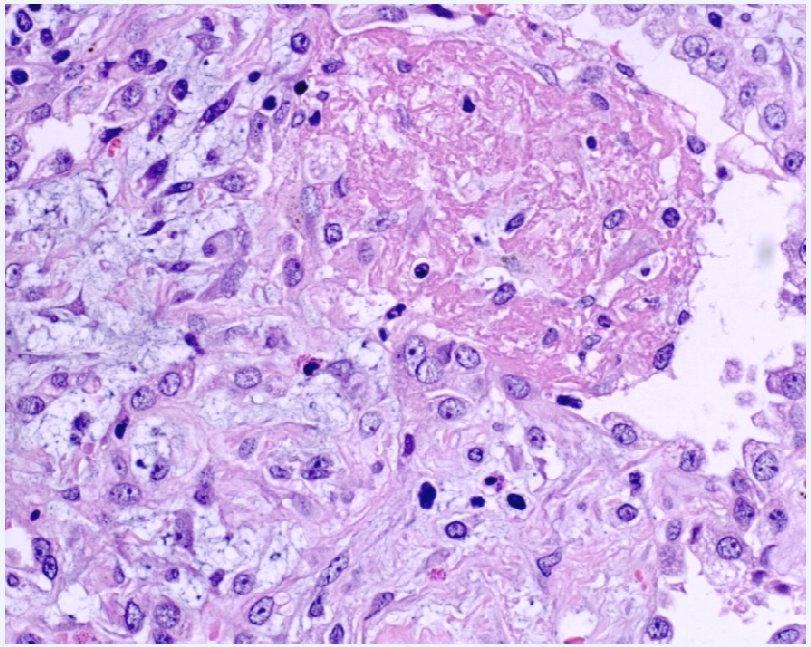
Macrophage-mediated degradation of fibro-inflammatory endoluminal aggregates



AEP

- AEP may be mistaken for other diseases, particularly community-acquired pneumonia, resulting in delayed or missed diagnosis. As defined by the North American-European Consensus Committee, many patients with AEP meet the criteria for acute lung injury (ALI), including its most severe subset - acute respiratory distress syndrome (ARDS). Distinguishing AEP and ARDS is critical because of differences in response to corticosteroids and outcome. Although both present with rapidly progressive respiratory failure, patients with AEP characteristically show a rapid response to corticosteroids and have an excellent prognosis resulting in a favorable outcome in most patients, whereas patients with ARDS do not always benefit from corticosteroid therapy and have a poor prognosis with mortality rates reported to range from 10% to 90%.





Arch Pathol Lab Med. 2002 Sep;126(9):1064-70. Acute fibrinous and organizing pneumonia: a histological pattern of lung injury and possible variant of diffuse alveolar damage. Beasley MB, Franks TJ, Galvin JR, Gochuico B, Travis WD. Department of Pulmonary, Armed Forces Institute of Pathology, Washington, DC 20306, USA.

Acute Fibrinous and Organizing Pneumonia

A Histologic Pattern of Lung Injury and Possible Variant of Diffuse Alveolar Damage

Mary Beth Beasley, MD; Teri J. Franks, MD; Jeffrey R. Galvin, MD; Bernadette Gochuico, MD; William D. Travis, MD

• *Context.*—The histologic patterns of diffuse alveolar damage (DAD), bronchiolitis obliterans with organizing pneumonia (BOOP), and eosinophilic pneumonia (EP) are well-recognized histologic patterns of lung injury associated with an acute or subacute clinical presentation. We have recognized acute fibrinous and organizing pneumonia (AFOP) as a histologic pattern, which also occurs in this clinical setting but does not meet the classic histologic criteria for DAD, BOOP, or EP and may represent an underreported variant.

Objectives.—To investigate the clinical significance of the AFOP histologic pattern and to explore its possible relationship to other disorders, including DAD and BOOP.

Design.—Open lung biopsy specimens and autopsy specimens were selected from the consultation files of the Armed Forces Institute of Pathology, which showed a dominant histologic pattern of intra-alveolar fibrin and organizing pneumonia. Varying amounts of organizing pneumonia, type 2 pneumocyte hyperplasia, edema, acute and chronic inflammation, and interstitial widening were seen. Cases with histologic patterns of classic DAD, BOOP, abscess formation, or eosinophilic pneumonia were excluded. To determine the clinical behavior of patients with this histologic finding, clinical and radiographic information and follow-up information were obtained. Statistical analysis was performed using Kaplan-Meier and χ^2 analysis.

Results.—Seventeen patients (10 men, 7 women) with a mean age of 62 years (range, 33–78 years) had acute-onset symptoms of dyspnea (11), fever (6), cough (3), and hemoptysis (2). Associations believed to be clinically related to the lung disease included definitive or probable collagen vascular disease (3), antidefective (1), sputum culture positive for *Haemophilus influenza* (1), lung culture positive for *Acinetobacter* sp. (1), lymphoma (1), hairspray (1), construction work (1), coal mining (1), and zoological work (1). Six patients had no identifiable origin or association. Follow-up revealed 2 clinical patterns of disease progression: a fulminate illness with rapid progression to death (n = 9; mean survival, 0.1 year) and a more subacute illness, with recovery (n = 8). Histologic analysis and initial symptoms did not correlate with eventual outcome, but 5 of the 5 patients who required mechanical ventilation died ($P = .007$).

Conclusions.—Acute fibrinous and organizing pneumonia is a histologic pattern associated with a clinical picture of acute lung injury that differs from the classic histologic patterns of DAD, BOOP, or EP. Similar to these patterns of acute lung injury, the AFOP pattern can occur in an idiopathic setting or with a spectrum of clinical associations. The overall mortality rate is similar to DAD and therefore may represent a histologic variant; however, AFOP appears to have 2 distinct patterns of disease progression and outcome. The need for mechanical ventilation was the only parameter that correlated with prognosis. None of the patients with a subacute clinical course required mechanical ventilation.

(*Arch Pathol Lab Med.* 2002;126:1064–1070)

Classification of biopsy specimens from patients with a clinical picture of acute lung injury often presents a difficult diagnostic challenge. Diffuse alveolar damage (DAD) and bronchiolitis obliterans with organizing pneumonia (BOOP) are well-recognized histologic patterns associated with an acute or subacute clinical presentation, respectively. Both the DAD and BOOP patterns may be associated with known causes, such as collagen vascular diseases, or they may be idiopathic.[1] In a recently proposed multidisciplinary consensus classification sponsored jointly by the American Thoracic Society (ATS) and the European Respiratory Society (ERS), idiopathic DAD may be referred to as acute interstitial pneumonia and idiopathic BOOP may be referred to as cryptogenic organizing pneumonia (COP).[2] This ATS/ERS statement also recommended using the term *organizing pneumonia* (OP) for the histologic pattern, rather than BOOP.[2] Eosinophilic pneumonia (EP) may also present clinically as an acute or subacute illness in the form of acute EP and chronic EP, respectively.[3]

We have encountered a histologic pattern associated with an acute or subacute clinical presentation, which does not meet the criteria for the patterns of DAD, OP or EP, but instead is composed of predominantly intra-alve-

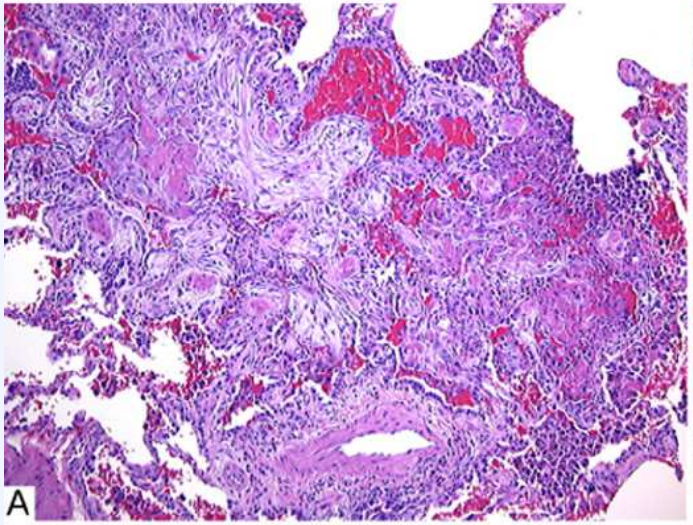
Accepted for publication April 24, 2002.

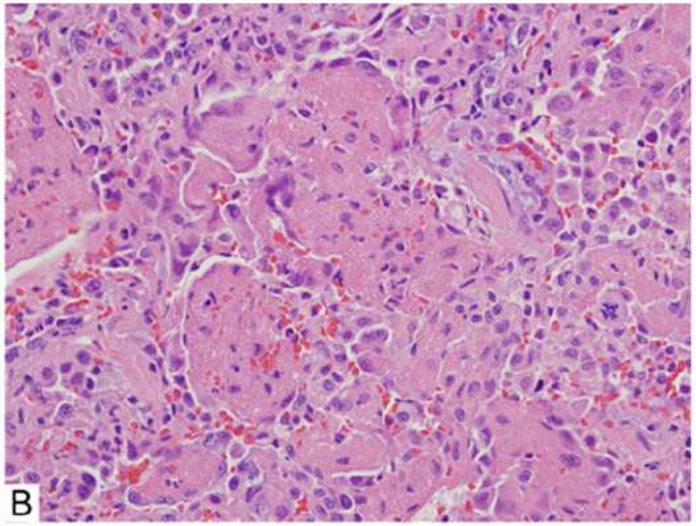
From the Departments of Pulmonary and Mediastinal Pathology (Drs Beasley, Franks, and Travis) and Radiologic Pathology (Dr Galvin), Armed Forces Institute of Pathology, and Pulmonary-Critical Care Medicine Branch of the National Heart, Lung, and Blood Institute (Dr Gochuico), Washington, DC.

Reprints: William D. Travis, MD, Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology, 6825 16th St, Washington, DC 20306-6000 (e-mail: travis@afip.osd.mil).

AFOP

Acute fibrinous and organizing pneumonia is a histologic pattern associated with a clinical picture of acute lung injury that differs from the classic histologic patterns of DAD, BOOP, or EP. Similar to these patterns of acute lung injury, the AFOP pattern can occur in an idiopathic setting or with a spectrum of clinical associations. The overall mortality rate is similar to DAD and therefore may represent a histologic variant; however, AFOP appears to have 2 distinct patterns of disease progression and outcome. The need for mechanical ventilation was the only parameter that correlated with prognosis. None of the patients with a subacute clinical course required mechanical ventilation





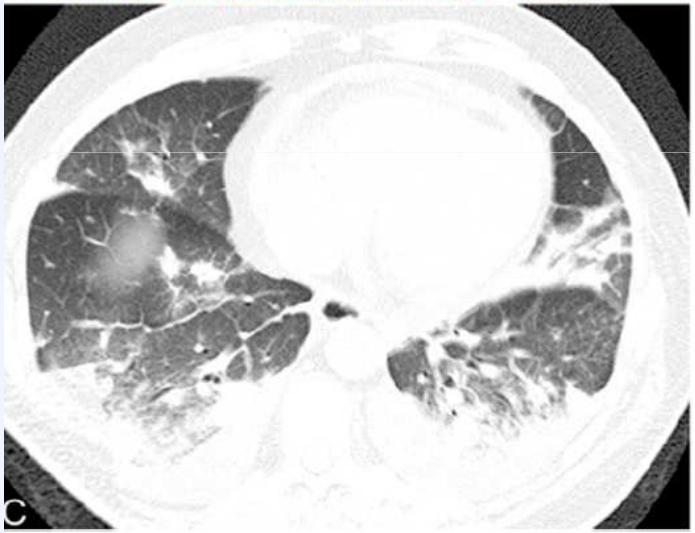


Figure 9. Acute fibrinous and organizing pneumonia. Computed tomography (CT) features. (*A*) Axial CT through the lung bases shows multiple poorly defined nodules and areas of consolidation, with peribronchovascular and basal predominance. Pleural and pericardial effusions are present. Histologic features: (*B*) Biopsy shows nodules of alveolar fibrin and organizing pneumonia. (*C*) The histology is dominated by intraalveolar plugs of alveolar fibrin.

American Thoracic Society Documents

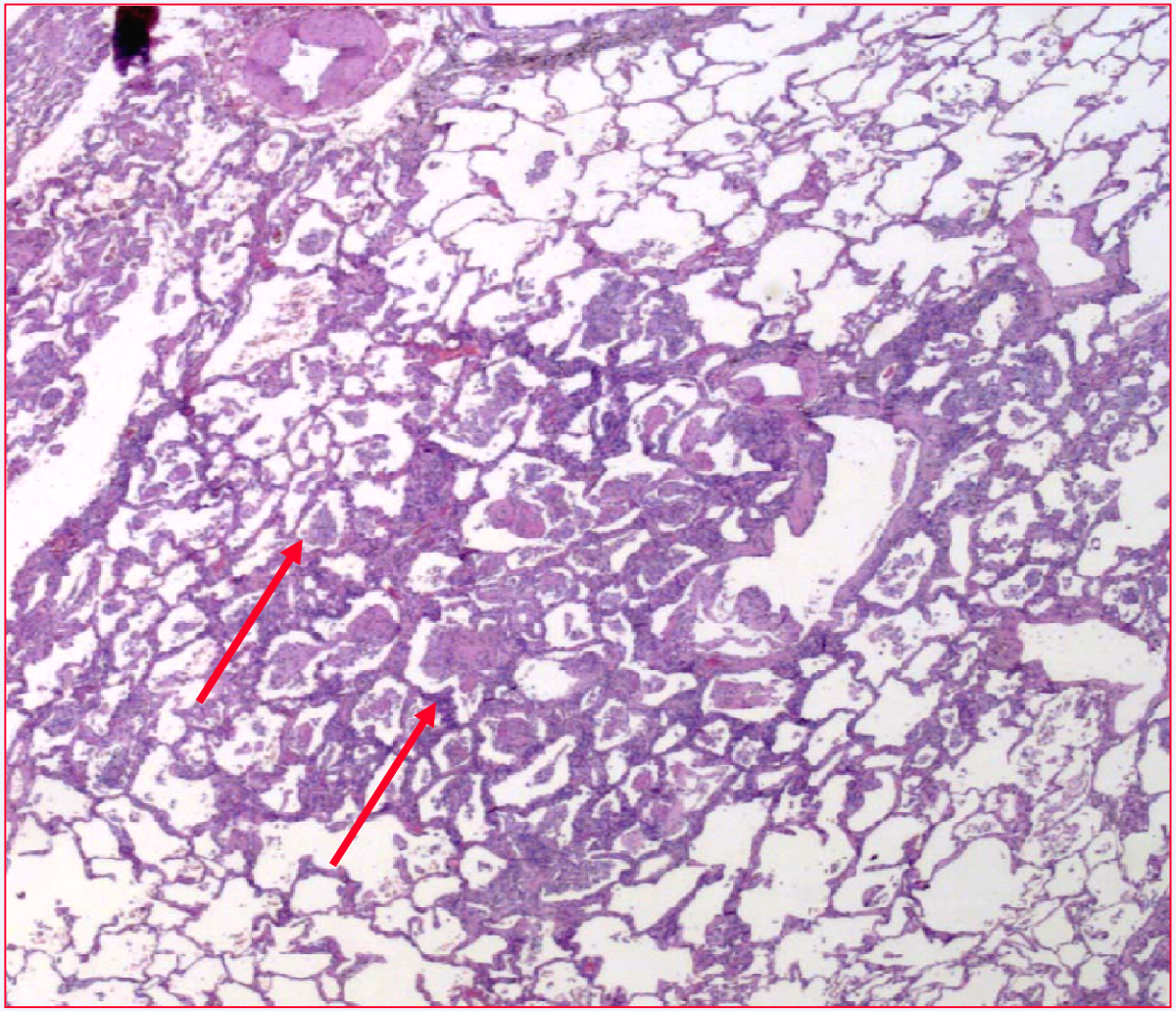
An Official American Thoracic Society/European Respiratory Society Statement: Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias

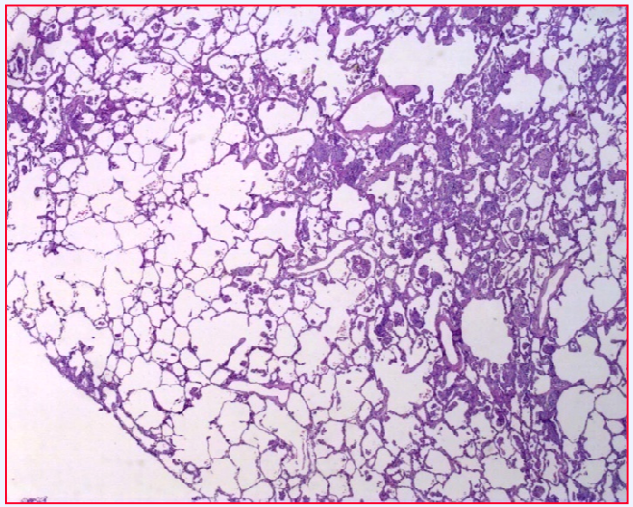
William D. Travis, Ulrich Costabel, David M. Hansell, Talmadge E. King, Jr., David A. Lynch, Andrew G. Nicholson, Christopher J. Ryerson, Jay H. Ryu, Moisés Selman, Athol U. Wells, Jurgen Behr, Demosthenes Bouros, Kevin K. Brown, Thomas V. Colby, Harold R. Collard, Carlos Robalo Cordeiro, Vincent Cottin, Bruno Crestani, Marjolein Drent, Rosalind F. Dudden, Jim Egan, Kevin Flaherty, Cory Hogaboam, Yoshikazu Inoue, Takeshi Johkoh, Dong Soon Kim, Masanori Kitaichi, James Loyd, Fernando J. Martinez, Jeffrey Myers, Shandra Protzko, Ganesh Raghu, Luca Richeldi, Nicola Sverzellati, Jeffrey Swigris, and Dominique Valeyre; on behalf of the ATS/ERS Committee on Idiopathic Interstitial Pneumonias

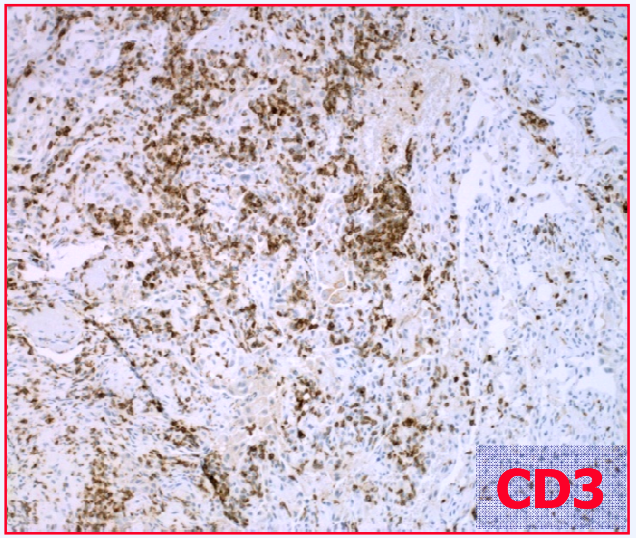
Am J Respir Crit Care Med Vol 188, 6, 733–748, 2013

