Acuerdos de Cooperación TEC – MD Anderson
Historia, Resultados y Oportunidades

Roberto Adachi, MD
Associate Professor, Department of Pulmonary Medicine
The University of Texas MD Anderson Cancer Center

Texas Medical Center

- Instituciones 54
  - Hospitales 21
  - Escuelas/Universidades 17
- Edificaciones 162
  - 5.4 Km^2 built: 4.6 million m^2
- Empleados 106,000
  - Profesionales 21,000
- Voluntarios 12,000
- Estudiantes 72,000
- Pacientes 10 million/y
  - Camas hospitalarias 9,200
  - Cirugías 180,000/y
  - Visitantes 160,000/d
- Presupuesto $25 billion/y
  - Investigación 4 billion/y

TMC 2016 Economic Report

U.T. MD Anderson Cancer Center

- Buildings 25
- Empleados 21,000
  - Profesores 1,700
- Voluntarios 3,000
- Estudiantes 7,000/y
  - Resident/fellows 1,500/y
- Pacientes 1.5 million/y
  - Camas hospitalarias 800
- Presupuesto $4.5 billion/y
  - Investigación 700 million/y
- Otros locales
  - Houston 12
  - Partners 5
  - Certified 15
  - International 5

UT MD Anderson Cancer Center 2016 Year Report

Medicine ≠ Science

- Planteamiento de la pregunta
- Evaluación crítica
- Desdén de la autoridad
- Generación de la hipótesis
- Falsificación de la teoría

Programas de Investigación TEC – MD Anderson:

Verano – Ciencias básicas – Medicina	(2005)	113
Verano – Ciencias clínicas – Medicina	(2016)	5
Verano – Ciencias básicas – Biotecnología	(2011)	10
Electivo – Ciencias básicas – Medicina	(2008)	16
Elective – Ciencias clínicas – Medicina	(2015)	2
Elective – Ciencias básicas – Biotecnología	(2011)	1
Servicio Social – Ciencias básicas – Medicina	(2009)	17
Servicio Social – Ciencias clínicas – Medicina	(2017)	2
M. en Ciencias (MS)	(2016)	2
M. en Salúd Pública (MPH)	(2019?)	?
MD/PhD	(2010)	1
PhD	(2010)	1
Post-Doctoral Fellows	(2004)	17
TOTAL		**187**

Post-Doctoral Fellows

Ernestina Melicoff	(2004 – 2007)
John Manllo	(2009 – 2011)
Cesar Ochoa	(2009 – 2012)
Daniel Moreira	(2010 – 2011)
Miguelina de la Garza	(2010 – 2012)
Alfredo Dávalos	(2011 – 2013)
Elsa Rodarte	(2012 – 2016)
Elizabeth Sánchez	(2012 – 2016)
Francisco Guzmán	(2013 – 2014)
David Moreno	(2014 – 2015)
Marco Ramos	(2014 – 2015)
Alejandro Tortoriello	(2015 – 2016)
José Flores	(2015 – 2017)
Miguel Leiva	(2015 – 2017)
Lisandra Sanchez	(2016 – 2018)
Miguel Chavez	(2017 – 2018)
Jazreel Pantaleon	(2018 –)

TEC – MD Anderson Presentaciones Orales y Posters (113)

2005. *Defective regulated exocytosis in mast cell from Synaptotagmin-2 knockout mice*. American Society of Hematology Annual Meeting, Atlanta, Georgia. Melicoff E.

2005. *Mast cell-specific gene targeting*. American Society of Hematology Annual Meeting, Atlanta, Georgia. Melicoff E.

2006. *Membrane tethers formed from mast cells of the mouse with laser tweezers elucidate tension of plasma membrane*. Biophysical Society Meeting (Exocytosis/Endocytosis), Salt Lake City, Utah. Melicoff E.

2006. *Specific defect in regulated exocytosis in mast cells from Synaptotagmin-2 knockout mouse. Its importance in allergy and infection*. American Thoracic Society International Conference, San Diego, California. Melicoff E.

2006. *Evaluation of the function of mast cell tryptases using recombinant material and novel transgenic mice*. The 26th Symposium of the Collegium Internationale Allergologicum "Cellular and molecular targets in allergy and clinical immunology", St. Julian's, Malta. Melicoff E, Moreira DC. **(ORAL PRESENTATION)**

2007. *Selective defect in mast cell regulated exocytosis. Its importance in allergy and infection*. Keystone Symposia: Mast Cells, Basophils, and IgE: Host defense and Disease, Cooper Mountain, Colorado. Melicoff E, Gomez A. **(ORAL PRESENTATION)**

2007. *Mouse mast cell protease 6 is essential in defense against Gram-negative bacterial infection*. Keystone Symposia: Mast Cells, Basophils, and IgE: Host defense and Disease, Cooper Mountain, Colorado. Melicoff E, Moreira DC. **(ORAL PRESENTATION)**

2007. *IP3 Receptors and Synaptotagmin-2 couple calcium signaling to mucin secretion in airway epithelium*. Gordon Research Conference: Cilia, Mucus and Mucociliary Interactions. Ventura, California. Melicoff E. **(ORAL PRESENTATION)**

2007. *Decreased survival of mice deficient in mouse mast cell protease-6 in a model of Gram-negative bacterial infection*. American Thoracic Society International Conference, San Francisco, California. Melicoff E, Moreira DC.

2007. *Mucin production in the airways of antigen challenged Muc5ac knockout mice.* American Thoracic Society International Conference, San Francisco, California. Perez G, Suarez A. **(ORAL PRESENTATION)**

2007. *Mast cell tryptase has an ancient origin and plays and essential role in defense against bacterial infections*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Melicoff E, Moreira DC. **(ORAL PRESENTATION, AWARD FOR BEST BASIC RESEARCH PRESENTATION)**

2008. *Curcumin inhibits airway inflammation and inflammation-induced lung cancer in mice*. American Thoracic Society International Conference, Toronto, Ontario, Canada. Torrez-Garza N.

2008. *Control of mast cell exocytosis by Munc18-2*. American Thoracic Society International Conference, Toronto, Ontario, Canada. Valdez G, Rodarte E, Castro FJ.

2008. *The mouse mast cell-restricted tetramer-forming tryptases mMCP-6 and MMCP-7 are critical mediators in inflammatory arthritis.* The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Moreira DC, Gomez A. **(ORAL PRESENTATION, FINALIST FOR BEST BASIC RESEARCH PRESENTATION)**

2008. *Curcumin inhibits airway inflammation and inflammation-induced lung cancer in mice*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Torrez-Garza N.

2009. *Mucin production in the airways of antigen challenged Muc5ac knockout mice*. American Thoracic Society International Conference, San Diego, California. Andaluz AC, Gonzalez D. **(ORAL PRESENTATION)**

2009. *Mast cell exocytosis, inflammation and cancer*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Moreira DC. **(ORAL PRESENTATION, FINALIST FOR BEST BASIC RESEARCH PRESENTATION)**

2010. *Hypoxia-inducible factor-1alpha is a key player in promotion of lung cancer by COPD-like airway inflammation in mice*. American Thoracic Society International Conference, New Orleans, Louisiana. Ochoa-Perez C.

2010. *Interleukin-6 is involved in the promotion of lung cancer by COPD-like airway inflammation in mice*. American Thoracic Society International Conference, New Orleans, Louisiana. Ochoa-Perez C. **(ORAL PRESENTATION)**

2010. *Mast cell proteases and lung cancer.* American Thoracic Society International Conference, New Orleans, Louisiana. Moreira DC, Davalos A, Manllo J. **(ATS TRAVEL AWARD WINNER)**

2010. *Role of Munc18-2 in mast cell exocytosis.* American Thoracic Society International Conference, New Orleans, Louisiana. Moreira DC, Gomez A, Manllo J, Moreno DS.

2010. *Role of Syntaxin 4 in mast cell exocytosis*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Manllo J, Moreira DC. **(AWARD FOR BEST POSTER PRESENTATION)**

2010. *Hypoxia-inducible factor-1alpha is a key player in promotion of lung cancer by COPD-like airway inflammation in mice*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Ochoa-Perez C.

2010. *Interleukin-6 is involved in the promotion of lung cancer by COPD-like airway inflammation in mice*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Ochoa-Perez C. **(ORAL PRESENTATION, AWARD FOR BEST BASIC RESEARCH PRESENTATION)**

2010. *NF-κB is the essential regulator of lung cancer promotion by COPD-like airway inflammation in mice*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Ochoa-Perez C.

2011. *Role of Syntaxin-4 in mast cell exocytosis*. American Thoracic Society International Conference, Denver, Colorado. Manllo J, Moreira DC, Dávalos AJ, Sánchez E, Tortoriello A, Moreno DS.