#00-2422

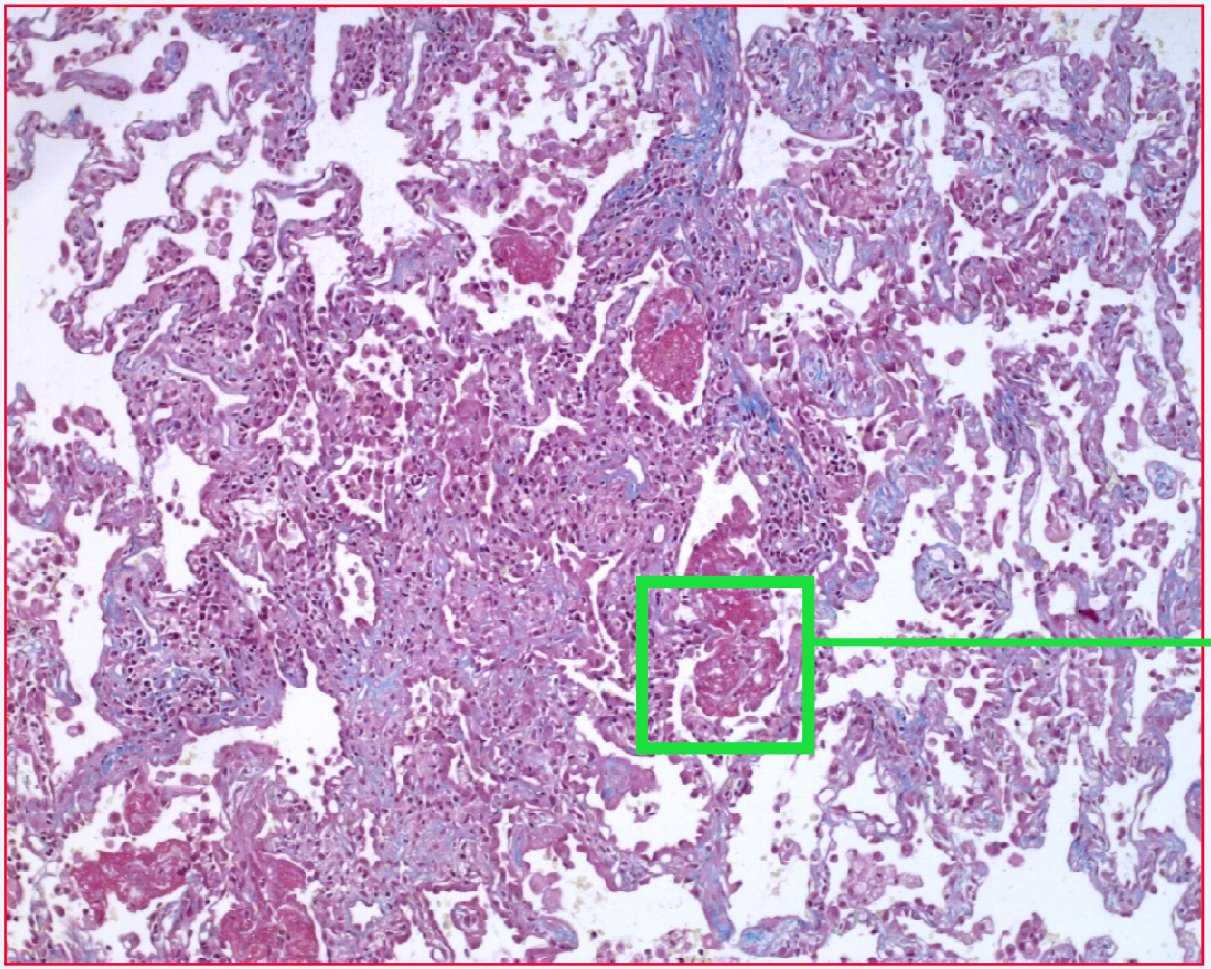
Localised AFOP pattern

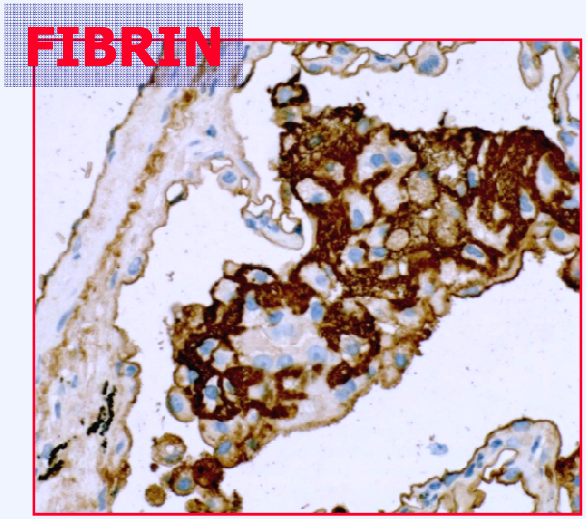


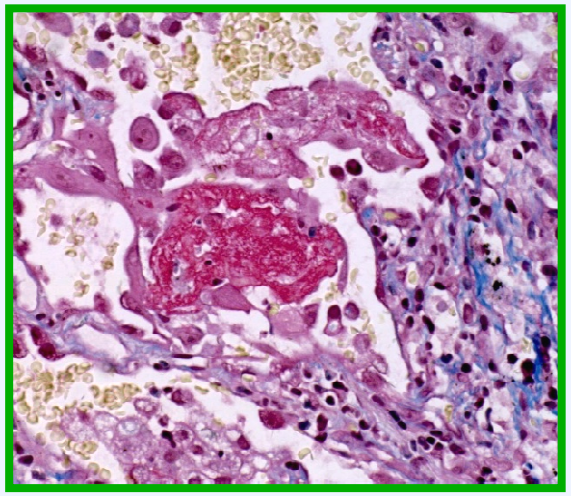




AFOP pattern





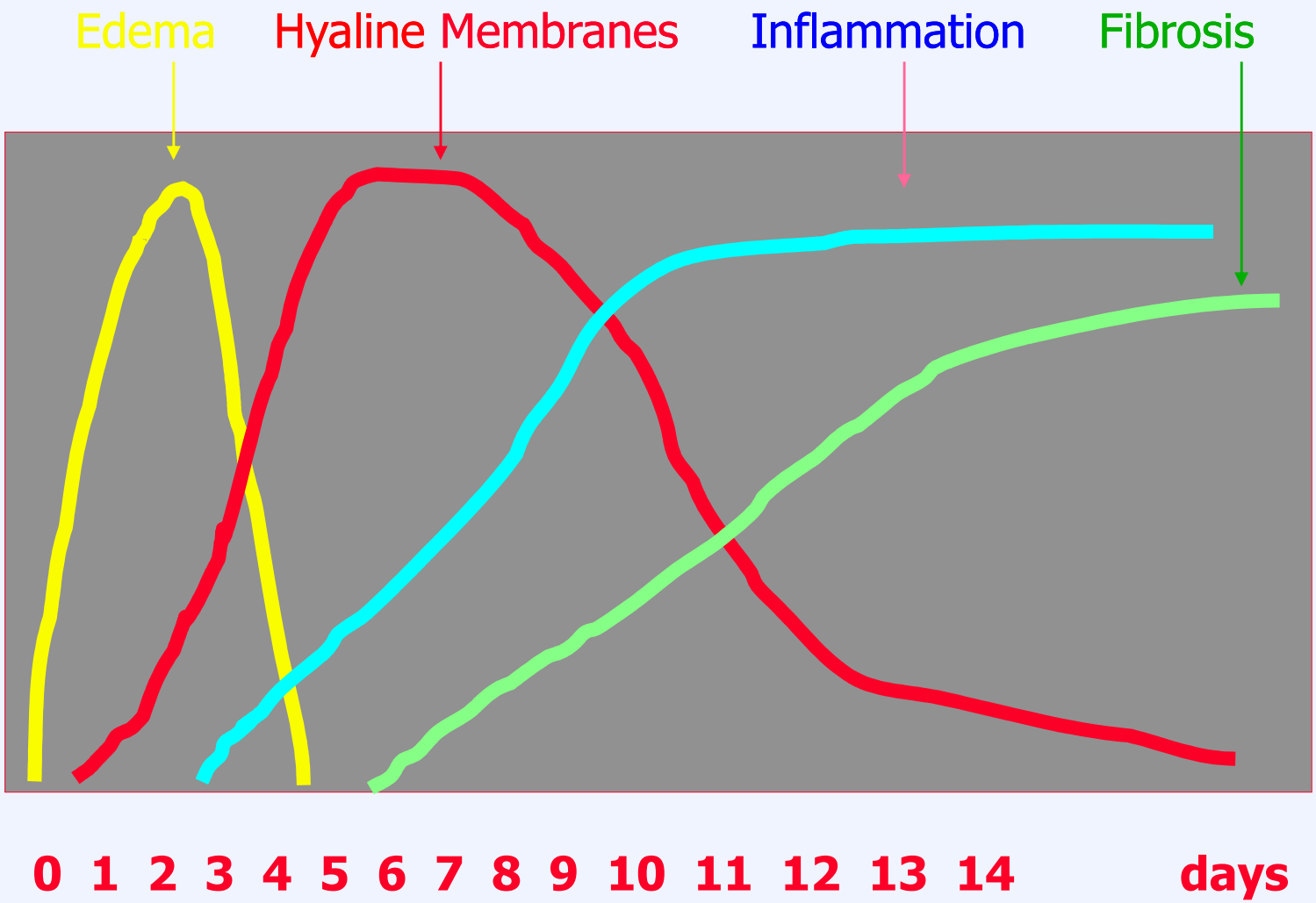


#00-2422

Acute Lung Injury/DAD

- Diffuse alveolar damage (DAD) is a form of acute lung injury that progresses through an exudative phase, characterised by pneumocyte and endothelial necrosis, edema, and formation of hyline membranes, to an organising phase with alveolar septal organising interstitial fibrosis and prominent type 2 pneumocyte proliferation.

Diffuse Alveolar Damage



Early phase

In the early (or exudative) phase, an intraalveolar and interstitial exudate composed of plasmatic proteins, fibrin and inflammation cells, essentially polynuclear neutrophilic and eosinophilic cells, appears in the first hours and persists for at least 1 week.

During this phase, the alveolar surfactant is altered by a modification in its composition and its functional capacities (alteration of its tensio-active properties). At this stage, there can also be morphological lesions of the alveolo-capillary barrier with a denudation of the endothelial and epithelial basal membranes.

Proliferative phase

The interstitial and alveolar exudate can then combine with macrophagic and fibroblastic infiltration controlled, among others, by activated proteases (neutrophil elastase, gelatinases). These proteases subsequently damage the basal membrane and the extracellular matrix. This phase, called the proliferative or organization phase, is characterized by the proliferation of type II pneumocytes (which differentiate in type I on the damaged zones of the alveolar epithelium), endothelial cells and fibroblasts.

Fibroblastic proliferation is mediated by signal molecules (fibronectin, collagen fragments, fibrin, elastin), tumor necrosis factor-*a and growth factors.*

Alveolar collapse

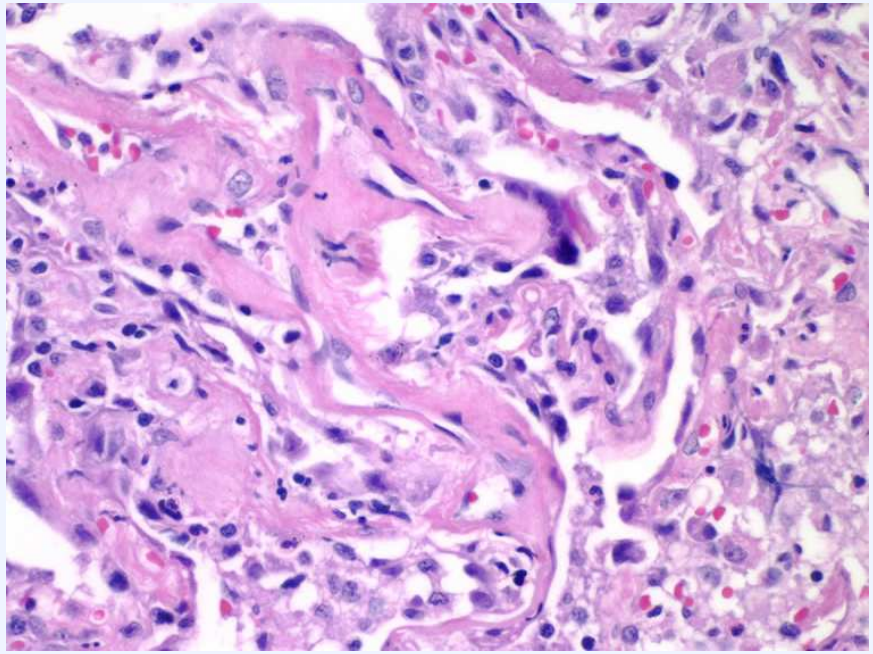
occurs in the most damaged zones with the occasional appearance of actual endoalveolar buds. Macroscopically, the lung has a pale grey color and a smooth surface. This is linked to the presence of young connective tissue.

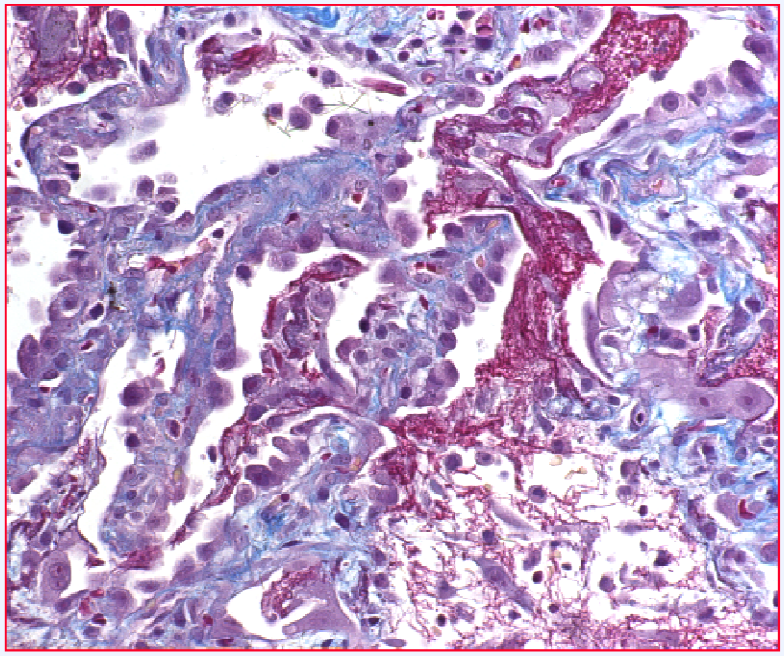
Fibrotic phase

Fibrosis is composed of an anarchic endoalveolar and septal deposit of type III collagen, followed by type I collagen, all secreted by fibroblasts. The alveolar and capillary spaces then become obstructed, which considerably disturbs pulmonary architecture and function.

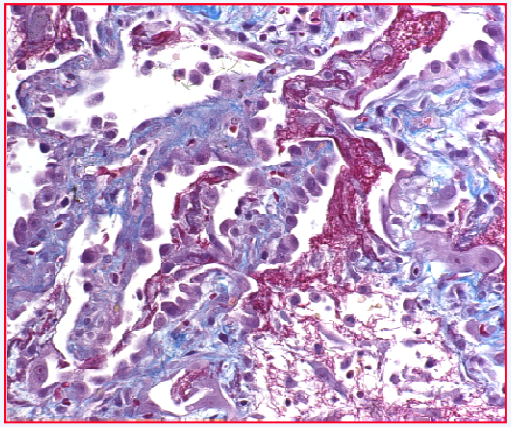
At this stage, the edema has usually disappeared owing to the effects of liquid, then proteic epithelial resorption. Moreover, intraalveolar angiogenesis appears in response to excessive growth factor products.

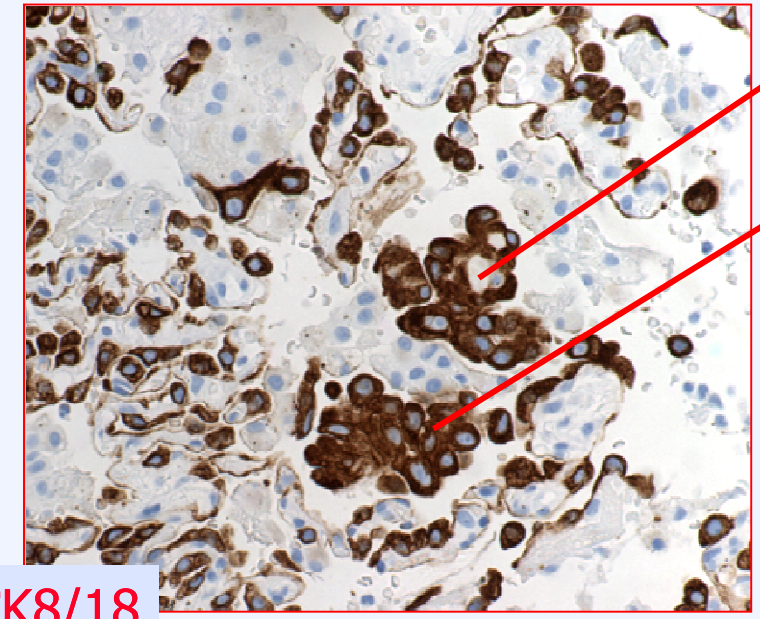
Hyaline membranes



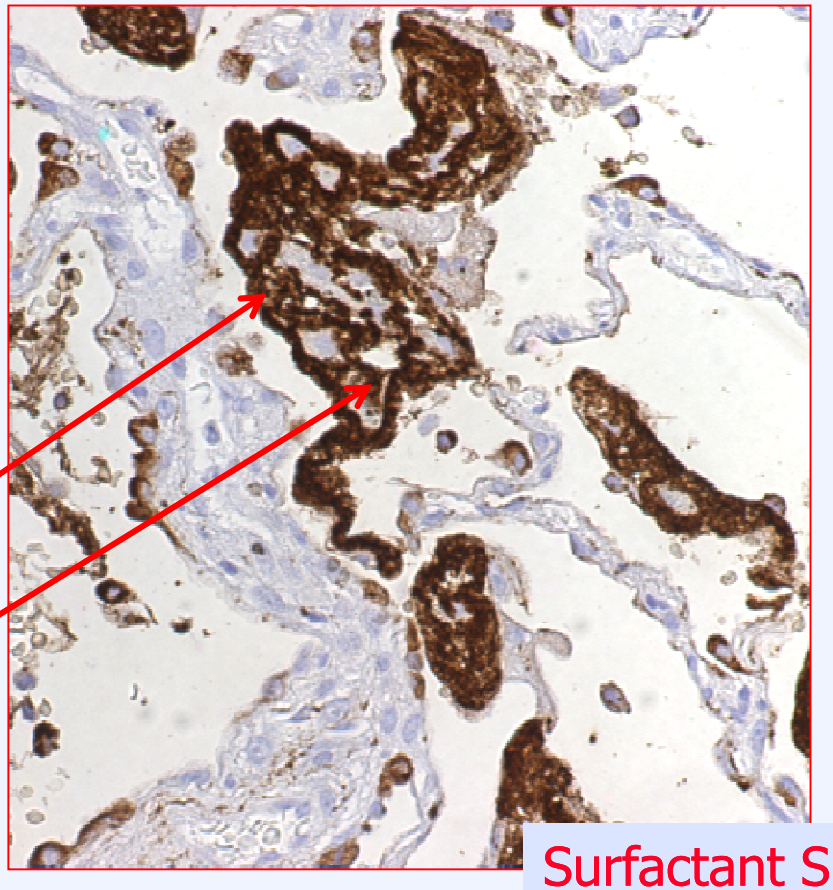


hyaline membranes

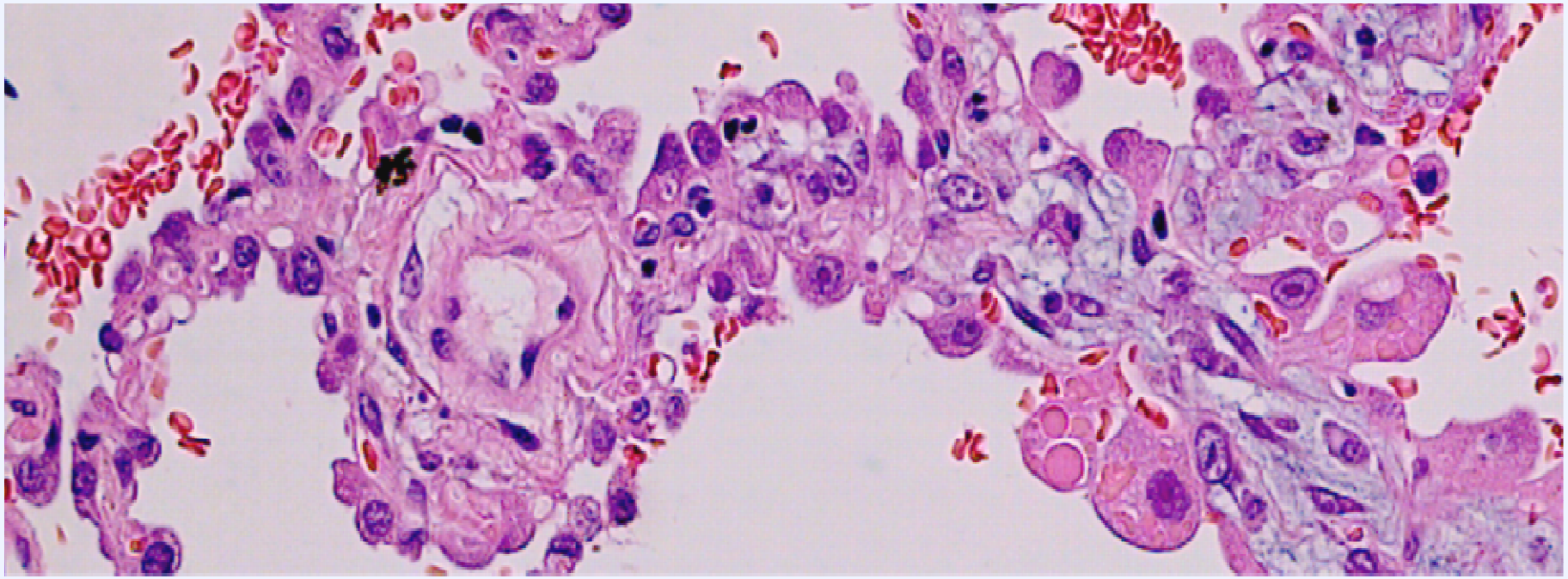




CK8/18



Surfactant SPA1



Pneumocyte type II hyperplasia and atypia

Response to extreme damage in DAD

LETTER

doi:10.1038/nature13173

Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq

Barbara Treutlein[1]*, Doug G. Brownfield[2]*, Angela R. Wu[1], Norma F. Neff[1], Gary L. Mantalas[1], F. Hernan Espinoza[2], Tushar J. Desai[3], Mark A. Krasnow[2] & Stephen R. Quake[1]

The mammalian lung is a highly branched network in which the distal regions of the bronchial tree transform during development into a densely packed honeycomb of alveolar air sacs that mediate gas exchange. Although this transformation has been studied by marker expression analysis and fate-mapping, the mechanisms that control the progression of lung progenitors along distinct lineages into mature alveolar cell types are still incompletely known, in part because of the limited number of lineage markers[1–3] and the effects of ensemble averaging in conventional transcriptome analysis experiments on cell populations[1–5]. Here we show that single-cell transcriptome analysis circumvents these problems and enables direct measurement of the various cell types and hierarchies in the developing lung. We used microfluidic single-cell RNA sequencing (RNA-seq) on 198 individual cells at four different stages encompassing alveolar differentiation to measure the transcriptional states which define the developmental and cellular hierarchy of the distal mouse lung epithelium. We empirically classified cells into distinct groups by using an unbiased genome-wide approach that did not require a priori knowledge of the underlying cell types or the previous purification of cell populations. The results confirmed the basic outlines of the classical model of epithelial cell-type diversity in the distal lung and led to the discovery of many previously unknown cell-type markers, including transcriptional regulators that discriminate between the different populations. We reconstructed the molecular steps during maturation of bipotential progenitors along both alveolar lineages and elucidated the full life cycle of the alveolar type 2 cell lineage. This single-cell genomics approach is applicable to any developing or mature tissue to robustly delineate molecularly distinct cell types, define progenitors and lineage hierarchies, and identify lineage-specific regulatory factors.

In mice, alveolar epithelial cells differentiate between embryonic days (E)16.5 and 18.5: distal airway tips expand into sac-like configurations ('sacculation') as a morphologically uniform population of columnar progenitors proceeds towards the fate of either flat alveolar type 1 (AT1) cells specialized for gas exchange or surfactant-secreting cuboidal alveolar type 2 (AT2) cells (Extended Data Fig. 1). At each time point during sacculation, progenitors, intermediates and recently differentiated cells coexist (Fig. 1a)[6]. To resolve the cellular composition of the developing bronchio-alveolar epithelium, we initially sequenced transcriptomes of 80 individual live cells of the developing mouse lung epithelium late in sacculation (E18.5; three biological replicates). Single-cell suspensions of micro-dissected distal lung regions were purified by magnetic-activated cell sorting (MACS) to deplete leukocytes and alveolar macrophages and enrich for epithelial cells ($CD45^-/EpCAM^+$) (Extended Data Fig. 2). An automated microfluidic platform was used to capture and lyse individual epithelial cells, reverse transcribe RNA and amplify complementary DNA.

RNA-seq libraries from the amplification products of single cells as well as bulk control samples were sequenced to a depth of $(2–5) \times 10^6$ reads per library (Methods). Saturation analysis confirmed that this sequencing depth is sufficient to detect most genes expressed by single cells (Extended Data Fig. 3a). Technical noise and dynamic range were

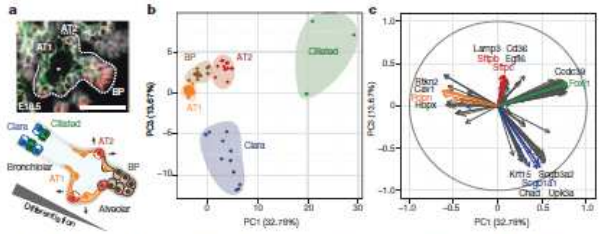


Figure 1 | Single-cell RNA-seq of 80 embryonic (E18.5) mouse lung epithelial cells enables unbiased identification of alveolar, bronchiolar and progenitor cell populations. a, Spatially heterogeneous differentiation of distal lung epithelium. The micrograph of a newly forming alveolar sac (asterisk) and the diagram below illustrate cell types and the gradient of developmental intermediates comprising the distal lung epithelium during sacculation (E18.5). Micrograph: green, Pdpn, alveolar type 1 (AT1) marker; red, Sftpc, AT2 marker; white, E-cadherin, pan-epithelial marker. BPs are characterized by co-expression of some AT1 and AT2 markers. In the diagram, BPs (brown) persist at the tip, and nascent AT2 (red) and AT1 (orange) cells are located more proximally. Ciliated (green) and Clara (blue) cells are located in the bronchiolar epithelium (not labelled in the micrograph). Scale bar, 75 μm. **b**, PCA of 80 single-cell transcriptomes (three biological replicates) at E18.5 distinguishes between major bronchiolar and alveolar cell lineages. PC, principal component. **c**, Distinct gene groups characterize each cell population on the basis of differential correlation with PC1 and PC3. The arrow tip denotes the correlation coefficient of the respective gene with each principal component.

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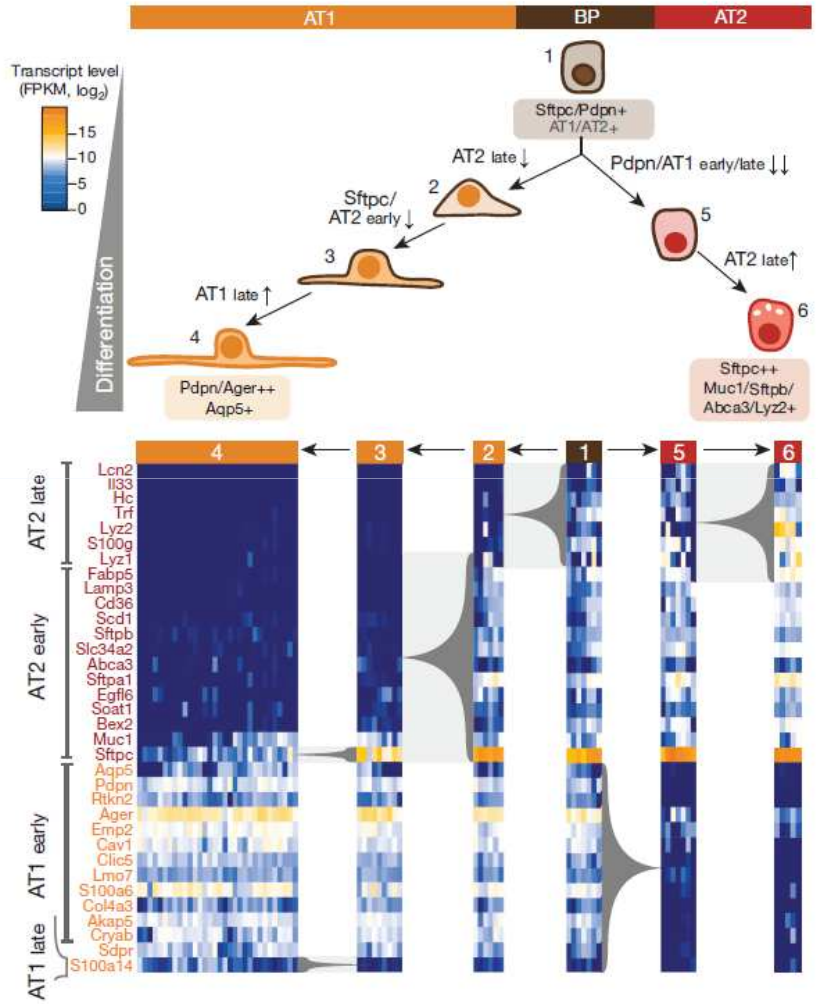
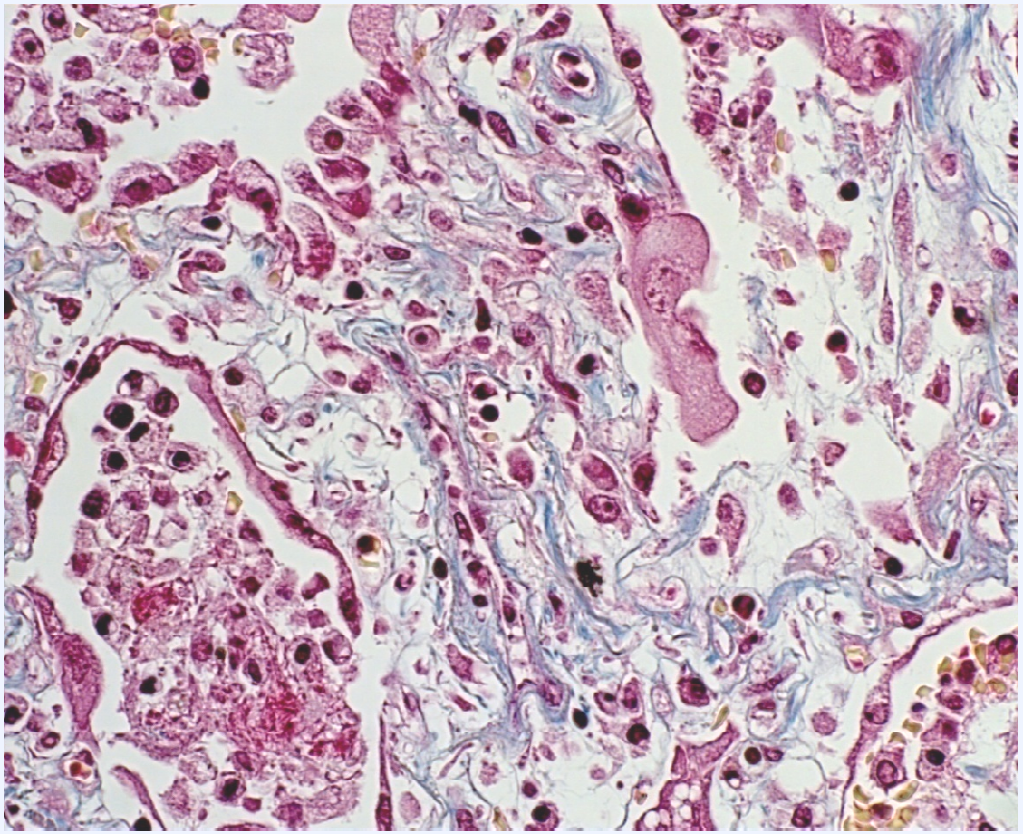
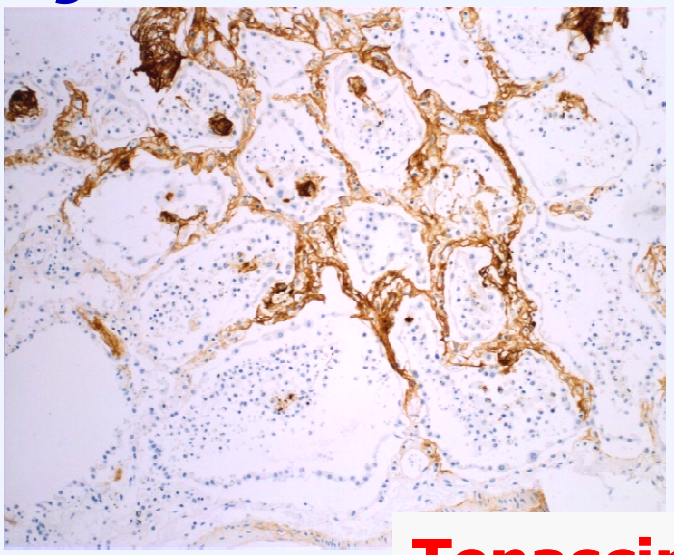
*These authors contributed equally to this work.



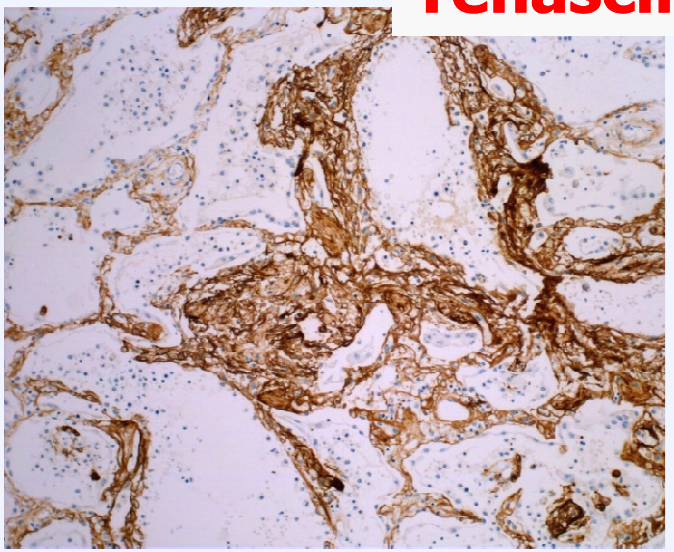
DAD

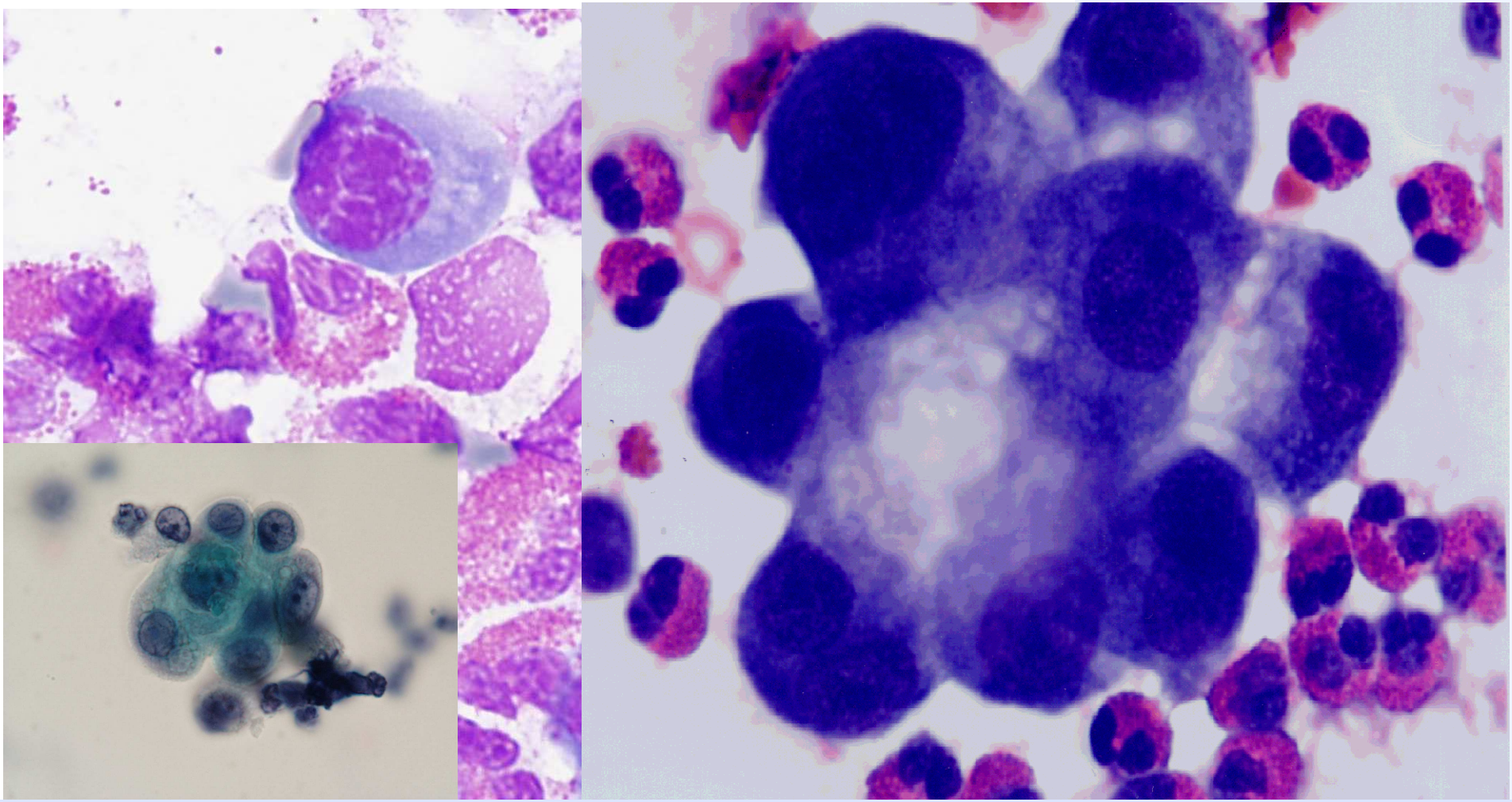
Pneumocyte damage, interstitial myofibroblastic activation and matrix remodeling





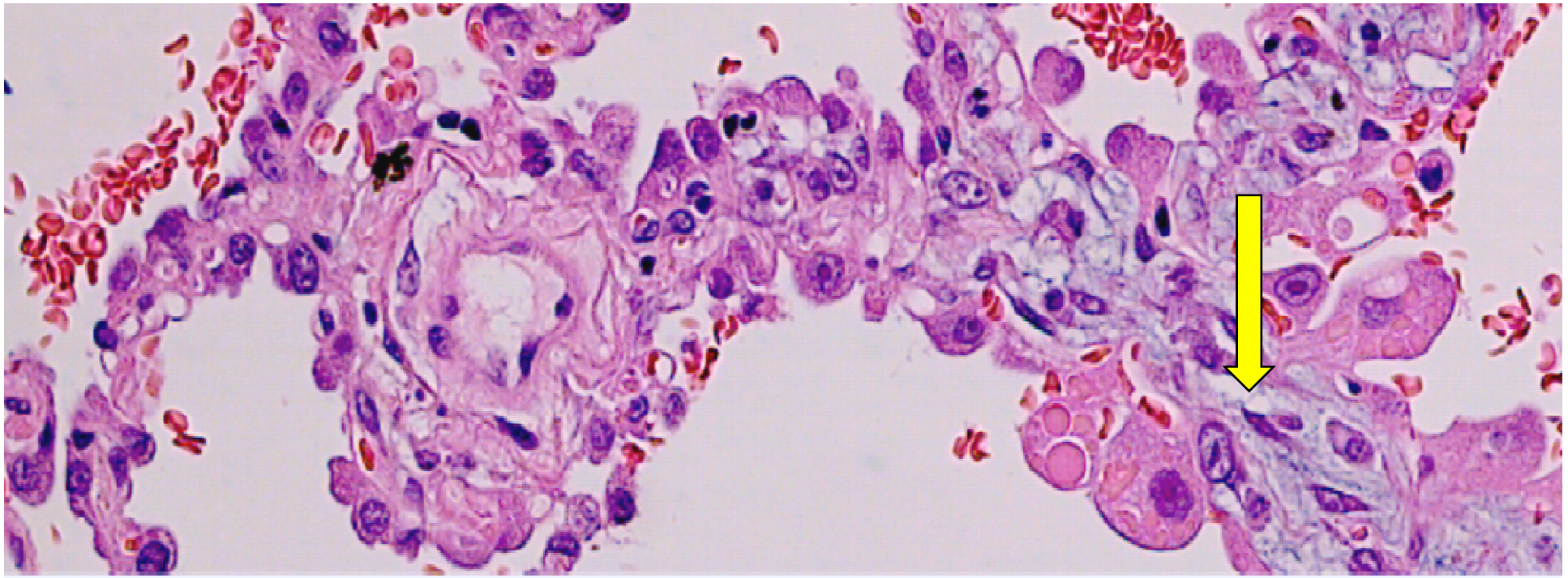
Tenascin





BRONCHOALVEOLAR LAVAGE SUGGESTING DIFFUSE ALVEOLAR DAMAGE IN A PATIENT WITH AEP.

Cells/ml=750,000; M=34%;L=6%;Eo=60%; CD4/CD8=1.31.
CD3+CD25+=39%; CD16+CD56+=18%; CD3+HLADR+=1%



Pneumocyte type II hyperplasia and atypia

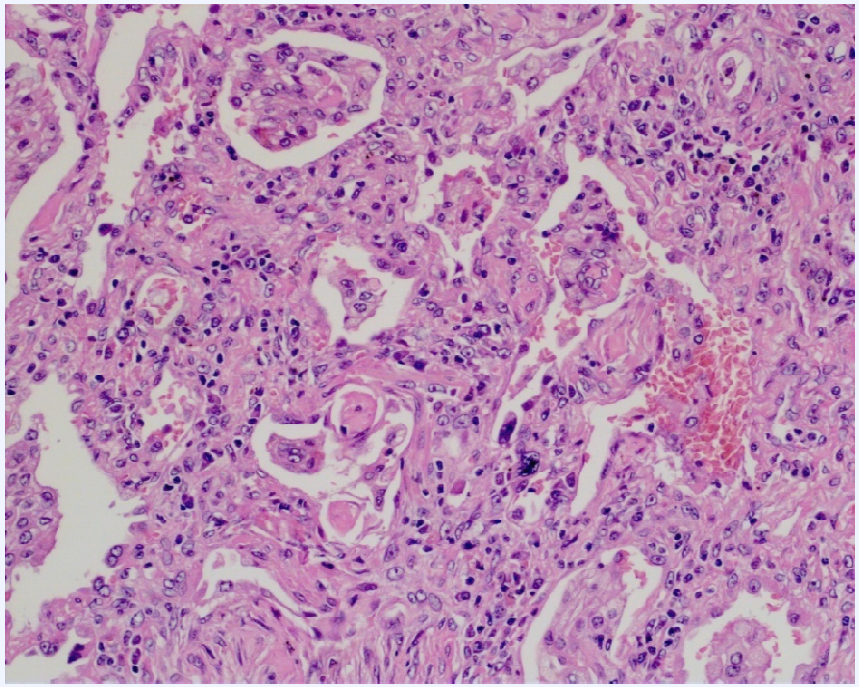
Targeted Injury of Type II Alveolar Epithelial Cells Induces Pulmonary Fibrosis

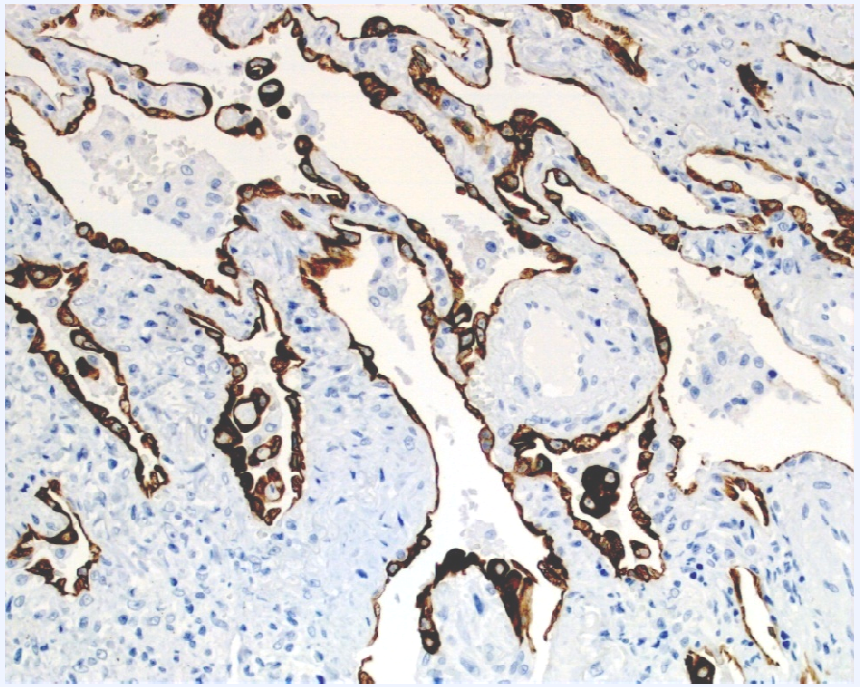
Am J Respir Crit Care Med Vol 181. pp 254–263, 2010

Thomas H. Sisson[1], Michael Mendez[2], Karen Choi[1], Natalya Subbotina[1], Anthony Courey[1], Andrew Cunningham[1], Aditi Dave[1], John F. Engelhardt[3], Xiaoming Liu[3], Eric S. White[1], Victor J. Thannickal[1], Bethany B. Moore[1], Paul J. Christensen[2], and Richard H. Simon[1]