2011. *NF-κB is the essential regulator of lung cancer promotion by COPD-like airway inflammation in mice*. American Thoracic Society International Conference, Denver, Colorado. De la Garza MM, Ochoa CE, Beltran E. **(ORAL PRESENTATION, ATS TRAVEL AWARD WINNER)**

2011. *Targeted overexpression of tumor necrosis factor (TNF) induces airway inflammation and promotes lung carcinogenesis*. American Thoracic Society International Conference, Denver, Colorado. Ochoa CE, Garza Flores A. **(ORAL PRESENTATION)**

TEC – MD Anderson Presentaciones Orales y Posters (113)

2011. *Targeted overexpression of tumor necrosis factor (TNF) induces airway inflammation and promotes lung carcinogenesis*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Ochoa CE, Garza Flores A. **(AWARD FOR BEST POSTER PRESENTATION)**

2011. *Role of airway inflammation in lung carcinogenesis: a mechanistic dissection.* 14th World Conference on Lung Cancer, Amsterdam, Netherlands. Ochoa CE, De la Garza MM, Torres Garza N, Garza Flores A.

2012. *Electrophysiologic assessment of the role of Munc13-4 in mast cell regulated exocytosis*. Biophysical Society, San Diego, California. Davalos AJ, Rodarte EM, Moreira DC, Sanchez E, Tortoriello A, Manllo J.

2012. *Role of Munc18-3 in mast cell exocytosis*. American Thoracic Society International Conference, San Francisco, California. Rodarte EM, Davalos AJ, Sanchez E, Tortoriello A.

2012. *Anaphylaxis driven by mast cell regulated exocytosis requires Munc13-4*. American Thoracic Society International Conference, San Francisco, California. Rodarte EM, Moreira DC, Davalos AJ, Sanchez E, Tortoriello A, Manllo J.

2012. *A protective role for toll-like receptor 2 in lung cancer promotion*. American Thoracic Society International Conference, San Francisco, California. De la Garza MM, Beltran E, Ochoa CE. **(ORAL PRESENTATION)**

2012. *An essential role for T-helper 17 (Th17) immune response in lung cancer promotion by inflammation*. American Thoracic Society International Conference, San Francisco, California. Ochoa CE. **(ORAL PRESENTATION)**

2013. *Reduced workflow 96 well plate solid phase extraction strategies for the determination of histamine and serotonin in mouse plasma prior to UPLC-ESI-MS/M*. 14th Annual Land O'Lakes Bioanalytical Conference – Emerging Challenges and Evolving Technologies for Bioanalysis, Madison, Wisconsin. Rodarte EM.

2013. *Synergistic inducible resistance against influenza pneumonia requires canonical TLR signaling in the lung epithelium*. American Thoracic Society International Conference, Philadelphia, Pennsylvania. Guzman FA. **(ORAL PRESENTATION)**

2013. *Canonical TLR signaling is required, but cannot fully explain synergistic inducible resistance to bacterial pneumonia.* American Thoracic Society International Conference, Philadelphia, Pennsylvania. Guzman FA. **(ORAL PRESENTATION)**

2013. *An essential role for neutrophils in lung cancer promotion*. Annual Meeting of the American Association for Cancer Research, Washington, DC. Ochoa CE, De la Garza MM

2013. *Toll like receptor 4 but not 2 promotes lung cancer in a K-ras mutant mouse model*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Torres Garza N, De la Garza MM, Beltran E. **(AWARD FOR POSTER PRESENTATION)**

2013. *Pharmacologic targeting of interleukin 6 as a therapeutic strategy for lung cancer*. American Thoracic Society International Conference, Philadelphia, Pennsylvania. Ochoa CE. **(ORAL PRESENTATION)**

2014. *Molecular machinery mediating mucin secretion in the airways*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Flores JR

2014. *Toll like receptor 4 but not 2 promotes lung cancer in a K-ras mutant mouse model*. American Thoracic Society International Conference, San Diego, California. Torres Garza N, De la Garza MM, Beltran E. **(ORAL PRESENTATION, ATS TRAVEL AWARD WINNER)**

2014. *A promoting role for MyD88 signaling in lung tumorigenesis*. American Thoracic Society International Conference, San Diego, California. De la Garza MM, **(ORAL PRESENTATION, ATS TRAVEL AWARD WINNER)**

2014. *Tumor necrosis factor promotes lung cancer through induction of a myeloid-derived suppressor cell response*. American Thoracic Society International Conference, San Diego, California. Garza Flores A, Ochoa CE. **(ORAL PRESENTATION, ATS TRAVEL AWARD WINNER)**

2014. *Pharmacologic targeting of interleukin 6 as a therapeutic strategy for lung cancer*. *American Association for Cancer Research - International Association for the Study of Lung Cancer* Joint Meeting on Molecular Origin of Lung Cancer, San Diego, California. Ochoa CE. **(TRAINEE AWARD WINNER)**

2015. *Re-educating lung tumor microenvironment through blockade of IL-6 as a preventive and therapeutic strategy for K-ras mutant lung cancer*. American Thoracic Society International Conference, Denver, Colorado. Bugarin E. **(ORAL PRESENTATION, ATS TRAVEL AWARD WINNER)**

2015. *Re-educating lung tumor microenvironment through blockade of IL-6 as a preventive and therapeutic strategy for K-ras mutant lung cancer*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Bugarin E.

2015. *Inducible epithelial resistance protects mice against pneumonia in the presence of acute leukemia and cytotoxic chemotherapy*. The University of Texas MD Anderson Cancer Center Trainee Research Day. Leiva-Juarez MM, Pantaleon-Garcia J, Martinez-Zayas G. (**ORAL PRESENTATION, AWARD IN TRANSLATIONAL RESEARCH)**

2015. *A promoting role for MyD88/IRAK4 signaling in lung fibrosis during COPD progression*. American Thoracic Society International Conference, Denver, Colorado. Nieto V, De La Garza MM.

2015. *Inducible epithelial resistance protects mice against pneumonia in the presence of acute leukemia and cytotoxic chemotherapy*. American Thoracic Society International Conference, Denver, Colorado. Leiva-Juarez MM, Pantaleon-Garcia J, Martinez-Zayas G. **(ORAL PRESENTATION)**

2015. *Inducible resistance to viral infection in primary lung epithelial cells*. American Thoracic Society International Conference, Denver, Colorado. Leiva-Juarez MM.

2015. *Inducible antiviral resistance enhances lung epithelial cell survival without modulating programmed cell death pathways*. American Thoracic Society International Conference, Denver, Colorado. Pantaleon-Garcia J, Martinez-Zayas G, Leiva-Juarez MM.

2015. *Lung epithelial cells are necessary and sufficient mediators of inducible antiviral resistance*. American Thoracic Society International Conference, Denver, Colorado. Pantaleon-Garcia J, Leiva-Juarez MM. **(TRAVEL AWARD)**

TEC – MD Anderson Presentaciones Orales y Posters (113)

2015. *Pneumonia prophylaxis by TLR 2/6 and TLR9 stimulation is associated with an early increase in transepithelial electrical resistance independent of PKCζ activation and a late increase in macromolecular transport in bronchial epithelial cells*. American Thoracic Society International Conference, Denver, Colorado. Pantaleon-Garcia J, Martinez-Zayas G, Leiva-Juarez MM. **(ABSTRACT AWARD)**

2015. *A promoting role for MyD88/IRAK4 signaling in lung fibrosis during COPD progression*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Nieto V, De La Garza MM.

2015. *Role of Syntaxin 3 in mast cell exocytosis*. American Thoracic Society International Conference, Denver, Colorado. Sánchez E, Moreno DS, Tortoriello A, Molina S, Ramos MA, Cárdenas E, Rodarte EM.

2015. *Role of Syntaxin 3 in mast cell exocytosis*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Sánchez E, Moreno DS, Tortoriello A, Molina S, Ramos MA, Cárdenas E, Rodarte EM.

2015. *SNAP23 is selectively expressed in airway secretory cells and mediates baseline and stimulated mucin secretion*. Gordon Research Conference: Cilia, Mucus and Mucociliary Interactions, Galveston, Texas. Flores JR

2015. *Munc18b selectively mediates stimulated more than basal mucin secretion*. Gordon Research Conference: Cilia, Mucus and Mucociliary Interactions, Galveston, Texas. Huerta AS.

2015. *Aerosolized hypertonic saline solution induces mucin secretion in mice*. Gordon Research Conference: Cilia, Mucus and Mucociliary Interactions, Galveston, Texas. Flores JR.

2015. *SNAP23 is selectively expressed in airway secretory cells and mediates baseline and stimulated mucin secretion*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Flores JR

2015. *Aerosolized hypertonic saline solution induces mucin secretion in mice*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Flores JR.

2015. *Lung vasculature growth and remodeling through intussuceptive angiogenesis Is coordinated with growth of alveolar type 1 cell*. The University of Texas MD Anderson Cancer Center, Genes and Development Program Retreat, Houston, Texas. Vila L.