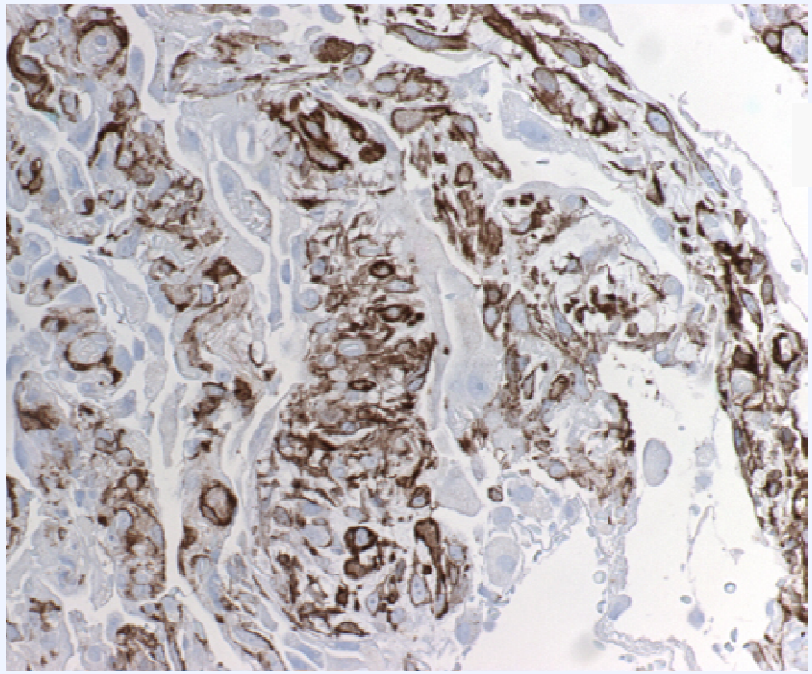
[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Hospital, Ann Arbor; [2]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Veterans Affairs Medical Center, Ann Arbor, Michigan; and [3]Department of Anatomy and Cell Biology, University of Iowa, Iowa City, Iowa

AIP-DAD

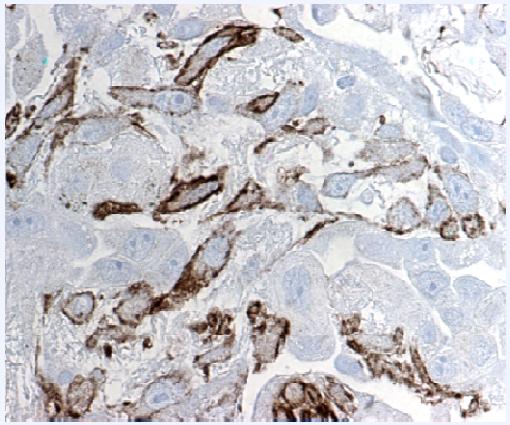


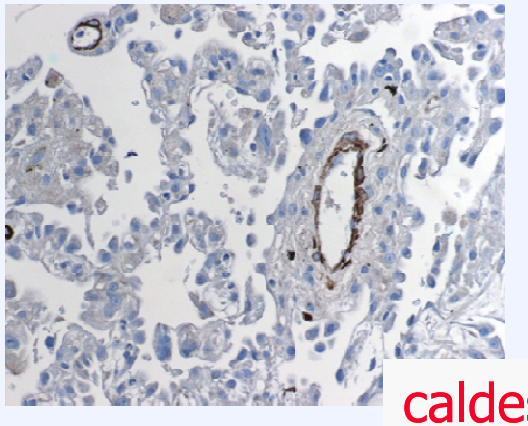


Myofibroblast interstitial accumulation

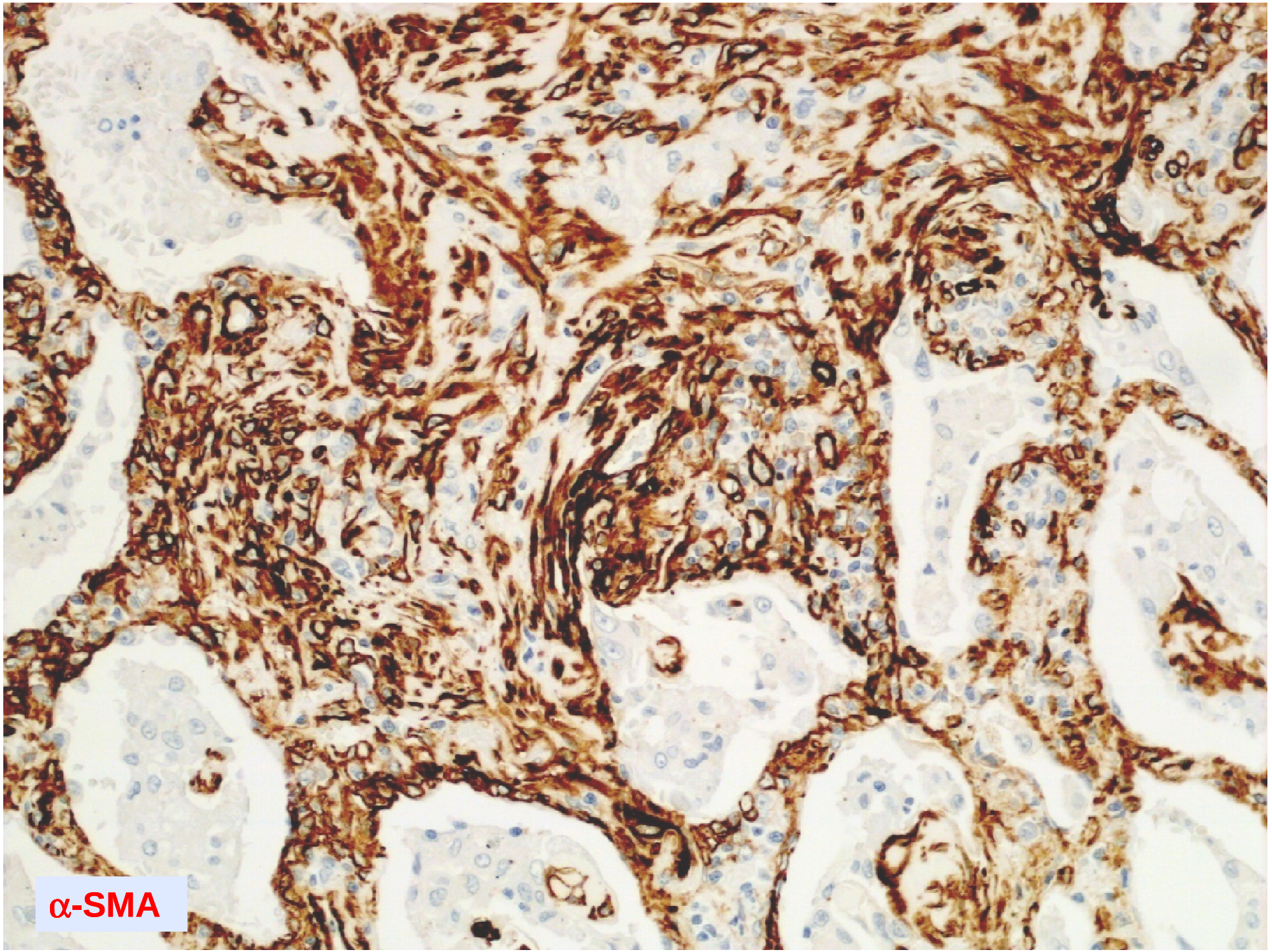


αSMA





caldesmon


α-SMA

Prognosis

Factors whose presence can be used to predict the risk of death at the time of diagnosis of acute lung injury and the acute respiratory distress syndrome include chronic liver disease, nonpulmonary organ dysfunction, sepsis, and advanced age.[6,7,10,30] Surprisingly, initial indexes of oxygenation and ventilation, including the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen and the lung-injury score, do not predict outcome. In three large studies, the mortality rate among patients with an initial ratio of partial pressure of arterial oxygen to fraction of inspired oxygen of 300 or less was similar to that among patients with a ratio of 200 or less.[6,7,30] However, the failure of pulmonary function to improve during the first week of treatment is a negative prognostic factor.[8]

Ware LB, Matthay MA. *The acute respiratory distress syndrome.*
N Engl J Med. 2000 May 4;342(18):1334-49.

Pulmonary Fibrosis Correlates With Outcome in Adult Respiratory Distress Syndrome*

A Study in Mechanically Ventilated Patients

Claude Martin, MD, FCCP†; Laurent Papazian, MD; Marie-Josée Payan, MD; Pierre Saux, MD; and François Gouin, MD

Study objective: The present study was carried out to evaluate the prognostic value of pulmonary fibrosis diagnosed on the basis of pulmonary samples obtained by fiberscopic transbronchial lung biopsy (TBLB) in patients treated for severe established adult respiratory distress syndrome (ARDS).
Design: Prospective cohort study.
Setting: Intensive Care Unit of a University Hospital.
Patients: Consecutive patients with a diagnosis of established ARDS.
Interventions: Samples of pulmonary tissue (3 to 6 in each patient) were obtained by fiberoptic TBLB. Severity of pulmonary fibrosis was assessed based on pathologic changes. Hematoxylin and eosin and Masson's trichrome stains were performed on each tissue sample.
Main results: Twenty-two lung specimens were obtained from 25 consecutive patients with ARDS of various origin (postsurgical complications, 7 patients; multiple trauma, 8 patients; medical problems, 7 patients). Transbronchial lung biopsy was complicated by small or moderate hemorrhage in three patients. No case of pneumothorax was identified. Pathologic findings showed that 14 patients (64%) had pulmonary fibrosis, either mild (9 patients) or moderate to severe fibrosis (5 patients). In the patients with pulmonary fibrosis, mortality rate was 57% (8 out of 14 patients), which was significantly different ($p < 0.02$) from the 0% mortality rate observed in patients without pulmonary fibrosis. Severity of pulmonary fibrosis (mild vs moderate and severe) did not influence outcome. With the exception of pathologic findings, characteristics of patients with and without pulmonary fibrosis (PaO_2, $PaCO_2$, the ratio of PaO_2 to fraction of inspired oxygen, and positive end-expiratory pressure) were not different.
Conclusion: In the study patients, pulmonary fibrosis diagnosed on the basis of TBLB was closely related to fatality in established ARDS.

(Chest 1995; 107:196-200)

Key words: adult respiratory distress syndrome (ARDS); fibrosis; transbronchial lung biopsy; prognosis

ARDS=adult respiratory distress syndrome; FIO_2=fraction of inspired oxygen; PEEP=positive end-expiratory pressure; TBLB=transbronchial lung biopsy

Role of open-lung biopsy in acute respiratory distress syndrome

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Current Opinion in Critical Care 2008, 14:75–79

Purpose of review

When classic examinations such as bronchoalveolar lavage are not contributory in the etiologic diagnosis of unresolving acute respiratory distress syndrome, surgical lung biopsy would appear to be useful to determine the specific cause, particularly infection or postaggressive fibrosis, which could benefit from an adapted treatment.

Recent findings

Postaggressive pulmonary fibrosis is a possible evolution for unresolving acute respiratory distress syndrome and its association with a poor prognosis has been demonstrated. The administration of corticoids would make it possible to improve certain ventilatory parameters as well as the prognosis in the fibroproliferation stage. No clinical or usual microbiological criterion can confirm both the existence of fibrosis and nosocomial pneumonia. Biological markers for fibrosis such as procollagen III are not validated to confirm the appearance of a postaggressive fibrosis. A recent study has shown that surgical lung biopsy performed in patients with unresolving acute respiratory distress syndrome led to a therapeutic modification in 78% of the cases and made it possible to avoid empiric corticotherapy in nearly 50% of the cases considering the absence of fibrosis.

Summary

Surgical lung biopsy could be proposed for patients with unresolving acute respiratory distress syndrome after 7–10 days of evolution despite well-conducted initial treatment when the etiology of acute respiratory distress syndrome has not been confirmed or when the appearance of postaggressive fibrosis is suspected.

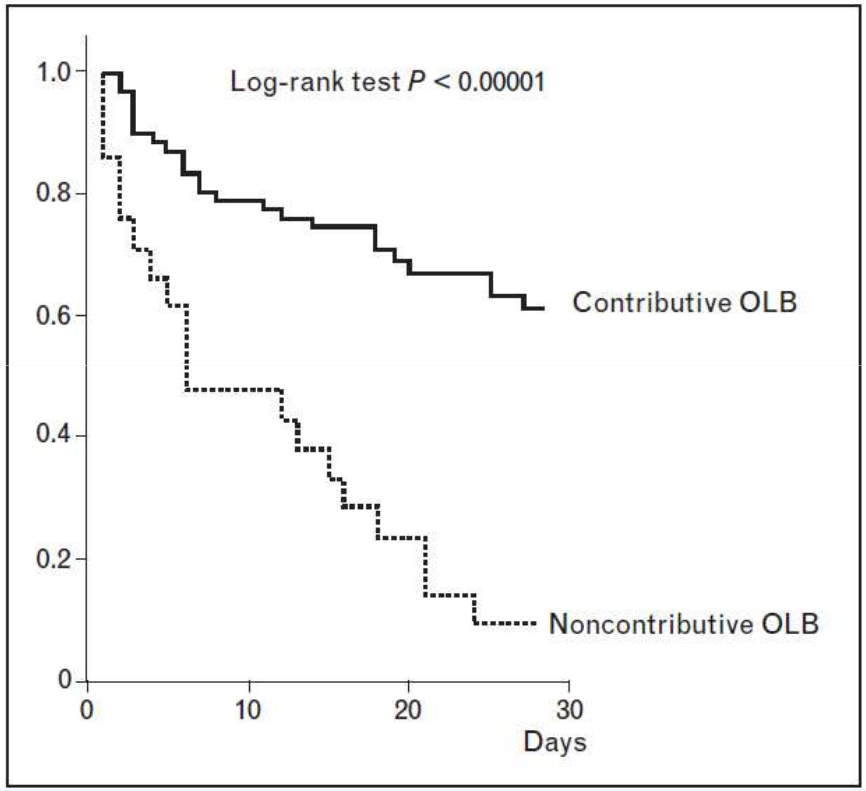
Clinical interest in surgical lung biopsy

In order to best treat unresolving ARDS (after making sure that prior treatment has been optimal with regard to plateau pressure, volemia, etc.), three questions must be asked.

1. ***Was the initial cause insufficiently treated?***
2. ***Is there an additional cause (nosocomial pneumonia)?***
3. ***Is postaggressive fibrosis developing?***

There are two essential reasons for the clinician to harvest a sample of the lung parenchyma: diagnose an etiology that is potentially curable when less-invasive examinations such as BAL were not contributory and/or reveal post-aggressive fibrosis in order to possibly administer anti-inflammatory treatment by corticoids which could improve survival or at least improve respiratory parameters .

Figure 1 Kaplan–Meier survival analysis comparing ARDS patients with a contributive OLB with ARDS patients with a noncontributive OLB

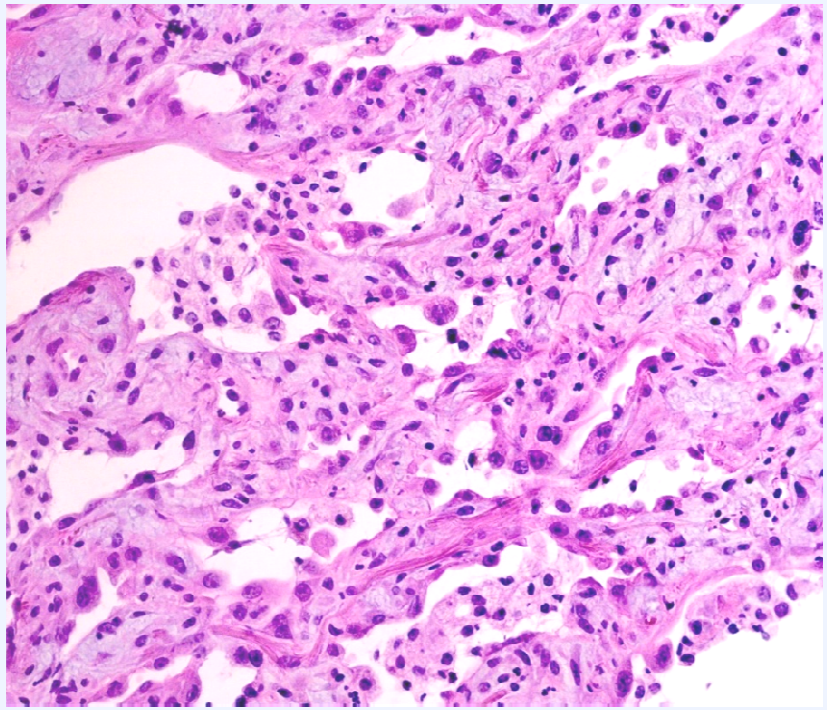


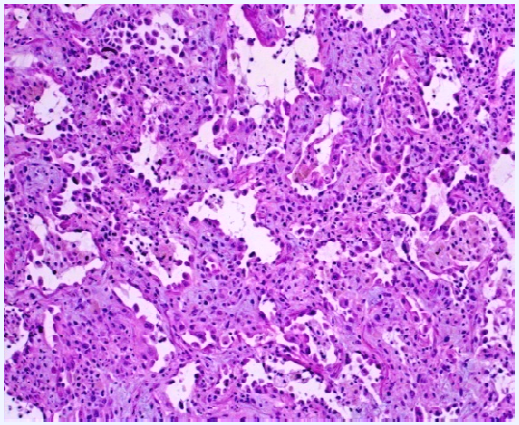
remains, pending less-invasive but equally specific examinations, the best diagnostic tool for unresolving ARDS.

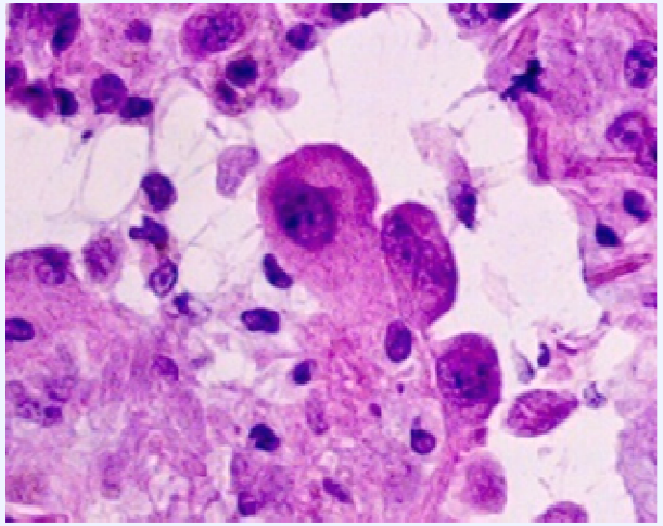
Conclusion

Unresolving ARDS presents the problem of evolving toward postaggressive fibrosis for which no noninvasive examination is both sensitive and specific enough. The classic strategy of administering corticosteroids on or about day 7 of the evolution of unresolving ARDS is hazardous in the absence of fibrosis and/or in the presence of infection. Surgical lung biopsy is the most specific examination for an etiologic diagnosis, and makes it possible to best adapt treatment and to avoid empiric corticotherapy which is sometimes useless and often dangerous. The contributory character of this biopsy, reported in the great majority of cases of unresolving ARDS, may be associated with better prognosis.

Frozen section analysis







Pathogenesis

These mechanisms differ according to the type of ARDS: primary with direct alveolar injury or secondary with indirect systemic injury.

The former essentially involves the alveolar epithelium and the latter, the endothelium.

Despite these physiopathological differences, the anatomopathological anomalies are quite similar.

Human Pathology (2009) 40, 1618–1627

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Original contribution

Two forms of diffuse alveolar damage in the lungs of patients with acute respiratory distress syndrome☆

Dedong Kang PhD, Tomoko Nakayama MD, PhD, Mayuko Togashi PhD, Masuki Yamamoto MD, PhD, Mikiko Takahashi MD, Shinobu Kunugi MD, PhD, Masamichi Ishizaki PhD, Yuh Fukuda MD, PhD*

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Received 17 November 2008; revised 31 March 2009; accepted 8 April 2009

Keywords:
Diffuse alveolar damage;
Multiple organ dysfunction Syndrome;
Myofibroblast

Summary Acute respiratory distress syndrome is a severe disease, the treatment and pathophysiology of which are not completely established. The pathology of acute respiratory distress syndrome involves diffuse alveolar damage, which comprises severe alveolar epithelial cell damage, hyaline membrane formation, and festinate myofibroblast proliferation and fibrosis in the intra-alveolar spaces. We performed a clinicopathologic investigation of 26 autopsy cases of diffuse alveolar damage. Three cases of them were diagnosed as acute interstitial pneumonia that is idiopathic illness and resembles pathologically organizing diffuse alveolar damage. Immunohistochemical staining for types I and IV collagen, α-smooth muscle actin, and Ki-67 was carried out, and the sites of myofibroblast proliferation and type I collagen production were examined. All diffuse alveolar damage cases in the proliferative phase showed intra-alveolar myofibroblast proliferation. When diffuse alveolar damage was diagnosed pathologically as being due to severe infection, all 7 patients showed multiple organ dysfunction syndrome, whereas only 2 of 7 patients showed interstitial myofibroblast proliferation. When diffuse alveolar damage was attributed to tumor treatment with chemotherapy or to drug toxicity, 3 of 16 patients showed multiple organ dysfunction syndrome; 15 of 16 showed interstitial myofibroblast proliferation, 3 of 3 acute interstitial pneumonia patients did not show multiple organ dysfunction syndrome; and 3 of 3 acute interstitial pneumonia showed marked interstitial myofibroblast proliferation. These results suggest that the pathophysiologic mechanism of diffuse alveolar damage caused by severe infection is one of systemic circulation disturbance, although the mechanism underlying diffuse alveolar damage due to tumor with chemotherapy or drug toxicity appears to involve interstitial pneumonia-like lesions that are similar to acute interstitial pneumonia.

Severe infection group:

Intraluminal myofibroblasts

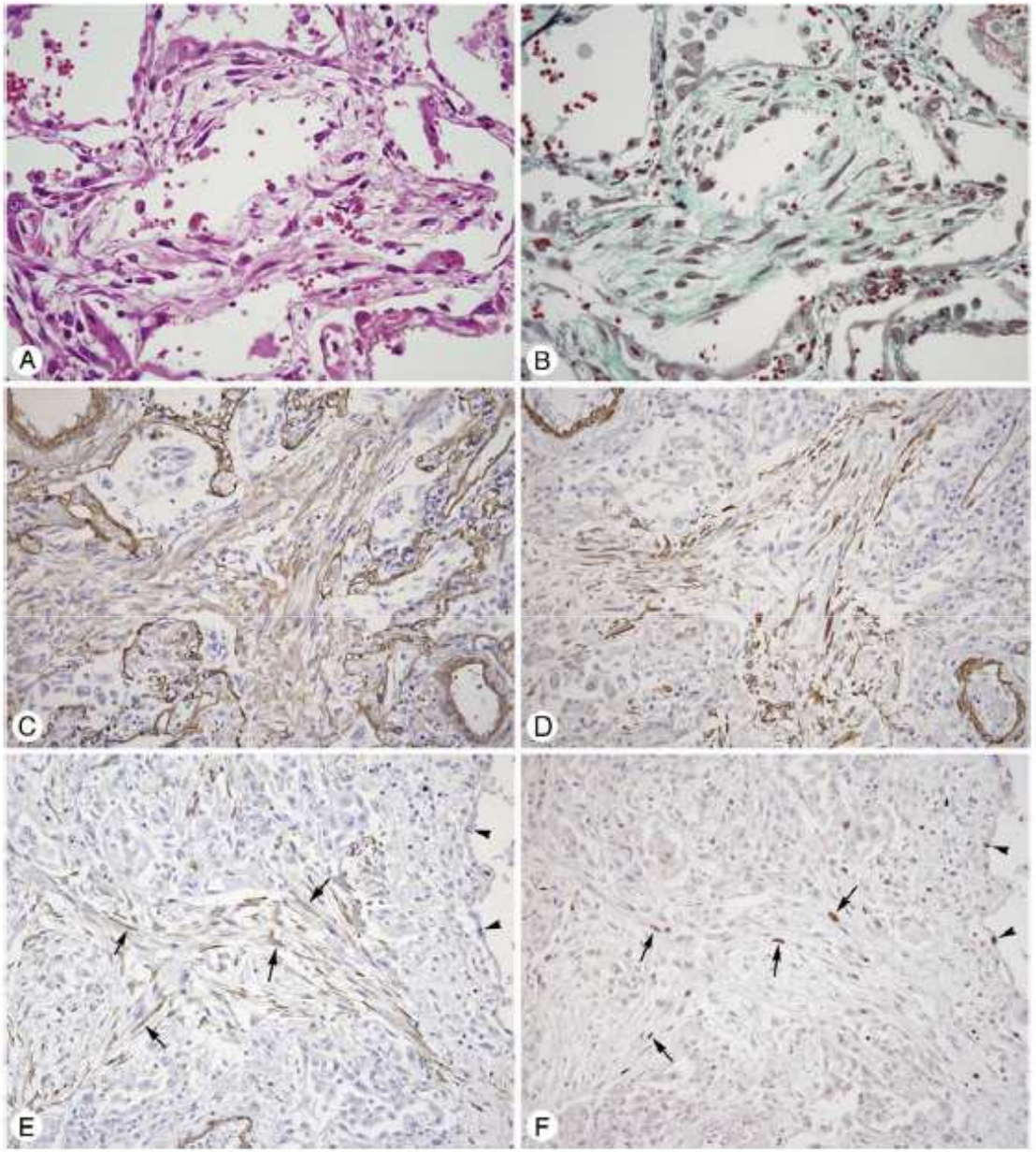


Fig. 1 Spindle-shaped cells appear prominently in the intra-alveolar spaces of cases from the severe infection group (A and B). Immunohistochemical staining of serial sections shows that many of the myofibroblasts that are positive for α-SMA (C) are located in the intra-alveolar space, as demonstrated by the distribution of type IV collagen (D). Some myofibroblasts (arrows) in the intra-alveolar space are positive for both α-SMA (E) and Ki-67 (F). Arrowheads show Ki-67-positive alveolar epithelial cells.

AIP

Interstitial myofibroblasts

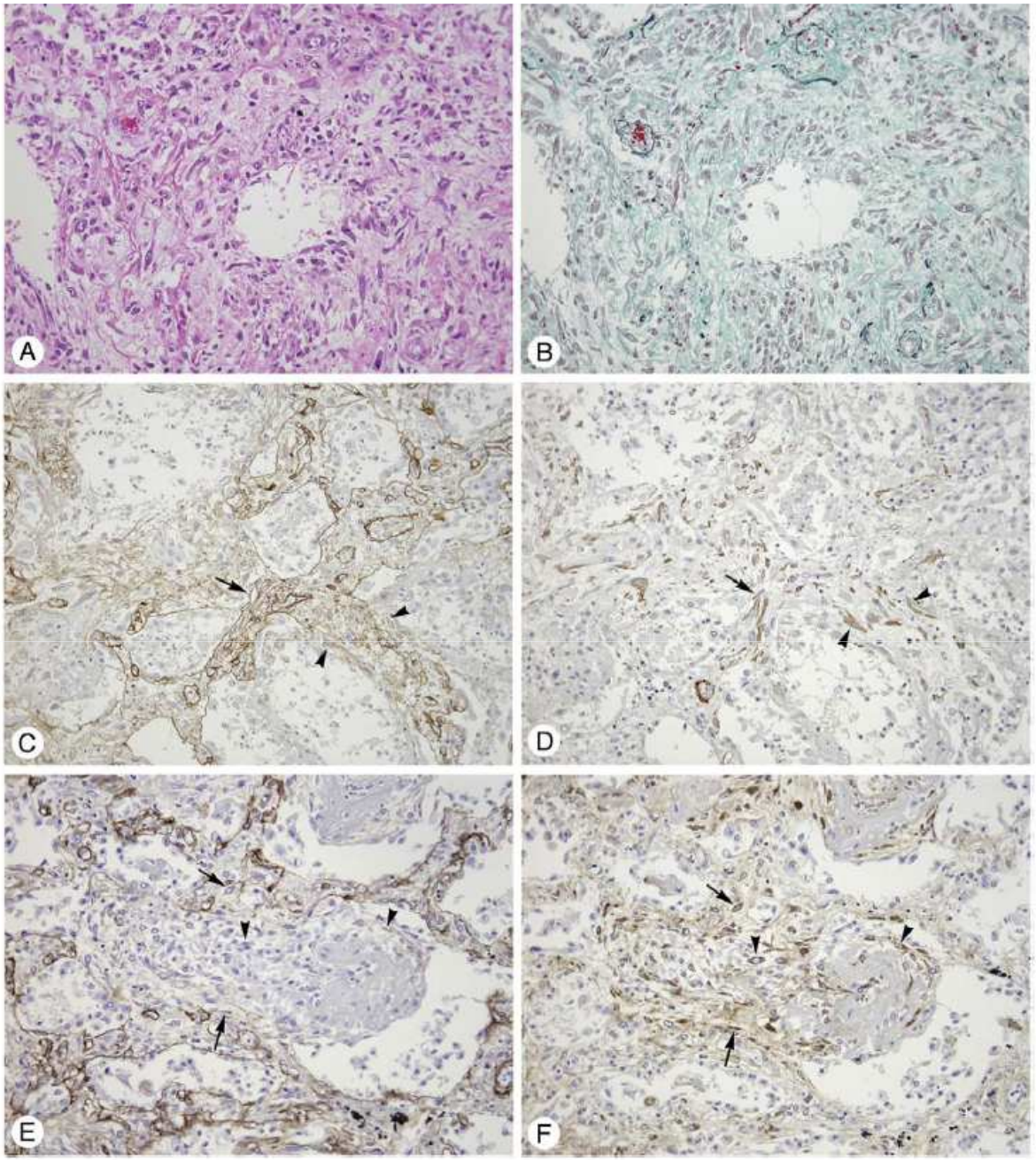


Fig. 3 In the AIP cases, many large spindle-shaped mesenchymal cells are found in the thickened alveolar interstitium (A and B). After serial section staining for type IV collagen and α-SMA (C and D), α-SMA-positive myofibroblasts (arrowheads) are located not only in the intra-alveolar space, but the myofobroblast (arrow) is also located in the interstitia of the alveolar wall. Some spindle-shaped cells in the intra-alveolar space (arrowheads) and the alveolar interstitium (arrows) also show the positive for the type I collagen in the serial sections (E and F).

Ware LB, Matthay MA. *The acute respiratory distress syndrome.* N Engl J Med. 2000 May 4;342(18):1334-49.

PATHOGENESIS

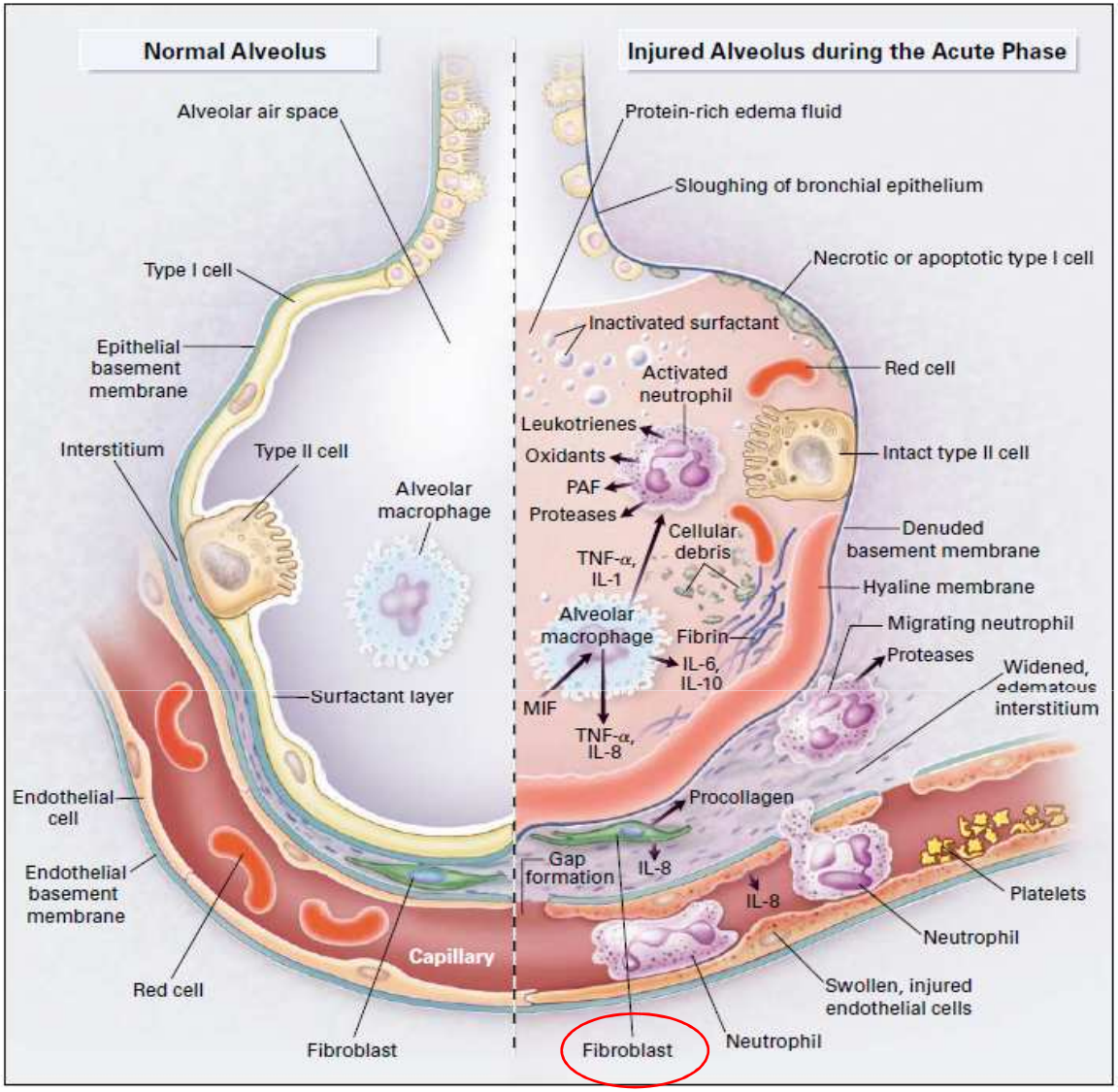
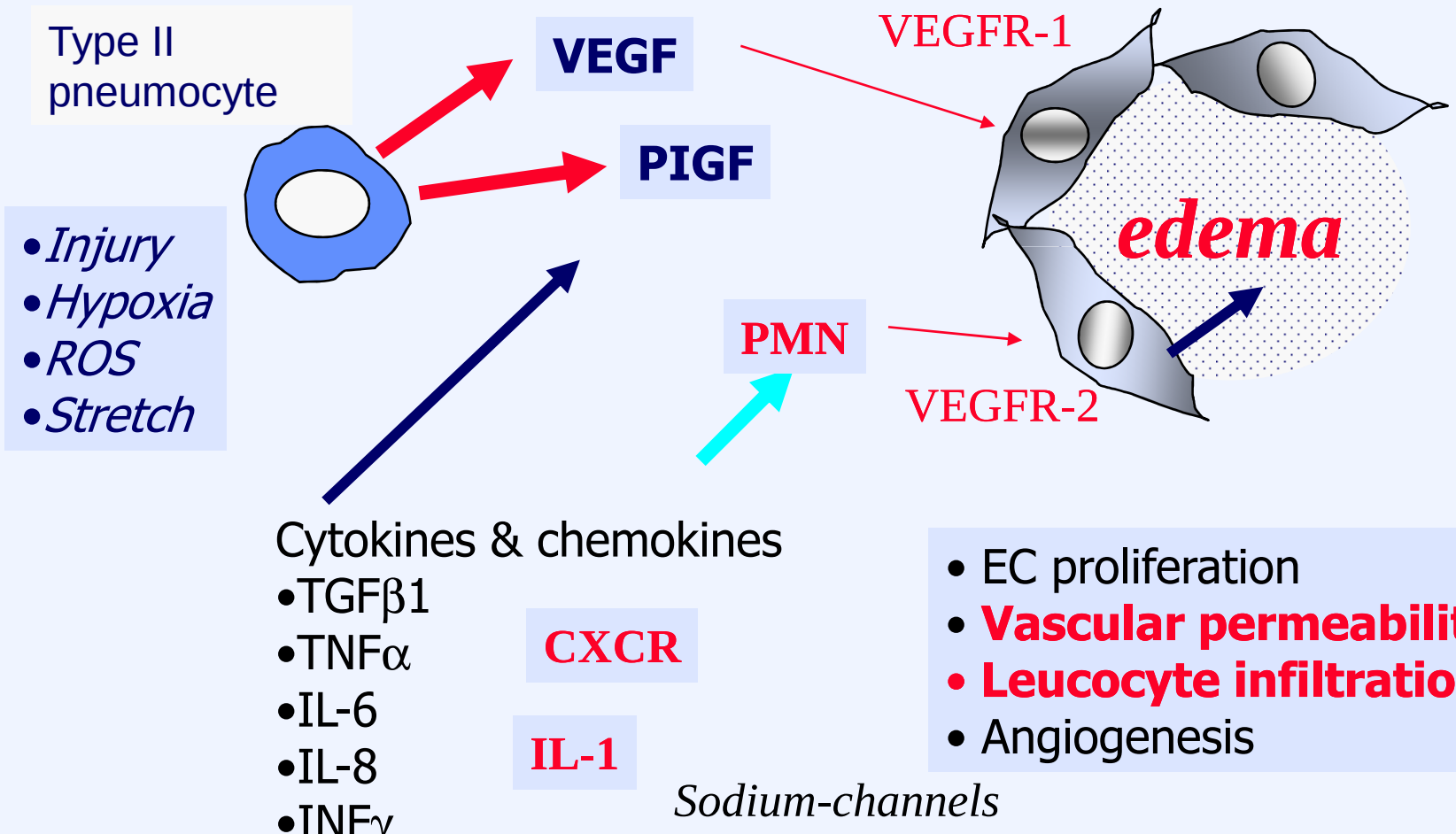


Figure 3. The Normal Alveolus (Left-Hand Side) and the Injured Alveolus in the Acute Phase of Acute Lung Injury and the Acute Respiratory Distress Syndrome (Right-Hand Side).

In the acute phase of the syndrome (right-hand side), there is sloughing of both the bronchial and alveolar epithelial cells, with the formation of protein-rich hyaline membranes on the denuded basement membrane. Neutrophils are shown adhering to the injured capillary endothelium and marginating through the interstitium into the air space, which is filled with protein-rich edema fluid. In the air space, an alveloar macrophage is secreting cytokines, interleukin-1, 6, 8, and 10, (IL-1, 6, 8, and 10) and tumor necrosis factor α (TNF-α), which act locally to stimulate chemotaxis and activate neutrophils. Macrophages also secrete other cytokines, including interleukin-1, 6, and 10. Interleukin-1 can also stimulate the production of extracellular matrix by fibroblasts. Neutrophils can release oxidants, proteases, leukotrienes, and other proinflammatory molecules, such as platelet-activating factor (PAF). A number of anti-inflammatory mediators are also present in the alveolar milieu, including interleukin-1–receptor antagonist, soluble tumor necrosis factor receptor, autoantibodies against interleukin-8, and cytokines such as interleukin-10 and 11 (not shown). The influx of protein-rich edema fluid into the alveolus has led to the inactivation of surfactant. MIF denotes macrophage inhibitory factor.

Molecular features in acute lung injury

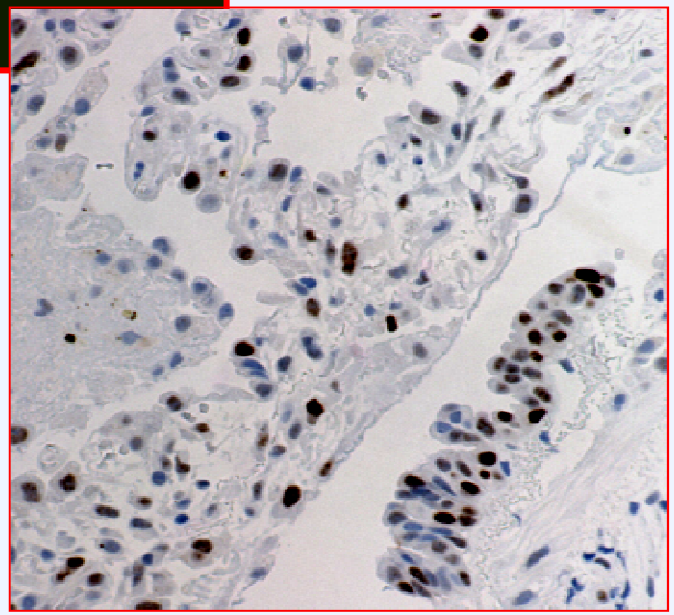


EPITHELIAL INJURY AND REPAIR

FREE RADICALS

Superoxide O_2^-

- H_2O_2
- OH^-
- $O_2^{\cdot}$



DNA Damage

p53

bcl-2

Bax

$p21^{waf1}$

Apoptosis

OPEN ACCESS Freely available online

PLoS GENETICS

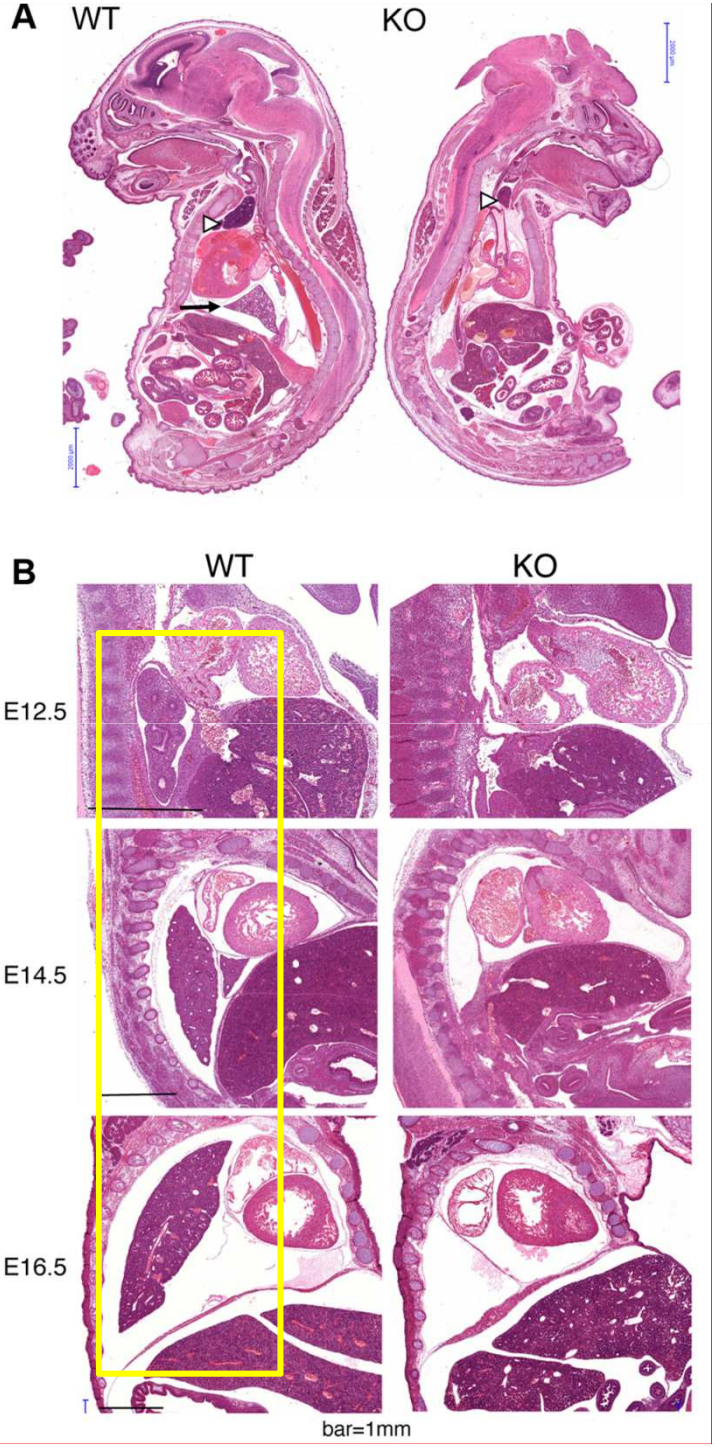
Dual Functions of ASCIZ in the DNA Base Damage Response and Pulmonary Organogenesis

Sabine Jurado[1,2☯], Ian Smyth[3☯], Bryce van Denderen[1,2☯], Nora Tenis[1], Andrew Hammet[1¤a], Kimberly Hewitt[1], Jane-Lee Ng[1], Carolyn J. McNees[1¤b], Sergei V. Kozlov[4], Hayato Oka[5¤c], Masahiko Kobayashi[6], Lindus A. Conlan[1], Timothy J. Cole[3], Ken-ichi Yamamoto[6], Yoshihito Taniguchi[5¤d], Shunichi Takeda[5], Martin F. Lavin[4,7], Jörg Heierhorst[1,2]*