2016. *Impact of interleukin-22 on K-ras mutant lung tumor microenvironment and stemness properties*. American Thoracic Society International Conference, San Francisco, California. De la Garza CC.

2016. *Gender–specific role of epithelial STAT3 in K-ras mutant lung tumorigenesis*. American Thoracic Society International Conference, San Francisco, California. Bugarin E.

2016. *The autocrine and paracrine roles of epithelial STAT3 in K-ras mutant lung cancer.* American Thoracic Society International Conference, San Francisco, California. Bugarin E. **(ORAL PRESENTATION)**

2016. *Impact of interleukin-22 on K-ras mutant lung cancer promotion and stemness properties*. *American Association for Cancer Research* Annual Meeting, New Orleans, Lousiana. De la Garza CC. **(ORAL PRESENTATION)**

2016. *Gender–specific role of epithelial STAT3 in K-ras mutant lung tumorigenesis*. *American Association for Cancer Research* Annual Meeting, New Orleans, Lousiana. Bugarin E.

2016. *A model to predict pleural effusion recurrence*. American Thoracic Society International Conference, San Francisco, California. Molina S.

2016. *Different Munc18 proteins mediate baseline and stimulated airway mucin secretion.* Gordon Research Conference: Proprotein Processing, Trafficking and Secretion, New London, New Hampshire. Huerta AS. **(POSTER AWARD)**

2016. *Cyclic GMP-AMP synthase (cGAS), stimulator of interferon genes (STING) and interferon activated gene 16 (IFI16) are essential for intrinsic lung epithelial responses against influenza A infection, but not for therapeutically induced antiviral resistance*. American Thoracic Society International Conference, San Francisco, California. Leiva-Juarez MM. **(TRAVEL AWARD)**

2016. *Mitochondrial reactive oxygen species-dependent inducible antiviral resistance in primary lung epithelial cells*. American Thoracic Society International Conference, San Francisco, California. Leiva-Juarez MM, Pantaleon-Garcia J.

2016. *Synergistic Toll-like receptor signaling-induced resistance protects mice against pneumonia in the presence of acute leukemia and cytotoxic chemotherapy*. American Thoracic Society International Conference, San Francisco, California. Leiva-Juarez MM. **(ABSTRACT AWARD, POSTER DISCUSSION)**

2016. *Synergistic Treatment with synergistic TLR ligands that protect against leukemia-associated pneumonias does not worsen tumor burden.* American Thoracic Society International Conference, San Francisco, California. Leiva-Juarez MM.

2016. *Aerosolized TLR Agonists Suppress Acute Sendai Virus Burden and Chronic Asthma-Like Lung Disease in Mice*. American Thoracic Society International Conference, San Francisco, California. Flores JR

2016. *Investigating Intussusceptive Angiogenesis in the Developing Mouse Lung.* The University of Texas MD Anderson Cancer Center, Genes and Development Program Retreat, Epigenetics and Molecular Carcinogenesis Retreat, Montgomery, Texas. Vila-Ellis L. **(ORAL PRESENTATION)**

2016. *A Novel Model of Angiogenesis in the Developing Mouse Lung.* Genes and Development. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Vila-Ellis L.

2016. *Formation and Disruption of Airway Cell Mosaicism*. Gordon Research Conference, Cilia, Mucus and Mucociliary Interactions, Galveston, Texas. Flores JR.

2016. *Aerosolized Hypertonic Saline Solution Increases Mucin Secretion in Mice*. Gordon Research Conference, Cilia, Mucus and Mucociliary Interactions, Galveston, Texas. Flores JR.

2017. *Distinct Munc18 Proteins Mediate Baseline and Stimulated Mucin Secretion*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Velasco P.

2017. *A Promoting Role for the Epithelial MyD88/IRAK4/NF-kB Signaling in K-ras Mutant Lung Tumorigenesis*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. De la Garza MM. **(BEST POSTER FINALIST)**

TEC – MD Anderson Presentaciones Orales y Posters (113)

2017. *Gender Specific Function of Epithelial IL-6/STAT3 Pathway in K-ras Mutant Lung Cancer*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Bugarin E.

2017. *Toll Like Receptors Mediated Inflammatory Signals Mediate Promotion of K-ras Mutant Lung Cancer by Chronic Obstructive Pulmonary Disease*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Torres-Garza N, De la Garza MM, Beltran E. **(BEST POSTER FINALIST)**

2017. *Control of Mast Cell Regulated Exocytosis by Munc18 proteins*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Gutierrez BA, Chavez MA, Ramos MA.

2017. *Munc13-4 regulates platelet dense granule release and hemostasis*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Cardenas EI, Ramos MA.

2017. *Platelet dense granule release contributes to allergic airway inflammation*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Cardenas EI, Flores JR. **(BEST POSTER AWARD)**

2017. *Control of Mast Cell Regulated Exocytosis by Munc18 proteins*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Gutierrez BA, Chavez MA, Ramos MA.

2017. *Protection Against Viral Pneumonia Following Inhalation of Synergistic TLR Agonists Is Not Mediated by Induction of Type I Interferons*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Leiva MM, Flores JR.

2017. *Aerosolized TLR Agonists Suppress Acute Sendai Virus Burden and Chronic Asthma-Like Lung Disease in Mice*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Flores JR.

2017. *Aerosolized Hypertonic Saline Solution Increases Mucin Secretion in Mice*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Flores JR.

2017. *Distinct Munc18 Proteins Mediate Baseline and Stimulated Mucin Secretion*. 38th Steenbock Symposium, Protein Trafficking in the Secretory Pathway, Madison, Wisconsin. Velasco P.

2017. *A Promoting Role for the Epithelial MyD88/IRAK4/NF-kB Signaling in K-ras Mutant Lung Tumorigenesis*. Annual Meeting of the American Association for Cancer Research, Washington, DC. De la Garza MM.

2017. *Gender Specific Function of Epithelial IL-6/STAT3 Pathway in K-ras Mutant Lung Cancer*. Annual Meeting of the American Association for Cancer Research, Washington, DC. Bugarin E.

2017. *Toll Like Receptors Mediated Inflammatory Signals Mediate Promotion of K-ras Mutant Lung Cancer by Chronic Obstructive Pulmonary Disease*. Annual Meeting of the American Association for Cancer Research, Washington, DC. Torres-Garza N, De la Garza MM, Beltran E.

2017. *Control of Mast Cell Regulated Exocytosis by Munc18 proteins*. American Thoracic Society International Conference, Washington, DC. Gutierrez BA, Chavez MA, Ramos MA.

2017. *Toll-like receptor-induced epithelial antiviral resistance affects the early influenza viral cycle with an associated upregulation in IFITM1 and IFITM2*. American Thoracic Society International Conference, Washington, DC. Leiva MM.

2017. *Treatment with synergistic TLR ligands that protect against leukemia-associated pneumonias does not worsen tumor burden*. American Thoracic Society International Conference, Washington, DC. Leiva MM.

2017. *Activation of lung epithelial NFkB and STAT3 signaling pathways is required for inducible resistance to pseudomonas infection*. American Thoracic Society International Conference, Washington, DC. Pantaleon J, Leiva MM.

2017. *Mitochondrial reactive oxygen species in lung epithelial cells are induced by oligodeoxynucleotides in a MyD88 and TRAF6-independent manner*. American Thoracic Society International Conference, Washington, DC. Leiva MM.

2017. *Myddosome-activated epithelial TRAF6 is required for synergistic TLR-induced resistance to pneumonia*. American Thoracic Society International Conference, Washington, DC. Pantaleon J, Leiva MM.

2017. *Protection against viral pneumonia following inhalation of synergistic TLR agonists is not mediated by induction of type I interferons*. American Thoracic Society International Conference, Washington, DC. Leiva MM, Flores JR. **(MINORITY TRAVEL AWARD)**

2017. *Investigating Alveolar Angiogenesis in the Developing Mouse Lung*. Keystone Symposia, Angiogenesis and Vascular Disease, Santa Fe, New Mexico. Vila-Ellis L. **(ORAL PRESENTATION and SCHOLARSHIP)**

2017. *A Model to Predict Pleural Effusion Recurrence in Patients with Malignancy*. American College of Chest Physicians, CHEST Annual Meeting, Toronto, Ontario, Canada. Molina S.

2017. *Predicting Postoperative Lung Function Following Lobectomy*. American College of Chest Physicians, CHEST Annual Meeting, Toronto, Ontario, Canada. Ontiveros N.

2018. *Synergistic Effect of Cigarette Smoke and Bacterial Induced Chronic Obstructive Pulmonary Disease Type Airway Inflammation on Promotion of K-ras Mutant Lung Cancer*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Ramos-Castaneda M, Gutierrez BA. **(BEST POSTER AWARD)**

2018. *Toll-Like Receptor Signaling Reveals Differential Gene Expression Profiles Involved in Prevention of Virus-Induced Asthma*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Flores JR.

2018. *Mechanisms of ODN-Induced Epithelial Reactive Oxygen Species Generation and Its Role in Inducible Resistance to Influenza Infection*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Leiva-Juarez MM, Chavez Cavazos MA.

2018. *Distinct Munc18 Proteins Mediate Baseline and Stimulated Mucin Secretion*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Flores JR.

2018. *Syntaxin 11 in mast cell and platelet degranulation*. The University of Texas MD Anderson Cancer Center Division of Internal Medicine Research Conference, Houston, Texas. Gonzalez-Delgado R, Cardenas EI, Gonzalez E, Rodarte AI.

TEC – MD Anderson Presentaciones Orales y Posters (113)

2018. *Synergistic Effect of Cigarette Smoke and Bacterial Induced Chronic Obstructive Pulmonary Disease Type Airway Inflammation on Promotion of K-ras Mutant Lung Cancer*. Annual Meeting of the American Association for Cancer Research, Chicago, IL. Ramos-Castaneda M, Gutierrez BA.

2018. *Toll-Like Receptor Signaling Reveals Differential Gene Expression Profiles Involved in Prevention of Virus-Induced Asthma*. American Thoracic Society International Conference, San Diego, CA. Flores JR.

2018. *Mechanisms of ODN-Induced Epithelial Reactive Oxygen Species Generation and Its Role in Inducible Resistance to Influenza Infection*. American Thoracic Society International Conference, San Diego, CA. Leiva-Juarez MM, Chavez Cavazos MA.

TEC – MD Anderson Publicaciones (34; 7 en revisión)

The Journal of Neuroscience, December 27, 2006 • 26(52):13493–13504 • 13493

Cellular/Molecular

Synaptotagmin-2 Is Essential for Survival and Contributes to Ca^{2+} Triggering of Neurotransmitter Release in Central and Neuromuscular Synapses

Zhiping P. Pang,[1] Ernestina Melicoff,[5] Daniel Padgett,[1] Yun Liu,[1] Andrew F. Teich,[5] Burton F. Dickey,[5] Weichun Lin,[1,3] Roberto Adachi,[5] and Thomas C. Südhof[1,2,4]

[1]Center for Basic Neuroscience, Departments of [2]Molecular Genetics and [3]Cell Biology, and [4]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75390, and [5]Department of Pulmonary Medicine, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Biochemical and genetic data suggest that synaptotagmin-2 functions as a Ca^{2+} sensor for fast neurotransmitter release in caudal brain regions, but animals and/or synapses lacking synaptotagmin-2 have not been examined. We have now generated mice in which the 5′ end of the synaptotagmin-2 gene was replaced by lacZ. Using β-galactosidase as a marker, we show that, consistent with previous studies, synaptotagmin-2 is widely expressed in spinal cord, brainstem, and cerebellum, but is additionally present in selected forebrain neurons, including most striatal neurons and some hypothalamic, cortical, and hippocampal neurons. Synaptotagmin-2-deficient mice were indistinguishable from wild-type littermates at birth, but subsequently developed severe motor dysfunction, and perished at ~3 weeks of age. Electrophysiological studies in cultured striatal neurons revealed that the synaptotagmin-2 deletion slowed the kinetics of evoked neurotransmitter release without altering the total amount of release. In contrast, synaptotagmin-2-deficient neuromuscular junctions (NMJs) suffered from a large reduction in evoked release and changes in short-term synaptic plasticity. Furthermore, in mutant NMJs, the frequency of spontaneous miniature release events was increased both at rest and during stimulus trains. Viewed together, our results demonstrate that the synaptotagmin-2 deficiency causes a lethal impairment in synaptic transmission in selected synapses. This impairment, however, is less severe than that produced in forebrain neurons by deletion of synaptotagmin-1, presumably because at least in NMJs, synaptotagmin-1 is coexpressed with synaptotagmin-2, and both together mediate fast Ca^{2+}-triggered release. Thus, synaptotagmin-2 is an essential synaptotagmin isoform that functions in concert with other synaptotagmins in the Ca^{2+} triggering of neurotransmitter release.

Key words: asynchronous release; endplate; neuromuscular junction; striatum; synapse; synaptotagmin

Introduction

Neurotransmitter release from presynaptic nerve terminals is triggered when an action potential gates Ca^{2+} influx into the terminal, and Ca^{2+} induces exocytosis of synaptic vesicles (Katz and Miledi, 1967). Neurotransmitter release occurs in two modes: fast synchronous release that is induced by brief transients of high Ca^{2+} concentrations, and slow asynchronous release that is induced at a slower rate by lower Ca^{2+} concentrations (Barrett and Stevens, 1972; Goda and Stevens, 1994; Cummings et al., 1996; Atluri and Regehr, 1998; Lu and Trussell, 2000; Hagler and Goda, 2001; Otsu et al., 2004). In the forebrain, synaptotagmin-1 functions as the Ca^{2+} sensor for fast synchronous release (Geppert et al., 1994; Fernandez-Chacon et al., 2001). Synaptotagmin-1 belongs to a large family of proteins (15 members in mouse) that contain similar domain structures, with an N-terminal transmembrane region, a linker sequence, and two C-terminal C_2 domains that bind Ca^{2+} in most but not all synaptotagmins (for review, see Südhof, 2002). Among synaptotagmins, synaptotagmin-2 shares the highest homology with synaptotagmin-1, has similar biochemical characteristics, and can functionally replace synaptotagmin-1 in neurons and chromaffin cells that lack synaptotagmin-1 (Stevens and Sullivan, 2003; Nagy et al., 2006). These experiments suggested that synaptotagmin-1 and -2 have similar functions. However, synaptotagmin-1 and -2 are not entirely functionally redundant because synaptotagmin-1 knock-out (KO) mice express normal levels of synaptotagmin-2 but nevertheless exhibit a severe phenotype that in hippocampal and cortical neurons manifests as a loss of fast synchronous release and perinatal lethality (Geppert et al., 1994; Nishiki and Augustine, 2004; Maximov and Südhof, 2005). A possible explanation for this finding is based on the differential expression of synaptotagmin-1 and -2, with the

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The Mast Cell-restricted Tryptase mMCP-6 Has a Critical Immunoprotective Role in Bacterial Infections*

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Although it has been shown that mast cell-deficient mice have diminished innate immune responses against bacteria, the most important immunoprotective factors secreted from activated mast cells have not been identified. Mouse mast cell protease 6 is a tetramer-forming tryptase. This serine protease is abundant in the secretory granules and is exocytosed upon bacterial challenge. Here we have described the generation of a mast cell protease-6-null mouse. Our discovery that mice lacking this neutral protease cannot efficiently clear *Klebsiella pneumoniae* from their peritoneal cavities reveals an essential role for this serine protease, and presumably its human ortholog, in innate immunity.

Approximately 50% of the weight of a mature tissue mast cell (MC)[2] consists of protease-serglycin proteoglycan complexes stored in the secretory granules. In humans, β tryptases are the most abundant MC-restricted neutral proteases (1–3). The corresponding tryptases in mice are mouse MC protease (mMCP)-6 (4, 5) and mMCP-7 (6), with mMCP-6 being the most similar in amino acid sequence and substrate specificity to human tryptase (hTryptase) β1 (7–9). MCs are the only cells that express mMCP-6, and this serine protease is particularly abundant in those MCs that reside in the peritoneal cavity, skin, and lung (4, 5, 10).

Numerous biochemical studies have been carried out to understand the biosynthesis and substrate preference of mMCP-6. This tryptase is initially translated as a zymogen with a 245-mer mature domain. When the signal and propeptides are proteolytically removed, the mature protease spontaneously forms tetramers with the active site of each monomer facing the central core of the tetramer unit, as first described for its human ortholog (11). A positively charged face forms on the surface of each monomer, thereby allowing mature mMCP-6 to interact with negatively charged serglycin proteoglycans in the Golgi complex. The resulting tryptase-serglycin macromolecular complexes are then targeted and packaged in the cell secretory granules. When exocytosed, these complexes are retained in connective tissues for hours because of their large sizes (12). Protease inhibitors are abundant in blood. Nevertheless, no circulating protease inhibitor has been identified that rapidly inactivates mMCP-6 or hTryptase β1. Substrate specificity studies carried out using varied peptide combinatorial libraries revealed that recombinant mMCP-6 (7) and hTryptase β1 (8, 9) prefer to cleave peptides having a Pro at residues P2 to P5 and a Lys or Arg at residue P1. However, due to the unique structural constraints of the tetramer unit, the abilities of mMCP-6 and hTryptase β1 to cleave large-sized proteins are very limited. Thus, the importance of these evolutionally conserved enzymes in MC-dependent reactions remains to be determined.