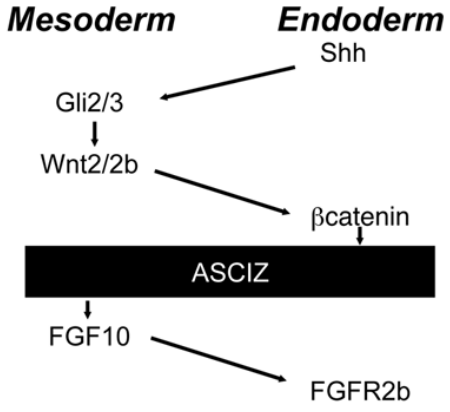
1 St. Vincent's Institute of Medical Research, Fitzroy, Australia, **2** Department of Medicine, St. Vincent's Hospital, The University of Melbourne, Fitzroy, Australia, **3** Department of Biochemistry and Molecular Biology and Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia, **4** Queensland Institute of Medical Research, Herston, Australia, **5** Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Kyoto, Japan, **6** Cancer Research Institute, Kanazawa University, Ishikawa, Japan, **7** Central Clinical Division, University of Queensland, Royal Brisbane Hospital, Herston, Australia

Abstract

Zn^{2+}-finger proteins comprise one of the largest protein superfamilies with diverse biological functions. The ATM substrate Chk2-interacting Zn^{2+}-finger protein (ASCIZ; also known as ATMIN and ZNF822) was originally linked to functions in the DNA base damage response and has also been proposed to be an essential cofactor of the ATM kinase. Here we show that absence of ASCIZ leads to *p53*-independent late-embryonic lethality in mice. *Asciz*-deficient primary fibroblasts exhibit increased sensitivity to DNA base damaging agents MMS and H_2O_2, but *Asciz* deletion or knock-down does not affect ATM levels and activation in mouse, chicken, or human cells. Unexpectedly, *Asciz*-deficient embryos also exhibit severe respiratory tract defects with complete pulmonary agenesis and severe tracheal atresia. Nkx2.1-expressing respiratory precursors are still specified in the absence of ASCIZ, but fail to segregate properly within the ventral foregut, and as a consequence lung buds never form and separation of the trachea from the oesophagus stalls early. Comparison of phenotypes suggests that ASCIZ functions between Wnt2-2b/ß-catenin and FGF10/FGF-receptor 2b signaling pathways in the mesodermal/endodermal crosstalk regulating early respiratory development. We also find that ASCIZ can activate expression of reporter genes via its SQ/TQ-cluster domain *in vitro*, suggesting that it may exert its developmental functions as a transcription factor. Altogether, the data indicate that, in addition to its role in the DNA base damage response, ASCIZ has separate developmental functions as an essential regulator of respiratory organogenesis.



	lung	trachea	oesophagus
Gli 2/3	-	-	-
Wnt 2/2b	-	-	+
Shh-Cre βcatenin	-	-	+
ASCIZ	-	(+)	+
FGF10	-	+	+
FGFR2b	-	+	+



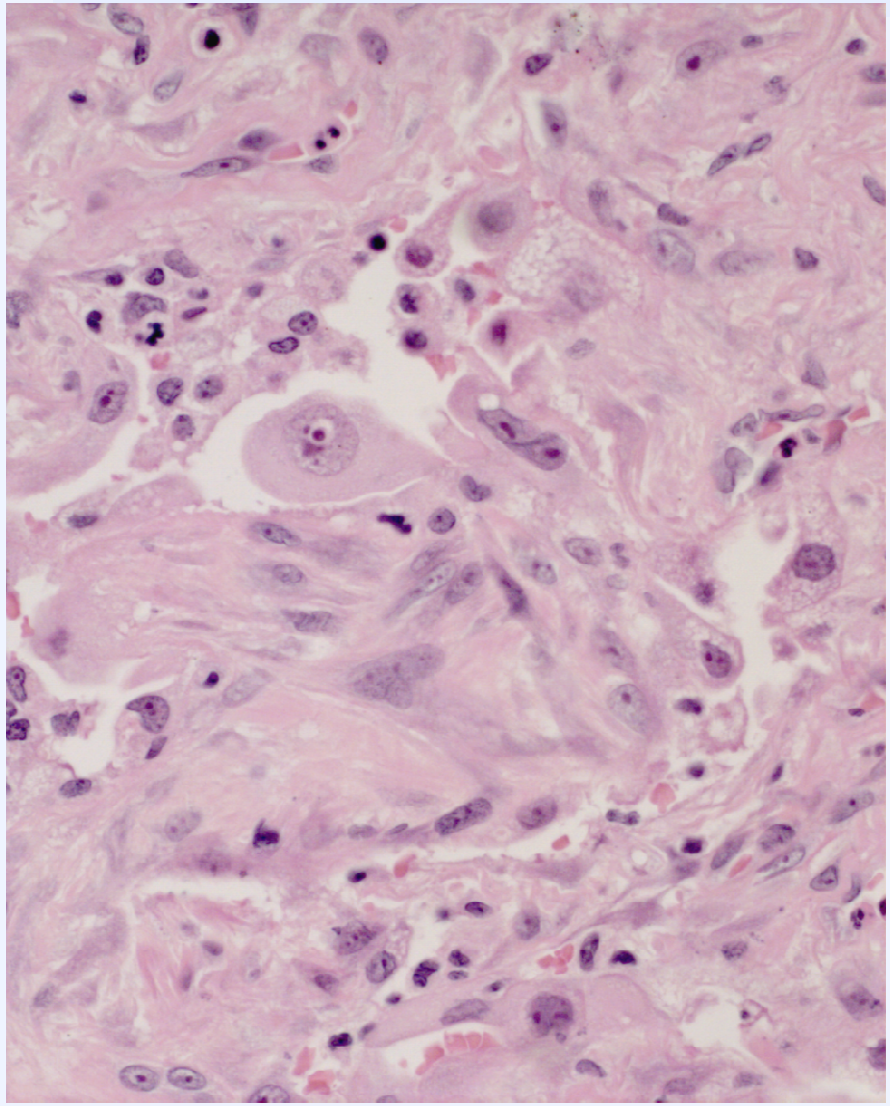
Keratinocyte Growth Factor Reduces Lung Damage Due to Acid Instillation in Rats

Toshiyuki Yano, Robin R. Deterding, W. Scott Simonet, John M. Shannon, and Robert J. Mason

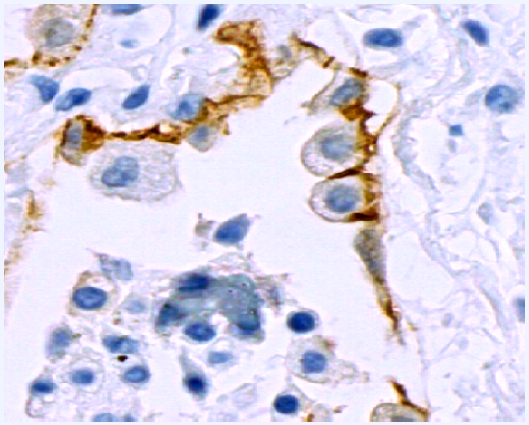
Departments of Medicine and Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado; and Amgen, Inc., Thousand Oaks, California

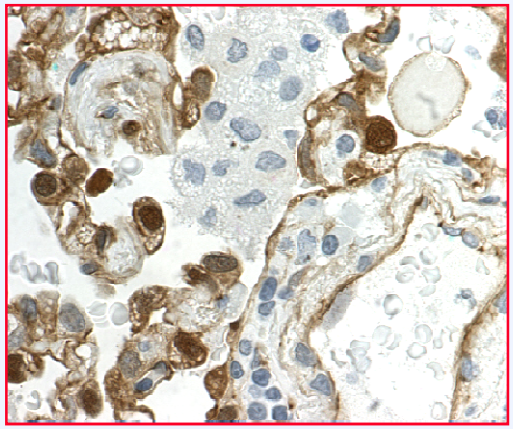
Acid aspiration is a serious complication of anesthesia and other forms of unconsciousness that can result in the adult respiratory distress syndrome (ARDS), which continues to have a very high mortality despite our current therapeutic interventions. This type of injury damages the alveolar epithelium, principally alveolar type I cells, and requires proliferation of alveolar type II cells to restore gas exchange units. Since keratinocyte growth factor (KGF) has been shown to be a potent mitogen for alveolar type II cells, we evaluated whether intrabronchial administration of KGF would minimize lung injury due to the unilateral instillation of 0.1 N hydrochloric acid (HCl). Rats were pretreated or post-treated by intrabronchial instillation of KGF (5 mg/kg) into the left lung before HCl instillation. All rats receiving KGF at 48 or 72 h before HCl instillation survived for the 7-day observation period, whereas the mortality rate for those receiving HCl alone or saline followed by HCl was 31% and 33%, respectively. Pretreatment with KGF at 72 h but not at 24 or 48 h considerably ameliorated morphologic damage produced by HCl. Inflammatory cells in bronchoalveolar lavage were markedly decreased 3 and 7 days after HCl instillation by the 72-h KGF pretreatment. Pretreatment with KGF at 72 h also attenuated the reduction of total lung capacity, decreased the α_1(I) procollagen mRNA levels, and diminished hydroxyproline accumulation due to HCl instillation. Saline pretreatment at 72 h had no significant effect on the HCl injury and subsequent physiologic abnormalities. Our attempts to improve outcome with post-treatment instillation of KGF were unsuccessful. We conclude that KGF pretreatment reduces lung injury due to acid instillation and can prevent subsequent pulmonary fibrosis. **Yano, T., R. R. Deterding, W. S. Simonet, J. M. Shannon, and R. J. Mason. 1996. Keratinocyte growth factor reduces lung damage due to acid instillation in rats. Am. J. Respir. Cell Mol. Biol. 15:433–442.**

Alveolar damage/repair



E-cadherin

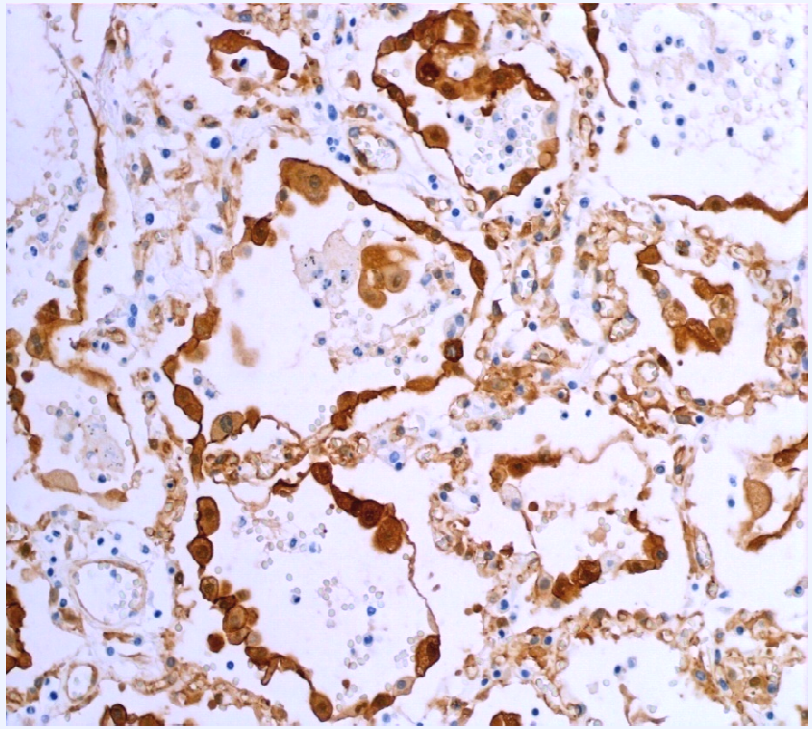


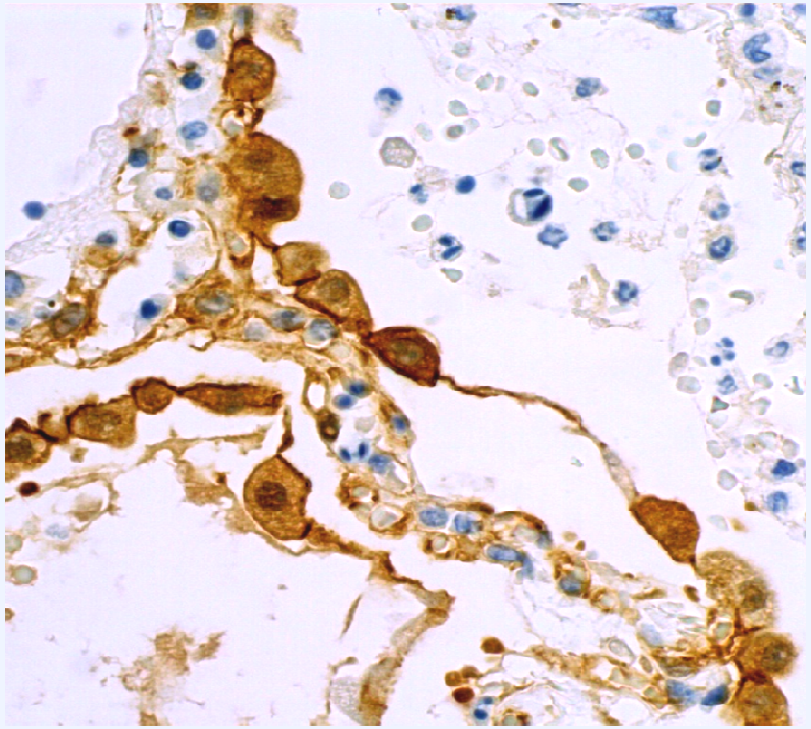


β-catenin - wnt

WNT-pathway activation

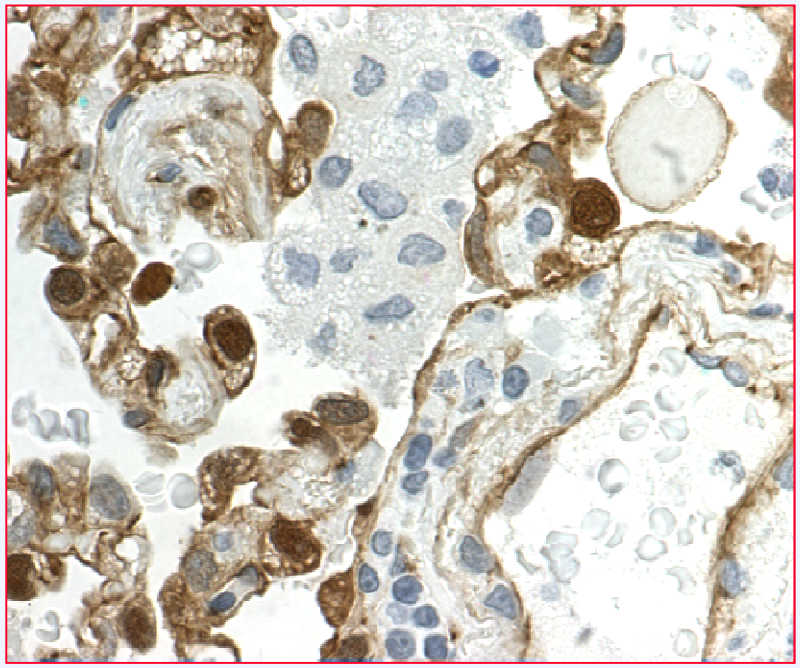
Nuclear localisation of β-catenin in regenerating pneumocytes





Isothiocyanate - DAD

b-catenin nuclear accumulation in type-II pneumocytes



β-catenin → • Cyclin-D1 • MMP7 • *c-myc* → Proliferation Apoptosis

Am J Pathol 2003 May;162(5):1495-502 Aberrant Wnt/beta-Catenin Pathway Activation in Idiopathic Pulmonary Fibrosis.

Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P, Pedron S, Bertaso M, Scarpa A, Murer B, Cancellieri A, Maestro R, Semenzato G, Doglioni C. Department of Pathology, University of Verona, Verona.

effects

Stem Cells. 2008 May ; 26(5): 1337–1346. doi:10.1634/stemcells.2008-0053.

Conditional Stabilization of β-Catenin Expands the Pool of Lung Stem Cells

Susan D. Reynolds[a], **Anna C. Zemke**[a], **Adam Giangreco**[a], **Brian L. Brockway**[a], **Roxana M. Teisanu**[a], **Jeffrey A. Drake**[a], **Thomas Mariani**[b], **Peter Y.P. Di**[a], **Mark M. Taketo**[c], and **Barry R. Stripp**[a]

[a]Center for Lung Regeneration, Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Abstract

Maintenance of classic stem cell hierarchies is dependent upon stem cell self-renewal mediated in part by Wnt/β-catenin regulation of the cell cycle. This function is critical in rapidly renewing tissues due to the obligate role played by the tissue stem cell. However, the stem cell hierarchy responsible for maintenance of the conducting airway epithelium is distinct from classic stem cell hierarchies. The epithelium of conducting airways is maintained by transit-amplifying cells in the steady state; rare bronchiolar stem cells are activated to participate in epithelial repair only following depletion of transit-amplifying cells. Here, we investigate how signaling through β-catenin affects establishment and maintenance of the stem cell hierarchy within the slowly renewing epithelium of the lung. Conditional potentiation of β-catenin signaling in the embryonic lung results in amplification of airway stem cells through attenuated differentiation rather than augmented proliferation. Our data demonstrate that the differentiation-modulating activities of stabilized β-catenin account for expansion of tissue stem cells.

Published in final edited form as:
Cell Stem Cell. 2009 September 4; 5(3): 279–289. doi:10.1016/j.stem.2009.06.017.

mTOR Mediates Wnt-Induced Epidermal Stem Cell Exhaustion and Aging

R. M. Castilho[1], **C. H. Squarize**[1], **L. A. Chodosh**[2], **B. O. Williams**[3], and **J. Silvio Gutkind**[1,*]

[1] Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA

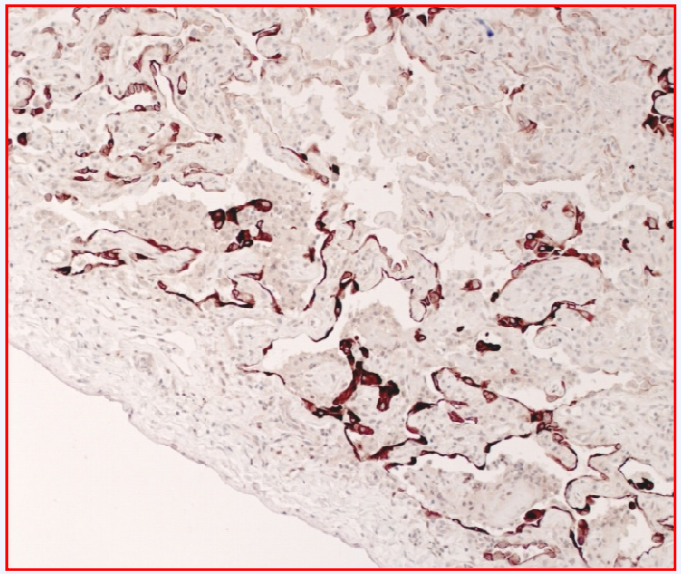
[2] Department of Cancer Biology and Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6160

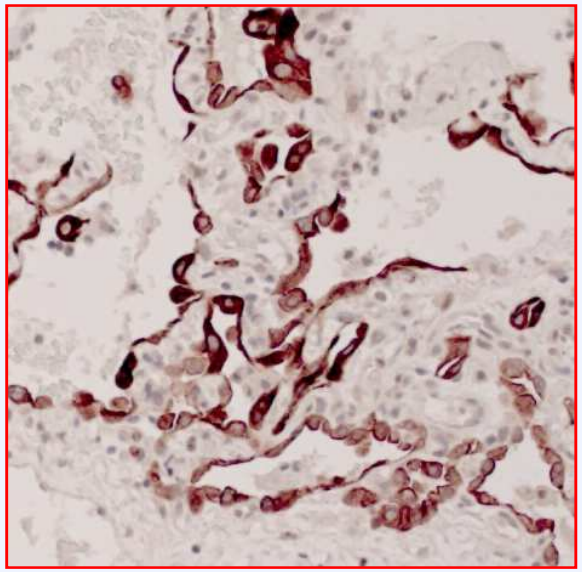
[3] Laboratory of Cell Signaling and Carcinogenesis, Van Andel Research Institute, Grand Rapids, Michigan 49503-2518, USA

Abstract

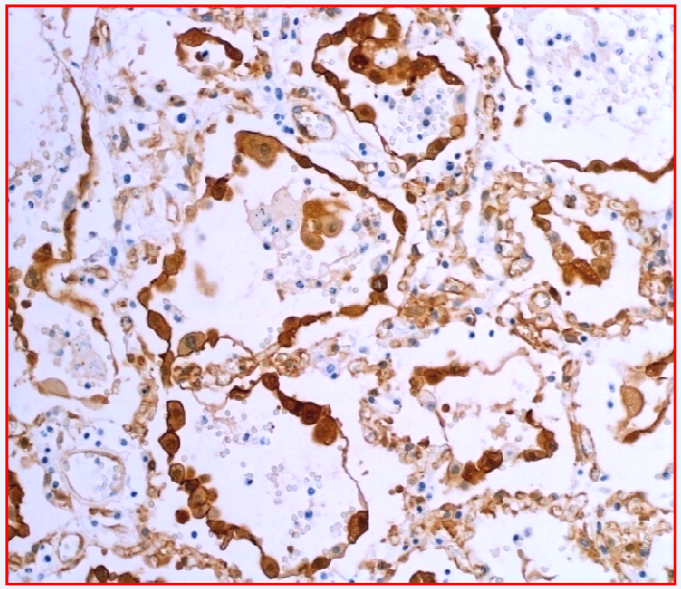
Epidermal integrity is a complex process established during embryogenesis and maintained throughout the organism lifespan by epithelial stem cells. While Wnt regulates normal epithelial stem cell renewal, aberrant Wnt signaling can contribute to cancerous growth. Here, we explored the consequences of persistent expressing Wnt1 in an epidermal compartment that includes the epithelial stem cells. Surprisingly, Wnt caused the rapid growth of the hair follicles, but this was followed by epithelial cell senescence, disappearance of the epidermal stem cell compartment, and progressive hair loss. While Wnt1 induced the activation of β-catenin and the mTOR pathway, both hair follicle hyperproliferation and stem cell exhaustion were strictly dependent on mTOR function. These findings suggest that whereas activation of β-catenin contributes to tumor growth, epithelial stem cells may be endowed with a protective mechanism that results in cell senescence upon the persistent stimulation of proliferative pathways that activate mTOR, ultimately suppressing tumor formation.