MC development *in vivo* is highly dependent on the cytokine kit ligand/stem cell factor on the surface of mesenchymal cells and its tyrosine kinase receptor c-Kit/CD117 on the surface of MC-committed progenitors. Signaling through c-Kit results in the translocation of microphthalmia transcription factor (MITF) into the nucleus. WBB6F_1-*Kit*^{W}/*Kit*$^{W-v}$ (*W*/*W*^{v}) mice are MC-deficient secondary to a point mutation in the intracellular domain of *c-kit*, which makes their MCs and progenitors less responsive to kit ligand. WBB6F_1-*tg/tg* (*tg/tg*) mice, on the other hand, have reduced numbers of MCs, because they express a mutated isoform of MITF. Thus, transcription of the *mMCP-6* gene and certain other MC-restricted genes are greatly diminished in MITF-deficient *tg/tg* mice (13).

In 1996, Echtenacher *et al.* (14) noted that *W*/*W*^{v} mice quickly die from septic peritonitis after their caecum is ligated and punctured. Malaviya *et al.* (15) reported at the same time that *W*/*W*^{v} mice cannot efficiently combat a *Klebsiella pneumoniae* infection of their peritoneal cavities or lungs. The same phenomenon was observed in *tg/tg* mice (16), confirming the importance of MCs in innate immunity. The latter data suggest that a MC-restricted gene whose transcription is highly dependent on MITF plays an essential immunoprotective role in bacterial infections. Although it has been concluded that MC-derived tumor necrosis factor-α (TNF-α) is needed to con-

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[2] The abbreviations and trivial names used are: MC, mast cell; CFU, colony-forming unit; DNP, 2,4-dinitrophenol; HSA, human serum albumin; ES, embryonic stem; hTryptase, human tryptase; MITF, microphthalmia transcription factor; mMCP, mouse MC protease; PCA, passive cutaneous anaphylaxis; *tg/tg*, WBB6F_1-*tg/tg* mouse; TNF-α, tumor necrosis factor-α; *W*^{sh}/*W*^{sh}, B6.Cg-*Kit*$^{W-sh}$/*Kit*$^{W-sh}$ mouse; *W*/*W*^{v}, WBB6F_1-*Kit*^{W}/*Kit*$^{W-v}$ mouse.

TEC – MD Anderson Publicaciones (34; 7 en revisión)

Central Role of Muc5ac Expression in Mucous Metaplasia and Its Regulation by Conserved 5′ Elements

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Mucus hypersecretion contributes to morbidity and mortality in many obstructive lung diseases. Gel-forming mucins are the chief glycoprotein components of airway mucus, and elevated expression of these during mucous metaplasia precedes the hypersecretory phenotype. Five orthologous genes (MUC2, MUC5AC, MUC5B, MUC6, and MUC19) encode the mammalian gel-forming mucin family, and several have been implicated in asthma, cystic fibrosis, and chronic obstructive pulmonary disease pathologies. However, in the absence of a comprehensive analysis, their relative contributions remain unclear. Here, we assess the expression of the entire gel-forming mucin gene family in allergic mouse airways and show that Muc5ac is the predominant gel-forming mucin induced. We previously showed that the induction of mucous metaplasia in ovalbumin-sensitized and -challenged mouse lungs occurs within bronchial Clara cells. The temporal induction and localization of Muc5ac transcripts correlate with the induced expression and localization of mucin glycoproteins in bronchial airways. To better understand the tight regulation of Muc5ac expression, we analyzed all available 5′-flanking sequences of mammalian MUC5AC orthologs and identified evolutionarily conserved regions within domains proximal to the mRNA coding region. Analysis of luciferase reporter gene activity in a mouse transformed Clara cell line demonstrates that this region possesses strong promoter activity and harbors multiple conserved transcription factor–binding motifs. In particular, SMAD4 and HIF-1α bind to the promoter, and mutation of their recognition motifs abolishes promoter function. In conclusion, Muc5ac expression is the central event in antigen-induced mucous metaplasia, and phylogenetically conserved 5′ noncoding domains control its regulation.

Keywords: mucin; metaplasia; airway; lung; epithelium

CLINICAL RELEVANCE

Mucus hypersecretion is associated with asthma and chronic obstructive pulmonary disease. MUC5AC is the most abundant gel-forming mucin present at the airway surface. Determining its expression and regulation in mice will allow us to identify its function and potentially useful and novel targets.

In the lungs, the conducting airways are lined by ciliated and nonciliated epithelial cells residing beneath a multiphase mucus film that has a superficial periciliary layer and an overlaying gel layer. Airway mucus is comprised of water, ions, polypeptides, cells, and cellular debris that are contained within a viscoelastic glycoprotein-rich gel (1). Functionally, airway mucus prevents desiccation of the underlying epithelium and traps inhaled particles and pathogens, allowing for their elimination by mucociliary clearance. Under healthy conditions, the steady-state regulation of the height and osmolarity of the periciliary layer and the thickness and composition of the gel layer allows for efficient mucociliary clearance. However, in obstructive lung diseases such as asthma, cystic fibrosis (CF), constrictive bronchiolitis, and chronic obstructive pulmonary disease (COPD), as well as in animal models of these diseases, mucus hypersecretion results in worsening of morbidity and mortality (for reviews, see Refs. 2 and 3). The hallmark of this mucous phenotype is elevated mucin production by surface epithelial cells, especially within the small (< 2 mm diameter) airways, by a process termed mucous (or goblet cell) metaplasia. In humans and in antigen-challenged mice, mucin production occurs via the induction of mucin gene expression within Clara cells (4, 5). In mice, this process occurs via activation of type 2 helper T-lymphocyte (Th2) (6) and epidermal growth factor (EGF) (7) signal transduction pathways.

Mucins are very high molecular weight glycoproteins that can either be membrane associated or secreted and released into the extracellular space. Membrane mucins are ubiquitously expressed by epithelia in respiratory mucosae, and they participate in cell adhesion and glycocalyx generation; they may also be secreted into the mucus layer as the result of shearing or the synthesis of splice variants lacking transmembrane domains (8, 9). Secreted mucins are expressed by nonciliated epithelial cells in the respiratory epithelium, and they are stored in intracellular secretory granules until stimulated for release by regulated exocytosis. A subset of secreted mucins, the gel-forming mucins, have large heavily O-glycosylated apoprotein cores (> 300 kD) as well as N- and C-terminal cysteine-rich von Willebrand Factor–like domains that participate in disulfide bond-mediated oligimerization. Once secreted, gel-forming mucins create very large (1 to > 10 MegaDalton) viscoelastic macromolecular complexes (10).

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Stimulation of Lung Innate Immunity Protects against Lethal Pneumococcal Pneumonia in Mice

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Rationale: **The lungs are a common site of serious infection in both healthy and immunocompromised subjects, and the most likely route of delivery of a bioterror agent. Since the airway epithelium shows great structural plasticity in response to inflammatory stimuli, we hypothesized it might also show functional plasticity.**
Objectives: **To test the inducibility of lung defenses against bacterial challenge.**
Methods: **Mice were treated with an aerosolized lysate of ultraviolet-killed nontypeable (unencapsulated) *Haemophilus influenzae* (NTHi), then challenged with a lethal dose of live *Streptococcus pneumoniae* (Spn) delivered by aerosol.**
Measurements and Main Results: **Treatment with the NTHi lysate induced complete protection against challenge with a lethal dose of Spn if treatment preceded challenge by 4 to 24 hours. Lesser levels of protection occurred at shorter (83% at 2 h) and longer (83% at 48–72 h) intervals between treatment and challenge. There was also some protection when treatment was given 2 hours after challenge (survival increased from 14 to 57%), but not 24 hours after challenge. Protection did not depend on recruited neutrophils or resident mast cells and alveolar macrophages. Protection was specific to the airway route of infection, correlated in magnitude and time with rapid bacterial killing within the lungs, and was associated with increases of multiple antimicrobial polypeptides in lung lining fluid.**
Conclusions: **We infer that protection derives from stimulation of local innate immune mechanisms, and that activated lung epithelium is the most likely cellular effector of this response. Augmentation of innate antimicrobial defenses of the lungs might have therapeutic value.**

Keywords: innate immunity; pneumonia; immunocompromised host; lung epithelium

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Antimicrobial proteins promote bacterial clearance from the lungs and are inducible in lung cells. However, the efficacy of stimulation of innate immunity in protection against lethal pneumonia is unknown.

What This Study Adds to the Field

Aerosolized treatment with a lysate from nontypeable *Haemophilus influenzae* induced protection against subsequent challenge with *Streptococcus pneumoniae*. These results indicate that augmentation of innate antimicrobial defenses of the lungs may have therapeutic benefit.

Pneumonia is the leading cause of death due to infection worldwide, and affects both healthy persons and those who are immunocompromised (1–3). The susceptibility of the lungs to infection derives from the architectural requirements of gas exchange, resulting in continuous exposure of a large surface area to the outside environment while imposing a minimal barrier to gas diffusion. This precludes protective strategies, such as encasement of the alveolar gas exchange surface in an impermeable barrier, as in the skin, or continuous generation of a heavy blanket of mucus, as in the gastrointestinal tract.

Despite their structural vulnerability, the lungs generally defend themselves successfully against infection through a variety of mechanical, humoral, and cellular mechanisms (4–8). First, most inhaled microbial pathogens fail to penetrate to the alveoli because of impaction or sedimentation against the walls of the conducting airways, where they are entrapped by mucus, then cleared by sneezing, coughing, or mucociliary action. Next, the airway lining fluid contains antibodies and antimicrobial peptides that limit the growth of pathogens that succeed in penetrating the mucus gel layer. Finally, alveolar macrophages that reside in the distal airspaces of the lungs ingest organisms that penetrate to that depth. When necessary, the parenchymal and resident inflammatory cells of the lungs release signaling molecules that result in exudation of plasma proteins and recruitment of leukocytes, although this impairs gas exchange and can be viewed as a defensive strategy of last resort (9).

In addition to defense mechanisms that function at baseline, the secretory cells of the airway epithelium are capable of a remarkable change in structure termed "inflammatory metaplasia." In response to viral, fungal, or allergic inflammation, these cells rapidly increase their height in association with filling of the apical cytoplasm with electron lucent secretory granules and conversion of apical smooth endoplasmic reticulum to rough endoplasmic reticulum (10, 11). Many of these structural changes can be ascribed to increased synthesis of the gel-forming mucin Muc5ac, a component of the innate immune system, although other molecular changes also occur (5, 11–14). The adaptive value of this structural and molecular plasticity of the airway epithelium is presumed to be augmented defense against microbial pathogens. This is supported by the inducible production of antimicrobial proteins by epithelial cells (15–20), which have been shown to contribute to bacterial clearance (21–25).

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Haemophilus influenzae Lysate Induces Aspects of the Chronic Obstructive Pulmonary Disease Phenotype

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Nontypeable *Haemophilus influenzae* (NTHi) commonly colonizes the lower airways of patients with chronic obstructive pulmonary disease (COPD). Whether it contributes to COPD progression is unknown. Here, we determined which aspects of the COPD phenotype can be induced by repetitive exposure to NTHi products. Mice were exposed weekly to an aerosolized NTHi lysate, and inflammation was evaluated by measurement of cells and cytokines in bronchoalveolar lavage fluid (BALF) and immunohistochemical staining; structural changes were evaluated histochemically by periodic acid fluorescent Schiff's reagent, Masson's trichrome, and Picrosirius red staining; mucin gene expression was measured by quantitative RT-PCR; and the role of TNF-α was examined by transgenic airway overexpression and use of an inhibitory antibody. NTHi lysate induced rapid activation of NF-κB in airway cells and increases of inflammatory cytokines and neutrophils in BALF. Repetitive exposure induced infiltration of macrophages, CD8+ T cells, and B cells around airways and blood vessels, and collagen deposition in airway and alveolar walls, but airway mucin staining and gel-forming mucin transcripts were not increased. Transgenic overexpression of TNF-α caused BALF neutrophilia and inflammatory cell infiltration around airways, but not fibrosis, and TNF-α neutralization did not reduce BALF neutrophilia in response to NTHi lysate. In conclusion, NTHi products elicit airway inflammation in mice with a cellular and cytokine profile similar to that in COPD, and cause airway wall fibrosis but not mucous metaplasia. TNF-α is neither required for inflammatory cell recruitment nor sufficient for airway fibrosis. Colonization by NTHi may contribute to the pathogenesis of small airways disease in patients with COPD.

Keywords: pulmonary disease, chronic obstructive; *Haemophilus influenzae*; bronchiolitis; inflammation; fibrosis

CLINICAL RELEVANCE

Nontypeable *Haemophilus influenzae* (NTHi) commonly colonizes the airways of patients with chronic obstructive pulmonary disease (COPD). Whether NTHi colonization contributes to COPD progression is unknown. Our findings define which aspects of COPD might be induced by repetitive exposure to NTHi to help future clinical research.

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is not fully reversible (1–4). COPD is thought to be caused by inflammation induced by inhaled smoke and particulates, and possibly by infecting pathogens as well, leading to the structural changes in airways and alveoli that result in airflow limitation. At the level of the conducting airways, there is metaplasia of the airway epithelium to a mucus hypersecreting phenotype that causes lumenal obstruction, thickening of the airway wall from increased deposition of matrix molecules and proliferation of mesenchymal cells, and narrowing of the airway from fibrosis. In the peripheral lung, there is destruction of alveolar walls leading to a reduction in the radial tethering that normally helps to hold conducting airways open and an enlargement of distal airspaces (5–8).

In histopathologic specimens of distal lung and in bronchoalveolar lavage fluid (BALF) from patients with COPD, macrophages, neutrophils, and CD8 + T cells are prominent (9–11). This cellular inflammation is accompanied by increased levels of inflammatory mediators, notably TNF-α, IL-6, IFN-γ, and the chemokine IL-8 (12–14). A striking feature of COPD is that even after withdrawal of the usual inciting stimulus, cigarette smoke, inflammation persists and lung function continues to deteriorate (15). Several possibilities have been proposed to explain the persistent inflammation: self-perpetuation of the immune response by autoantigens resulting from inflammatory and oxidative lung injury, persistent or recurrent infection of damaged airways as a co-stimulator, or antigenic mimicry or as a polyclonal activator, which could provide a persisting antigenic stimulus and maintain the inflammatory process (16, 17).

Nontypeable (unencapsulated) *Haemophilus influenzae* (NTHi) is present frequently in the airways of adults with COPD (18–21). In addition to colonization during clinically stable periods, acquisition of new strains of NTHi is an important cause of lower respiratory tract infection resulting in exacerbations of COPD (22–25). Incubation of cultured human bronchial epithelial cells with endotoxin from NTHi leads to markedly increased expression and release of proinflammatory mediators, including IL-6, IL-8, and TNF-α (26). Together, these findings suggest that persistent or repetitive exposure of the airway to NTHi products may contribute to airway inflammation in COPD (22).

Animal studies have been critical in shaping contemporary views of the pathogenesis of asthma and COPD. So far, animal models of experimentally induced COPD have included inhalation of noxious agents (cigarette smoke, SO_2, NO_2, and ozone), instillation of elastase, and generation of genetic models that mimic particular aspects of the complex pathogenesis of this disease (27). To help determine which aspects of the COPD phenotype can be ascribed to exposure to NTHi products, we established a mouse model of repetitive exposure to an aerosolized NTHi lysate and characterized the inflammatory and

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Synaptotagmin 2 Couples Mucin Granule Exocytosis to Ca^{2+} Signaling from Endoplasmic Reticulum*[S]

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Synaptotagmin 2 (Syt2) functions as a low affinity, fast exocytic Ca^{2+} sensor in neurons, where it is activated by Ca^{2+} influx through voltage-gated channels. Targeted insertion of *lacZ* into the mouse *syt2* locus reveals expression in mucin-secreting goblet cells of the airways. In these cells, rapid Ca^{2+} entry from the extracellular medium does not contribute significantly to stimulated secretion (Davis, C. W., and Dickey, B. F. (2008) *Annu. Rev. Physiol.* 70, 487–512). Nonetheless, $Syt2^{-/-}$ mice show a severe defect in acute agonist-stimulated airway mucin secretion, and $Syt2^{+/-}$ mice show a partial defect. In contrast to $Munc13\text{-}2^{-/-}$ mice (Zhu, Y., Ehre, C., Abdullah, L. H., Sheehan, J. K., Roy, M., Evans, C. M., Dickey, B. F., and Davis, C. W. (2008) *J. Physiol. (Lond.)* 586, 1977–1992), $Syt2^{-/-}$ mice show no spontaneous mucin accumulation, consistent with the inhibitory action of Syt2 at resting cytoplasmic Ca^{2+} in neurons. In human airway goblet cells, inositol trisphosphate receptors are found in rough endoplasmic reticulum that closely invests apical mucin granules, consistent with the known dependence of exocytic Ca^{2+} signaling on intracellular stores in these cells. Hence, Syt2 can serve as an exocytic sensor for diverse Ca^{2+} signaling systems, and its levels are limiting for stimulated secretory function in airway goblet cells.