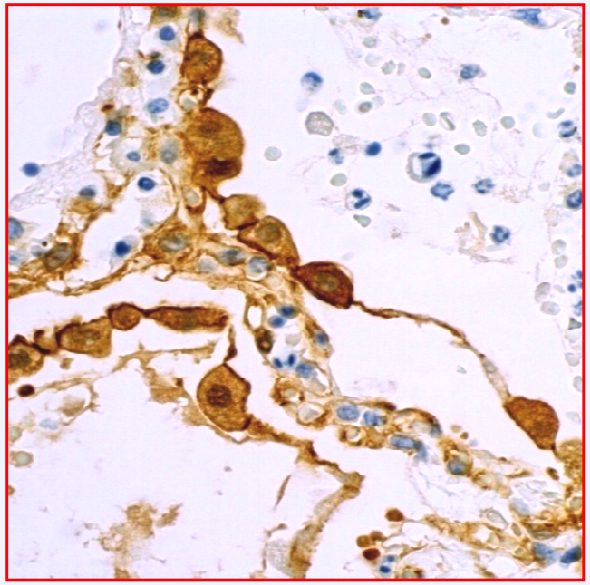
DAD/ARDS





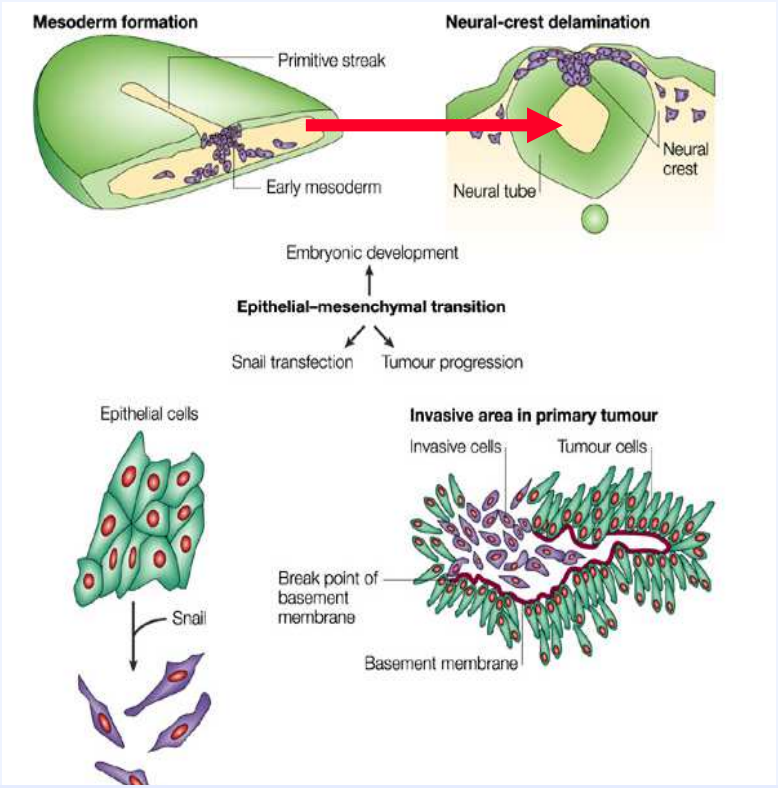
ph-mTOR

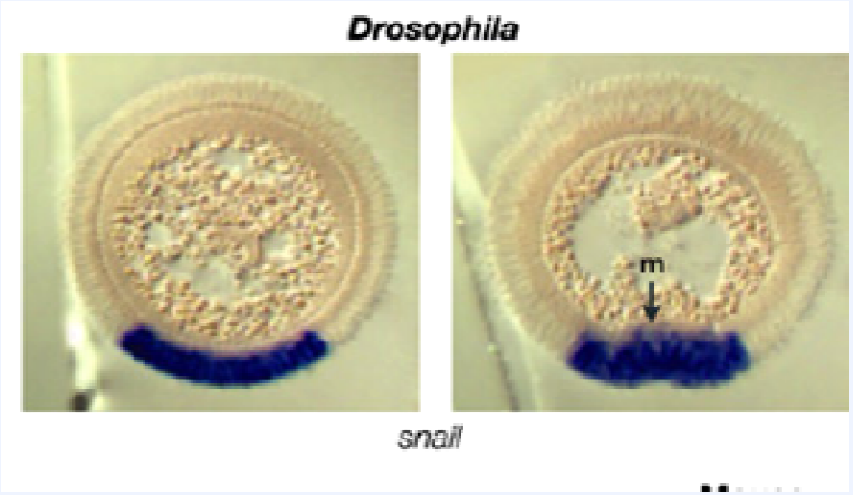




beta-Catenin

EMT in embriogenesis





J Biol Chem. 2003 Oct 10;278(41):40231-8.

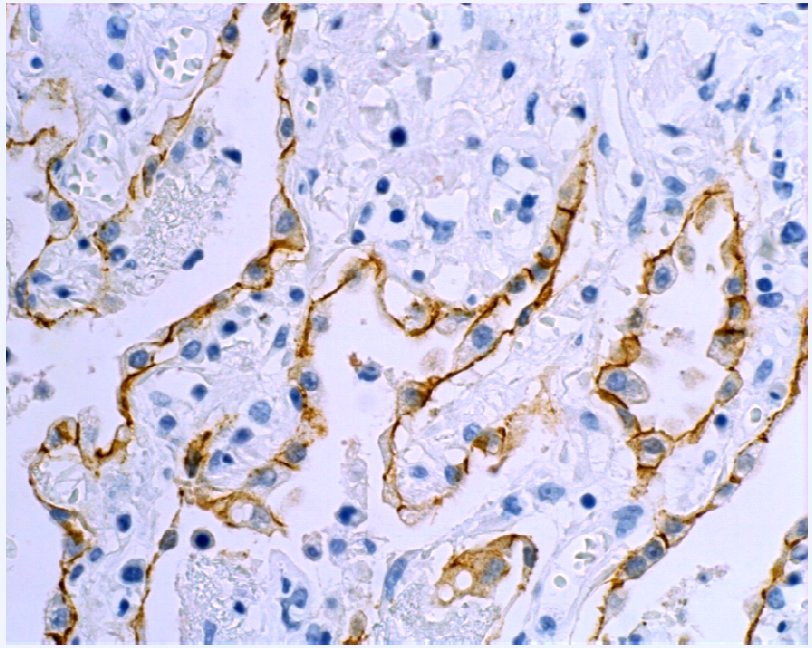
β-Catenin is required for specification of proximal/distal cell fate during lung morphogenesis. *Mucenski ML, Wert SE, Nation JM, Loudy DE, Huelsken J, Birchmeier W, Morrisey EE, Whitsett JA.* Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229-3039, USA.

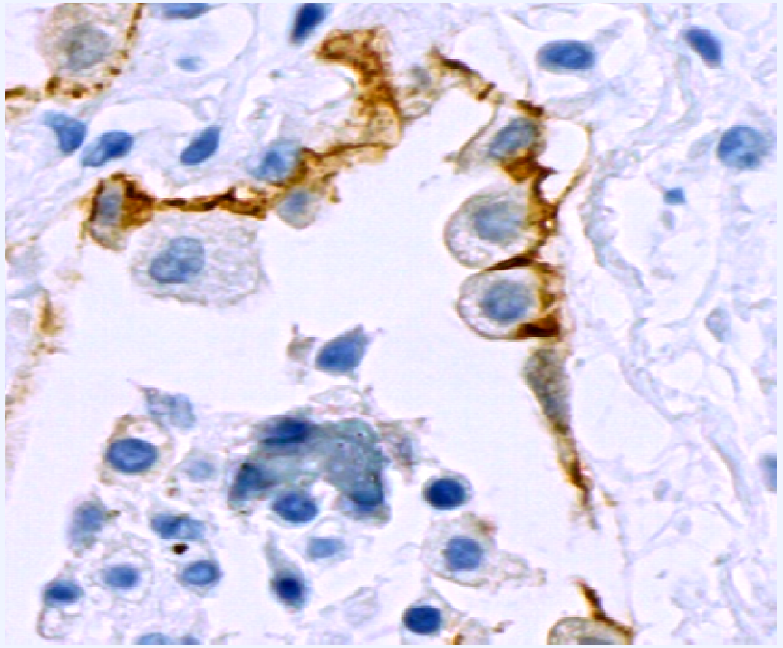
Markers of Acute lung injury

MOLECULAR MARKERS (in pneumocytes)

- LAM-5 γ-2chain
- hsp27
- P53-p21waf1
- β-catenin
- TA-p63
- Tubulin β3
- Ph-mTOR

Epithelial Mesenchymal Transition



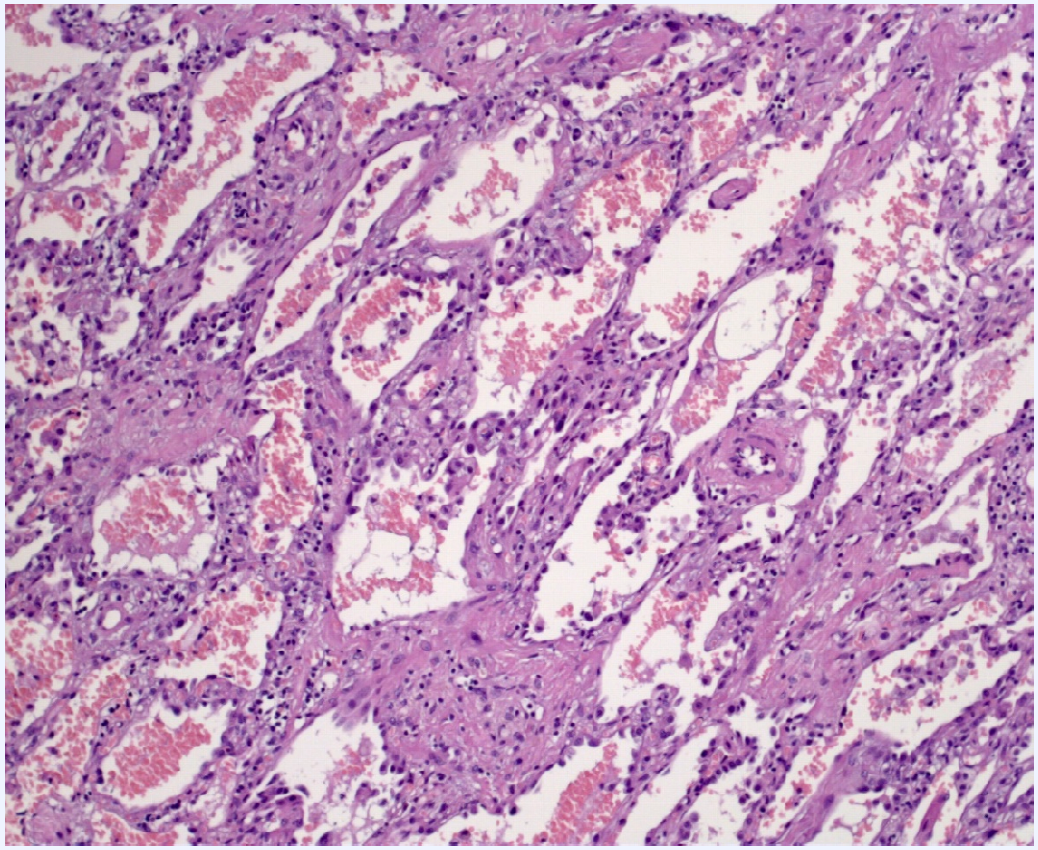


E-cadherin

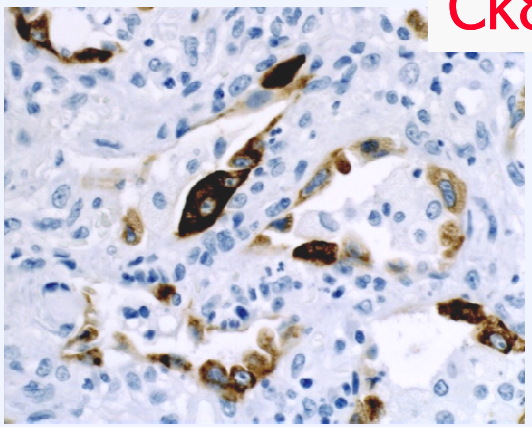
Potential mechanisms for myofibroblast recruitment

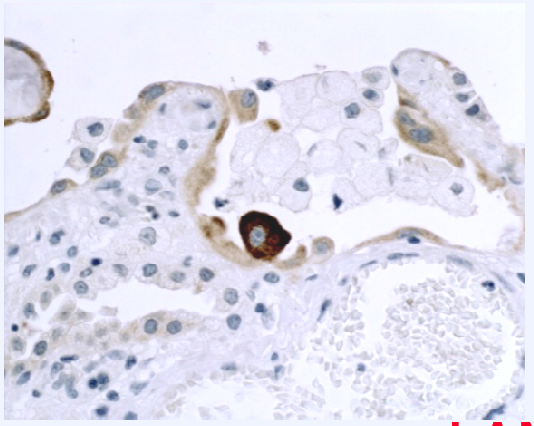
1. Local fibroblasts: proliferation, migration, activation
2. MSC recruitment from bone marrow
3. Epithelial-Mesenchymal Transition (EMT)

Artrite reumatoide
#04-3324

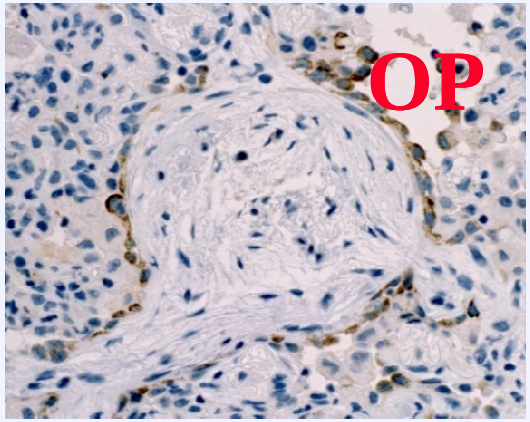


Ck8/18

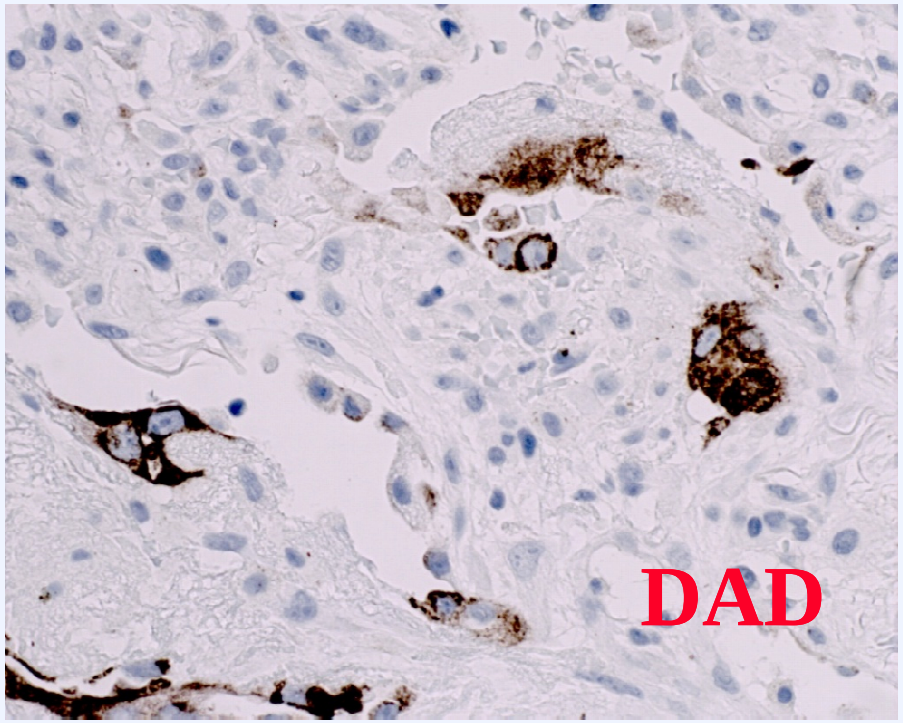


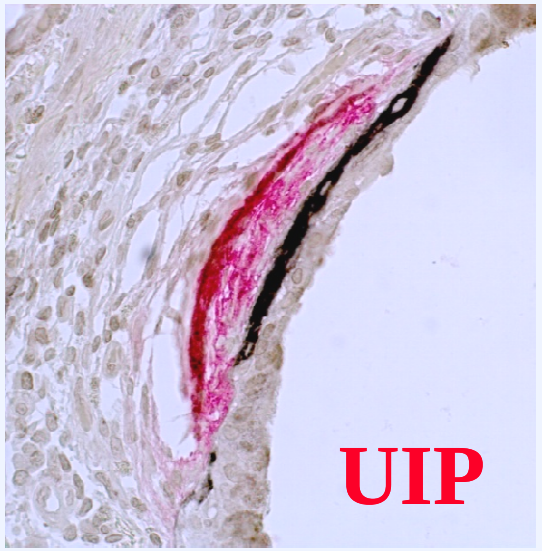


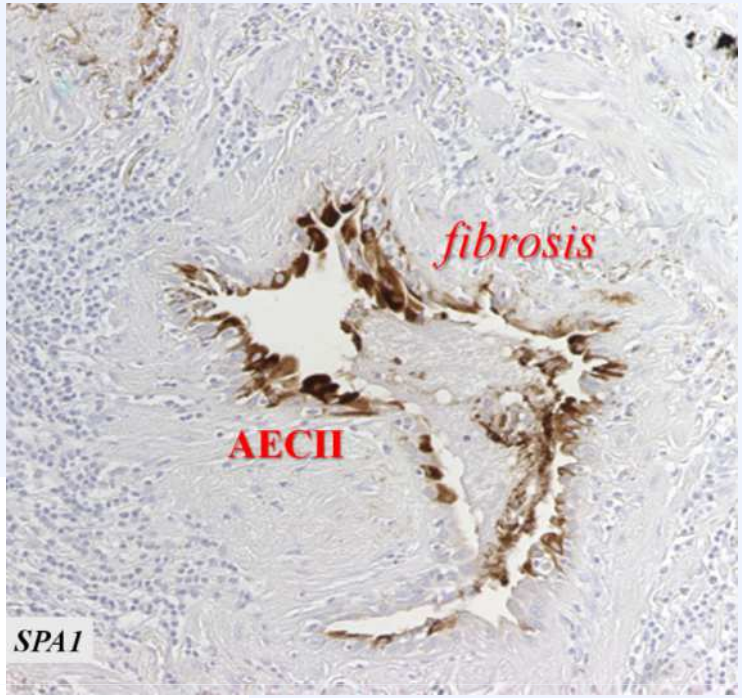
LAM5γ2



LAM5-2γ







Expression of Apoptotic and Antiapoptotic Markers in Epithelial Cells in Idiopathic Pulmonary Fibrosis*

Maria Plataki, MD; Anastassios V. Koutsopoulos, MD; Katherine Darivianaki, BS; George Delides, MD, PhD; Nikolaos M. Siafakas, MD, PhD, FCCP; and Demosthenes Bouros, MD, FCCP

CHEST 2005;127:266

Targeted Injury of Type II Alveolar Epithelial Cells Induces Pulmonary Fibrosis

Am J Respir Crit Care Med Vol 181. pp 254–263, 2010

Thomas H. Sisson[1], Michael Mendez[2], Karen Choi[1], Natalya Subbotina[1], Anthony Courey[1], Andrew Cunningham[1], Aditi Dave[1], John F. Engelhardt[3], Xiaoming Liu[3], Eric S. White[1], Victor J. Thannickal[1], Bethany B. Moore[1], Paul J. Christensen[2], and Richard H. Simon[1]

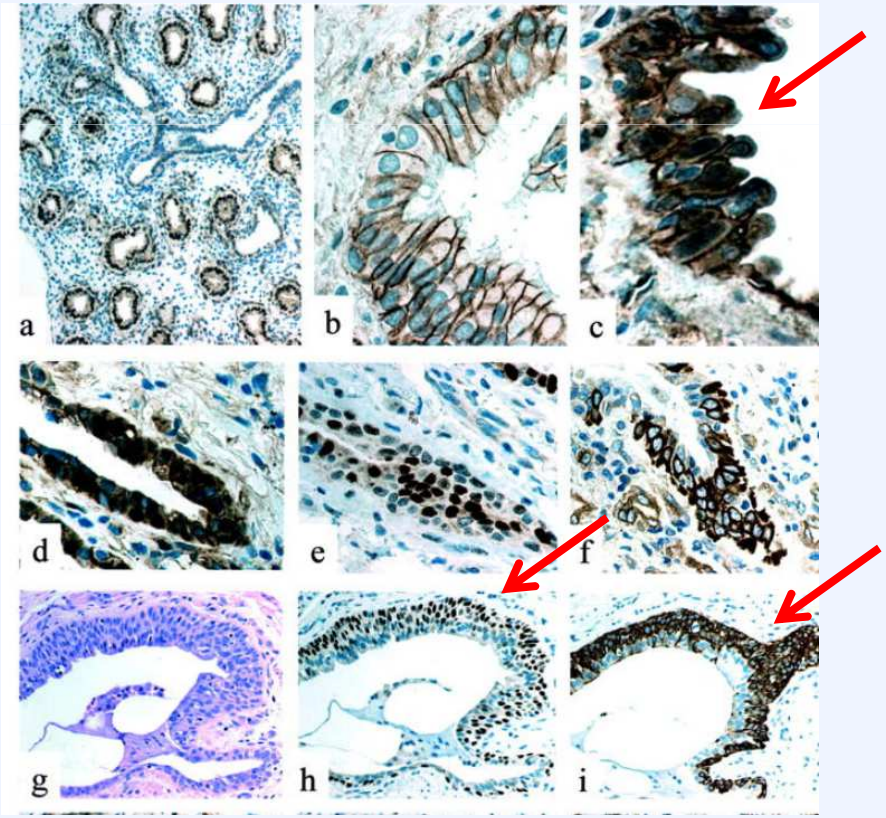
[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Hospital, Ann Arbor; [2]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Veterans Affairs Medical Center, Ann Arbor, Michigan; and [3]Department of Anatomy and Cell Biology, University of Iowa, Iowa City, Iowa

Am J Pathol 2003 May;162(5):1495-502

Aberrant Wnt/beta-Catenin Pathway Activation in Idiopathic Pulmonary Fibrosis.