Mucin secretion in the airways of the lungs is crucial for clearance of inhaled particulates and pathogens (1). However, mucin hypersecretion is a leading cause of mortality in common diseases such as asthma and cystic fibrosis (2). Thus, tight control of mucin secretion is critical for lung homeostasis. Airway mucin secretion is stimulated by triphosphate nucleotides secreted into the extracellular lumenal liquid layer (3). These bind to epithelial apical $P2Y_2$ receptors that activate G_q, which in turn activates phospholipase $C\beta_1$ generating the intracellular second messengers diacylglycerol and inositol trisphosphate (IP_3).[3] Diacylglycerol directly induces mucin granule exocytosis by activating the priming protein Munc13-2 (4), and indirectly regulates exocytosis by activating protein kinase Cε (5). IP_3 induces the release of Ca^{2+} from intracellular stores, resulting in a rise in cytoplasmic Ca^{2+} that rapidly triggers mucin granule exocytosis (6–9). However, the precise mechanism by which a rise in cytoplasmic Ca^{2+} is coupled to exocytosis in goblet cells is not known.

Synaptotagmins (Syts) are a family of structurally related proteins of which several are known to mediate Ca^{2+}-dependent exocytosis. Syts are composed of a short intravesicular amino terminus, a transmembrane domain, a variable linker region, and two conserved C_2 domains near the carboxyl terminus (10, 11). There are at least 15 Syt family members encoded in mammalian genomes. Of these, eight (Syt1–3, -5–7, -9, and -10) display Ca^{2+}-dependent phospholipid binding that is thought to be essential for Ca^{2+}-dependent exocytosis (12–16). A subset of three of these (Syt1, -2, and -9) binds Ca^{2+} with low affinity (~10 μM) and high cooperativity ($n = 5$) and functions as fast, synchronous Ca^{2+} sensors in neurons (16). Syt1 mediates synchronous synaptic vesicle release in forebrain neurons, and also mediates rapid exocytosis in adrenal chromaffin cells (15, 16). Like neurons, chromaffin cells express voltage-gated Ca^{2+} channels activated by neurotransmitter-induced depolarization. Syt2 mediates synchronous synaptic vesicle release in hindbrain neurons and at the neuromuscular junction (14, 17), but it has not been previously known to function outside the nervous system. Syt9 mediates synchronous synaptic vesicle release from limbic and striatal neurons (16), and it also functions in dense core granule release from the PC12 chromaffin cell line (18–20) and insulin release from pancreatic islet cells (21, 22). In islet cells, membrane depolarization and opening of voltage-gated Ca^{2+} channels is induced by closure of K_{ATP} channels when blood glucose is elevated. To our knowledge, there has been no analysis of the function of a low Ca^{2+} affinity, fast Syt in a nonexcitable cell (*i.e.* a cell not expressing voltage-gated Ca^{2+} channels).

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[3] The abbreviations used are: IP_3, inositol trisphosphate; IP_3-R, inositol trisphosphate receptor; Syt2, synaptotagmin 2; CCSP, Clara cell secretory protein; PDI, protein disulfide isomerase; MUC5AC, human mucin 5AC; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; PAFS, periodic acid-fluorescent Schiff; WT, wild type; ER, endoplasmic reticulum; PBS, phosphate-buffered saline; IL, interleukin.

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LETTERS

Compound vesicle fusion increases quantal size and potentiates synaptic transmission

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Exocytosis at synapses involves fusion between vesicles and the plasma membrane[1]. Although compound fusion between vesicles[2,3] was proposed to occur at ribbon-type synapses[4,5], whether it exists, how it is mediated, and what role it plays at conventional synapses remain unclear. Here we report the existence of compound fusion, its underlying mechanism, and its role at a nerve terminal containing conventional active zones in rats and mice. We found that high potassium application and high frequency firing induced giant capacitance up-steps, reflecting exocytosis of vesicles larger than regular ones, followed by giant down-steps, reflecting bulk endocytosis. These intense stimuli also induced giant vesicle-like structures, as observed with electron microscopy, and giant miniature excitatory postsynaptic currents (mEPSCs), reflecting more transmitter release. Calcium and its sensor for vesicle fusion, synaptotagmin, were required for these giant events. After high frequency firing, calcium/synaptotagmin-dependent mEPSC size increase was paralleled by calcium/synaptotagmin-dependent post-tetanic potentiation. These results suggest a new route of exocytosis and endocytosis composed of three steps. First, calcium/synaptotagmin mediates compound fusion between vesicles. Second, exocytosis of compound vesicles increases quantal size, which increases synaptic strength and contributes to the generation of post-tetanic potentiation. Third, exocytosed compound vesicles are retrieved via bulk endocytosis. We suggest that this vesicle cycling route be included in models of synapses in which only vesicle fusion with the plasma membrane is considered[1].

We performed cell-attached capacitance recordings at the release face of calyces in brainstem (Fig. 1a)[6]. Brainstem slices were from rats, unless otherwise mentioned. Before KCl application (control), spontaneous up-steps occurred at 0.008 ± 0.001 Hz ($n = 78$ patches, Fig. 1a, see Methods). About 94% of up-steps were <220 aF (mean = 84 ± 4 aF, $n = 96$ up-steps). The remaining 6% were 220–380 aF (mean = 275 ± 22 aF, $n = 6$ up-steps, Fig. 1b). A regular vesicle's capacitance is ~23–220 aF, because the vesicle diameter is ~30–80 nm in calyces[7], and the specific membrane capacitance is 8–11 fF μm^{-2} (ref. 8). Thus, most up-steps in the control were caused by single vesicle fusion (see also ref. 6).

KCl application (~50–100 mM, unless mentioned) increased the up-step frequency to 0.16 ± 0.02 Hz ($n = 17$ patches, Fig. 1a, see Methods). The up-step amplitude distribution and cumulative probability curve were shifted to the right, compared to control (Fig. 1b, Kolmogorov–Smirnov test, $P < 0.001$). The mean of all up-steps was 193 ± 9 aF ($n = 857$), or ~203% of control (95 ± 5 aF, $n = 102$, $P < 0.01$). About 20% of up-steps were > 220 aF, a regular vesicle's maximal capacitance (Fig. 1a, b), and were called 'giant' up-steps. Their mean was 571 ± 37 aF ($n = 174$ up-steps) and the largest was 2,878 aF, or 34–40 times the mean of regular vesicles (73 aF in ref. 6, 84 aF here). Both the percentage and the mean size of giant up-steps were significantly higher than control (χ^2 test and *t*-test, $P < 0.01$). Even when up-steps between 100 and 220 aF were measured, KCl significantly increased the amplitude from 133 ± 6 aF ($n = 28$) in control to 144 ± 2 aF ($n = 294$, $P < 0.05$), suggesting compound fusion between 2–3 regular vesicles. Thus, up-step size increase was not limited to giant up-steps.

Giant up-steps were not caused by dense-core vesicle fusion, as dense-core vesicles are rare in calyces[7]. Giant up-steps were not composed of multiple small up-steps, because, even when the time resolution was increased to 0.6–0.9 ms, giant up-steps rose from 20% to 80% in one step ($n = 44$ giant up-steps, 3 patches, Fig. 1a). In 6% of giant up-steps, we detected an initial fusion pore conductance (G_p) of 162 ± 41 pS ($n = 10$ up-steps), which gradually increased for ~10–230 ms, and ended in rapid pore expansion (Fig. 1c). G_p in remaining giant up-steps was too large or too fast to resolve (Fig. 1a). Most giant up-steps could not be explained by independent fusion of multiple vesicles, because the up-step frequency (~0.16 Hz) was too low to account for the observed large percentage (~20%) of giant up-steps (Supplementary Information II-1). Thus, giant up-steps reflect fusion of a single structure larger than regular vesicles.

KCl application also increased the frequency of down-steps, which reflected endocytosis, from 0.006 ± 0.002 Hz in control ($n = 78$ patches) to 0.10 ± 0.01 Hz ($n = 17$ patches; $P < 0.01$, Fig. 1d), and shifted the down-step size distribution (Supplementary Information II-2) and cumulative probability curve to the right (Fig. 1e, Kolmogorov–Smirnov test, $P < 0.01$). The percentage (17%) and the mean amplitude (501 ± 42 aF, $n = 102$ down-steps; range: 220–2,943 aF) of giant down-steps during KCl application were significantly higher than control (8%, χ^2 test, $P < 0.01$; 331 ± 31 aF, $n = 6$, $P < 0.01$). In 5% of giant down-steps, we detected an initial fission pore conductance of 376 ± 113 pS ($n = 5$), corresponding to a pore with a diameter of 2.7 ± 1.5 nm, which reduced at 36 ± 11 pS ms^{-1} or 0.82 ± 0.46 nm ms^{-1} to an undetectable level (Fig. 1c). Thus, KCl induced more and larger giant down-steps, which reflect bulk endocytosis[9–11].