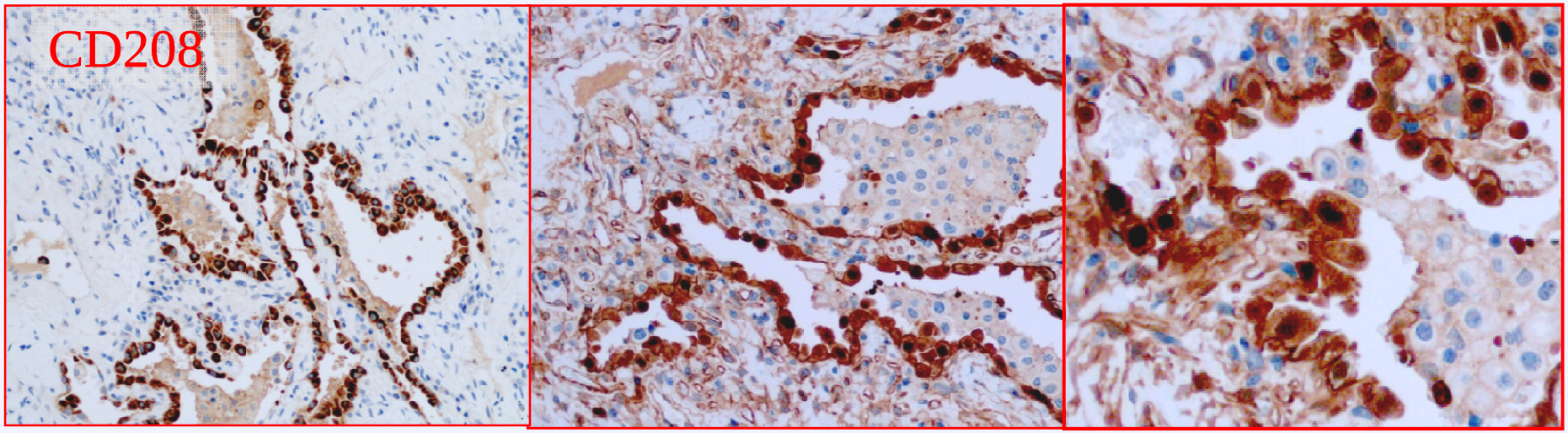
Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P, Pedron S, Bertaso M, Scarpa A, Murer B, Cancellieri A, Maestro R, Semenzato G, Doglioni C.
Department of Pathology, University of Verona, Verona.

the aberrant activation of Wnt/beta-catenin signaling could trigger a divergent epithelial regeneration at bronchiolo-alveolar junctions and epithelial-mesenchymal-transitions, leading to severe and irreversible remodeling of the pulmonary tissue.



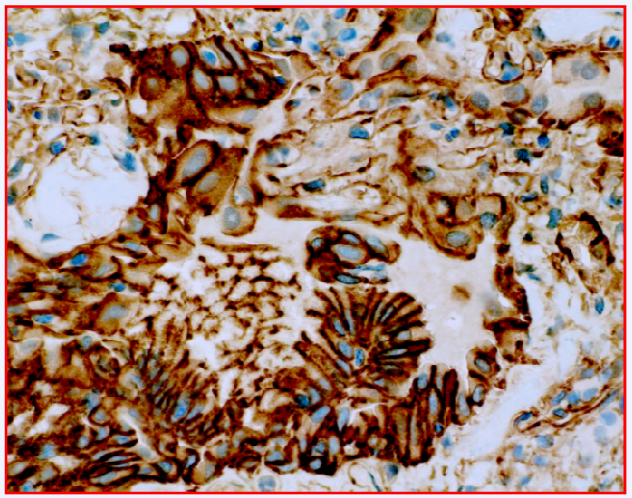
Wnt activtion in bronchiolar basal cells

DAD/ARDS



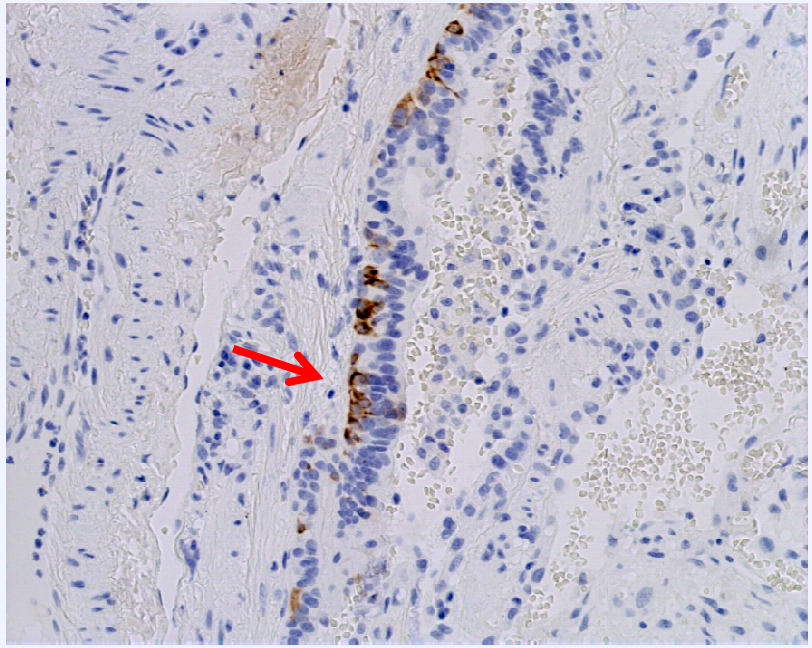
β-catenin nuclearization in AECII

β-catenin is not in the nuclei of bronchiolar cells

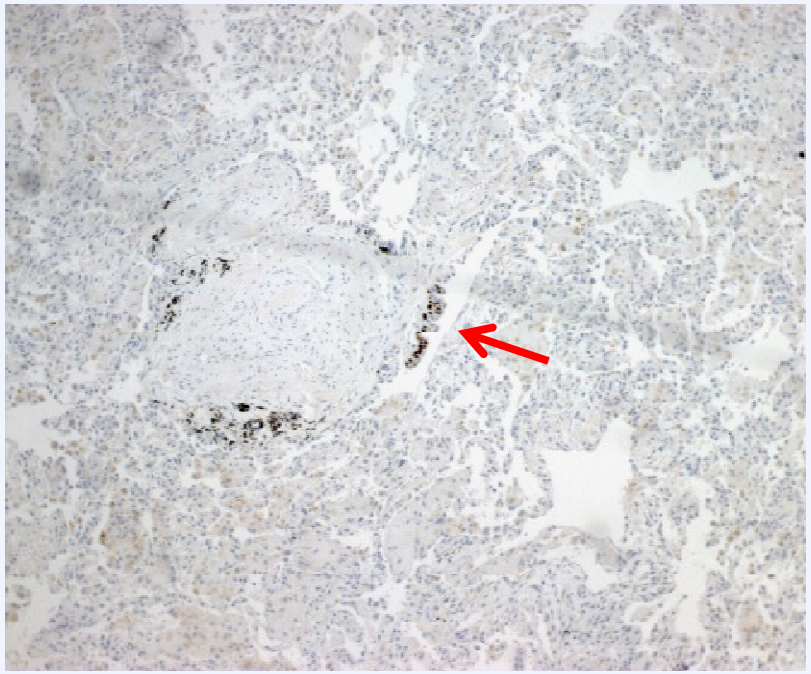


DAD/ARDS

Absence of bronchiolar proliferation



CK5



ΔN-p63

Am J Respir Crit Care Med. 2014 May 1;189(9):1142-5. doi: 10.1164/rccm.201312-2134LE.

Keratin-14 expression in pneumocytes as a marker of lung regeneration/repair during diffuse alveolar damage.

Ficial M[1], Antonaglia C, Chilosi M, Santagiuliana M, Tahseen AO, Confalonieri D, Zandonà L, Bussani R, Confalonieri M.

Author information

PMID: 24787069 [PubMed - indexed for MEDLINE]

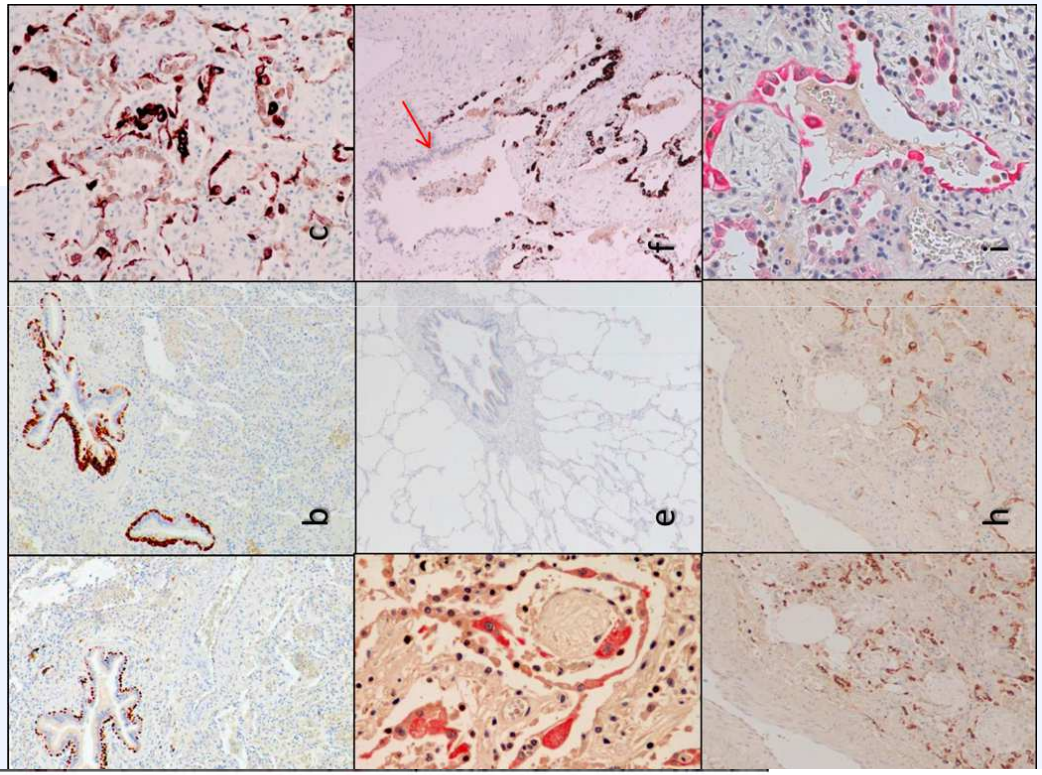


Figure 1. (*a–c*) Bronchiolar basal cells are positive for both (*a*) ΔN-p63 and (*b*) keratin-5 (Krt5), whereas both bronchiolar and alveolar cells express (*c*) 34βE12 cytokeratin in diffuse alveolar damage (DAD). (*d* and *e*) Immunohistochemical analysis with antibodies recognizing single keratins reveals that Krt14 is expressed in (*d*) alveolar epithelial cells type II (AECII) in DAD (*red*, immunoalkaline phosphatase staining); (*e*) alveolar cells in normal lung parenchyma are completely Krt14 negative. (*f–h*) Immunohistochemical characterization of Krt14-expressing cells, using pneumocyte-specific markers: (*f*) CD208, strongly expressed in AECII and absent in a bronchiole (*arrow*), and (*g*) ABCA3 expressed in (*h*) Krt14-positive AECII on serial sections. (*i*) Dual immunostaining for Krt14 (*red*, alkaline phosphatase immunostaining).

Table 1: Acute Respiratory Distress Syndrome Study Population: Timing of Sampling, Morphological Phase of Diffuse Alveolar Damage, Percentage of Krt14$^+$ Pneumocytes, Percentage of Krt14$^+$ Bronchiolar Cells, and Percentage of Proliferating Pneumocytes Expressing Krt14 among Krt14$^+$ Alveolar Epithelial Cells Type II

Patients with ARDS	Duration of MV (*d*)	Timing of Sampling from Hospital/ICU Admission*	DAD Morphological Phase	Percent Krt14$^+$ Alveolar Cells	Percent Krt14$^+$ Bronchiolar Cells	Ki67$^+$/Krt14$^+$ Cells: Percent among Krt14$^+$ AECII
1	10	11 d	Late exudative/ early proliferative	90% pos (50% +++; 30% ++; 10% +); 10% neg	NA	29 ± 3
2	19	19 d	Late proliferative/ early fibrotic	<5% +; >95% neg	NA	9 ± 4
3	4	4 d	Exudative	100% pos (60% +++; 15% ++; 25% +); 0% neg	84%	21 ± 5
4	21	22 d	Exudative, early and late proliferative	70% pos (60% ++, 10% +); 30% neg	90% pos	11 ± 4
5	16	16 d	Proliferative	90% pos (40% +++; 40% ++; 10% +); 10% neg	90% pos	18 ± 4
6	9	8 d	Proliferative	90% pos (50% +++; 20% ++; 20% +); 10% neg	70% pos	14 ± 6
7	10	6 d	Proliferative	95% pos (60% +++; 10% ++; 25% +); 5% neg	80% pos	30 ± 4
8	7	9 d	Proliferative	90% pos (20% +++; 50% ++; 20% +); 10% neg	70% pos	23 ± 2
9	17	3 mo	Proliferative	95% pos (30% +++; 50% ++; 15% +); 5% neg	90% pos	25 ± 9
10	12	12 d	Proliferative	90% pos (30% +++; 50% ++; 10% +); 10% neg	95% pos	32 ± 8
11	11	12 d	Proliferative	90% pos (60% +++; 20% ++; 10%); 10% neg	80% pos	18 ± 7
12	10	6 d	Exudative	95% pos (60% +++; 30% ++; 5% +); 5% neg	90% pos	NA
13	1	2 d	Exudative	90% pos (50% +++; 20% ++; 20% +); 10% neg	80% pos	NA
14	10	9 d	Exudative	95% pos (40% +++; 30% ++; 25% +); 5% neg	NA	NA
15	11	7 d	Exudative	90% pos (30% +++; 30% ++; 30% +); 10% neg	90% pos	NA

Definition of abbreviations: AECII = alveolar epithelial cells type II; ARDS = acute respiratory distress syndrome; DAD = diffuse alveolar damage; ICU = intensive care unit; MV = mechanical ventilation; NA = not available; neg = negative; pos = positive.
*See the online supplement for more details.

Author disclosures are available with the text of this letter at www.atsjournals.org.

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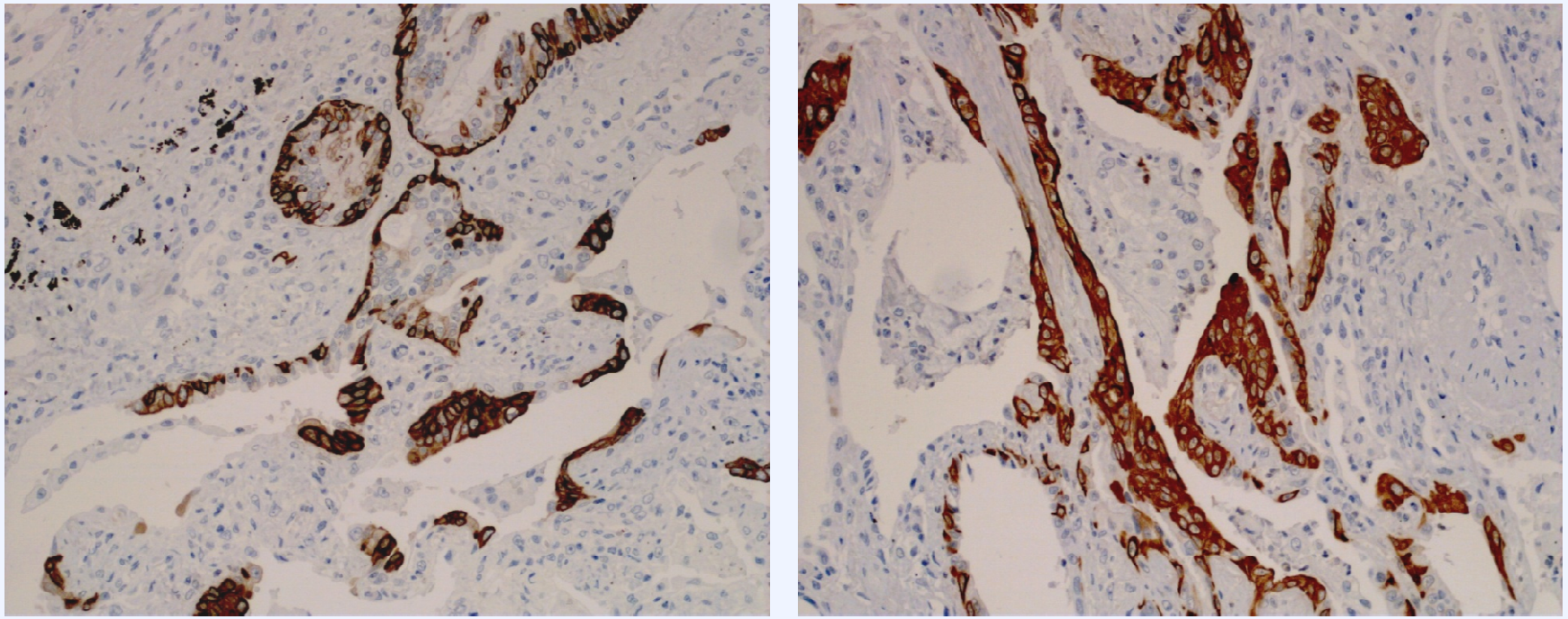

CK5

Squamous Metaplasia

Am J Physiol Lung Cell Mol Physiol 286: L643–L649, 2004.
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CALL FOR PAPERS | *Stem Cells in Lung Biology*

In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations

Kyung U. Hong,[1,2] Susan D. Reynolds,[1,2] Simon Watkins,[3] Elaine Fuchs,[4] and Barry R. Stripp[1,2,3]
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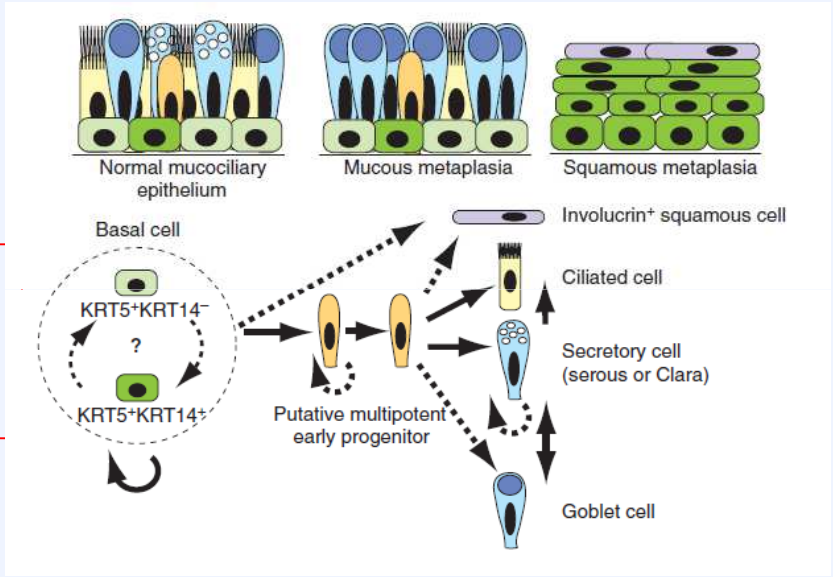
CK14 expressing cells include subsets capable of either multipotent or unipotent differentiation in vivo.



J Pathol. 1997 Jun;182(2):217-24.

Alterations in cytokeratin expression by the alveolar lining epithelial cells in lung tissues from patients with idiopathic pulmonary fibrosis.

Iyonaga K, Miyajima M, Suga M, Saita N, Ando M.
First Department of Internal Medicine, Kumamoto University School of Medicine, Japan.

Increased expression of CK14 in IPF

Cell

Distal Airway Stem Cells Yield Alveoli In Vitro and during Lung Regeneration following H1N1 Influenza Infection

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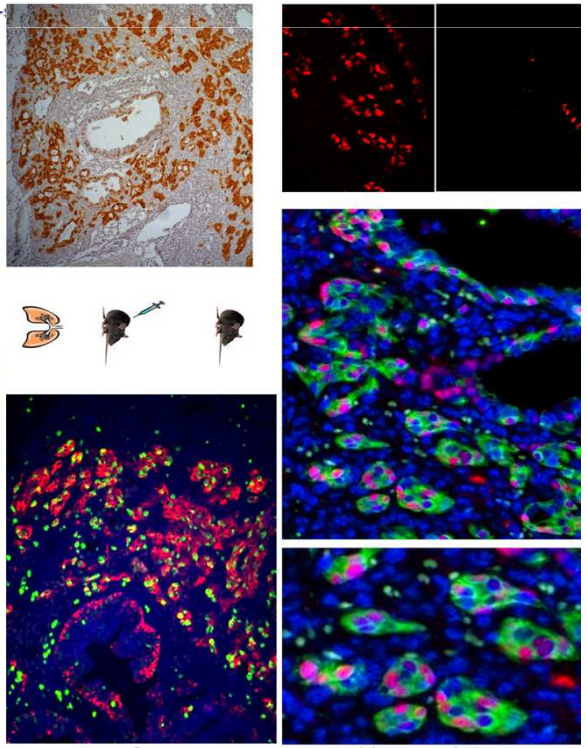
SUMMARY

The extent of lung regeneration following catastrophic damage and the potential role of adult stem cells in such a process remains obscure. Sublethal infection of mice with an H1N1 influenza virus related to that of the 1918 pandemic triggers massive airway damage followed by apparent regeneration. We show here that p63-expressing stem cells in the bronchiolar epithelium undergo rapid proliferation after infection and radiate to interbronchiolar regions of alveolar ablation. Once there, these cells assemble into discrete, Krt5+ pods and initiate expression of markers typical of alveoli. Gene expression profiles of these pods suggest that they are intermediates in the reconstitution of the alveolar-capillary network eradicated by viral infection. The dynamics of this p63-expressing stem cell in lung regeneration mirrors our parallel finding that defined pedigrees of human distal airway stem cells assemble alveoli-like structures in vitro and suggests new therapeutic avenues to acute and chronic airway disease.



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Basal Cells Are a Multipotent Progenitor Capable of Renewing the Bronchial Epithelium

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