Endosome-like structures formed by bulk endocytosis may undergo exocytosis[12]. For two reasons, this mechanism did not underlie giant up-steps. First, the first giant up-step occurred ~80 s earlier than the first giant down-step after KCl application ($n = 17$ patches, $P < 0.001$, Fig. 1f, g), and was no later than the first regular-sized down-step (Supplementary Information II-3), suggesting that it was not caused by re-exocytosis of endocytic structures. Second, when calyces were whole-cell dialyzed with GDPβS (0.3 mM) to block endocytosis[13], the up-step frequency (0.24 ± 0.07 Hz, $n = 6$ patches) and the percentage (28%) of giant up-steps were similar to those without GDPβS

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Synaptotagmin-2 Controls Regulated Exocytosis but Not Other Secretory Responses of Mast Cells*

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Mast cell degranulation is a highly regulated, calcium-dependent process, which is important for the acute release of inflammatory mediators during the course of many pathological conditions. We previously found that Synaptotagmin-2, a calcium sensor in neuronal exocytosis, was expressed in a mast cell line. We postulated that this protein may be involved in the control of mast cell-regulated exocytosis, and we generated Synaptotagmin-2 knock-out mice to test our hypothesis. Mast cells from this mutant animal conferred an abnormally decreased passive cutaneous anaphylaxis reaction on mast cell-deficient mice that correlated with a specific defect in mast cell-regulated exocytosis, leaving constitutive exocytosis and nonexocytic mast cell effector responses intact. This defect was not secondary to abnormalities in the development, maturation, migration, morphology, synthesis, and storage of inflammatory mediators, or intracellular calcium transients of the mast cells. Unlike neurons, the lack of Synaptotagmin-2 in mast cells was not associated with increased spontaneous exocytosis.

Mast cells (MCs)[2] participate in adaptive and innate immune responses. Their secreted products play important roles in immunoglobulin E (IgE)-dependent inflammatory reactions such as allergic asthma and anaphylaxis (1) and are also involved in other forms of inflammation such as immune arthritis (2, 3) and innate immune responses to bacterial infections (4, 5). Upon activation, MCs exhibit three main secretory responses: release of granule contents (*i.e.* degranulation), secretion of prostaglandins and leukotrienes, and secretion of cytokines and growth factors (6). The exocytic release of preformed mediators (*e.g.* histamine and proteases) stored in secretory granules is immediate and regulated at the step of fusion between the membrane of the granule and the plasma membrane. Thus, it is an example of regulated exocytosis, like neuronal synaptic neurotransmitter release and insulin secretion (7). Another early event is the release of metabolites of arachidonic acid (*e.g.* prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4)). These eicosanoids cross the plasma membrane using transmembrane transporters (8), and their production is regulated by the activation of their synthetic enzymes (9). A late response after MC activation is the secretion of cytokines and growth factors (*e.g.* tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4)). The gap in time of minutes to hours between stimulation and the secretion of these mediators is explained by the fact that regulation is at the transcriptional and post-transcriptional levels, with secretion occurring via constitutive exocytosis (10).

A common intracellular mediator linking the stimulation event to these three MC responses is calcium (Ca^{2+}) that is released into the cytoplasm from intracellular stores and introduced from the extracellular environment via specialized channels. Increase in the cytoplasmic concentration of Ca^{2+} ($[Ca^{2+}]_i$) is required for the activation of phospholipase A2 and other enzymes in the synthetic pathway of prostaglandins and leukotrienes, for the expression of cytokines and growth factors, and for the regulated exocytosis of MC secretory granules (11).

MC degranulation is a highly regulated process, and, like other exocytic events, it requires the participation of soluble NSF (*N*-ethylmaleimide-sensitive fusion protein) adaptor protein receptor (SNARE) proteins. These are present both on the cytoplasmic surfaces of secretory vesicles (v-SNAREs such as vesicle-associated membrane protein, VAMP) and of the plasma membrane (target or t-SNAREs such as Syntaxin and synaptosome-associated protein of 25 kDa, SNAP-25). Cognate v- and t-SNAREs form a highly stable quadruple α-helix complex (core complex) apposing the vesicle and target membranes. According to the "zipper hypothesis" the coiling of this complex drives the fusion between these membranes, opening a fusion pore and releasing the vesicle contents into the extracellular space (12). This step is Ca^{2+}-dependent and is under the control of multiple proteins such as Synaptotagmin (Syt) (13).

There are at least 15 Syts in mammals, and several of them mediate Ca^{2+}-dependent exocytosis. All Syts have a short

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[2] The abbreviations used are: MC, mast cell; BMMC, bone marrow-derived MC; $[Ca^{2+}]_i$, cytoplasmic Ca^{2+} concentration; C_m, plasma membrane capacitance; Δ, change; DNP, 2,4-dinitrophenol; EM, electron microscopy; FcεRIα, α-subunit of the high affinity receptor for IgE; FITC, fluorescein isothiocyanate; HSA, human serum albumin; IgE, immunoglobulin E; IL, interleukin; LTC_4, leukotriene C_4; NSF, *N*-ethylmaleimide-sensitive fusion protein; P16, postnatal day 16; PCA, passive cutaneous anaphylaxis; PGD_2, prostaglandin D_2; SCF, stem cell factor; SNAP-25, synaptosome-associated protein of 25 kDa; SNARE, soluble NSF adaptor protein receptor; Syt, Synaptotagmin; TNF-α, tumor necrosis factor α; VAMP, vesicle-associated membrane protein; W^{sh}/W^{sh}, MC-deficient B6.Cg-$Kit^{W\text{-}sh}$/$Kit^{W\text{-}sh}$ mouse; GTP-γS, guanosine 5′-3-*O*-(thio)triphosphate.

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Ca^{2+} and calmodulin initiate all forms of endocytosis during depolarization at a nerve terminal

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Although endocytosis maintains synaptic transmission, how endocytosis is initiated is unclear. We found that calcium influx initiated all forms of endocytosis at a single nerve terminal in rodents, including clathrin-dependent slow endocytosis, bulk endocytosis, rapid endocytosis and endocytosis overshoot (excess endocytosis), with each being evoked with a correspondingly higher calcium threshold. As calcium influx increased, endocytosis gradually switched from very slow endocytosis to slow endocytosis to bulk endocytosis to rapid endocytosis and to endocytosis overshoot. The calcium-induced endocytosis rate increase was a result of the speeding up of membrane invagination and fission. Pharmacological experiments suggested that the calcium sensor mediating these forms of endocytosis is calmodulin. In addition to its role in recycling vesicles, calcium/calmodulin-initiated endocytosis facilitated vesicle mobilization to the readily releasable pool, probably by clearing fused vesicle membrane at release sites. Our findings provide a unifying mechanism for the initiation of various forms of endocytosis that are critical in maintaining exocytosis.

After vesicle exocytosis, endocytosis generates new vesicles, which replenishes the vesicle pool and maintains exocytosis[1]. Five different kinetic forms of endocytosis have been reported. First, slow endocytosis, which takes tens of seconds, has been observed widely at synapses and non-neuronal secretory cells[2–7]. It is mediated by a classical, clathrin-dependent mechanism[5,8–10]. Second, rapid endocytosis, which takes a few seconds, has been observed at ribbon-type and calyx-type synapses[2,6], but its existence is debated at small synapses[1]. Rapid endocytosis may be clathrin independent in chromaffin cells and goldfish ribbon-type synapses[8,10]. Third, bulk endocytosis, which forms large endosome-like structures by retrieving large pieces of membrane, has been seen at many synapses after strong stimulation (for examples, see refs. 11,12). Fourth, endocytosis overshoot, which retrieves more membrane than was exocytosed by the stimulation, has been observed in non-neuronal secretory cells[13] and calyx-type synapses[14]. The mechanisms mediating bulk endocytosis and endocytosis overshoot are largely unclear. Finally, very slow or absent endocytosis has been observed[15,16].

Because of these various forms, endocytosis in different conditions differs markedly in speed, amount and vesicle size. The mechanisms that initiate these different forms and rates of endocytosis are unclear. It has been proposed that the same mechanism underlies rapid and slow rates, with individual events occurring stochastically[17]. Another view is that different rates depend on the ratio between simultaneously occurring rapid and slow endocytosis pathways[2,4,6,18], although what determines this ratio is somewhat controversial. For example, calcium facilitates rapid endocytosis at calyceal synapses[6], but was reported to facilitate or inhibit rapid endocytosis in different studies at ribbon synapses[2,4,18]. It has been suggested that calcium regulates the endocytic rate at many nerve terminals[3,19–25]. Whether calcium initiates endocytosis remains unclear, as quantitative measurements did not reveal a complete block of endocytosis when calcium influx was reduced[3,25]. Because of the difficulty in identifying the endocytic trigger, it is often assumed that endocytosis follows exocytosis automatically, perhaps through molecular coupling.

Wee studied the mechanisms that initiate various forms of endocytosis with capacitance measurements at a large nerve terminal, the calyx of Held. We found that calcium influx initiated slow endocytosis, bulk endocytosis, rapid endocytosis and endocytosis overshoot with increasingly higher thresholds. Pharmacological experiments suggested that the calcium sensor mediating these forms of endocytosis was calmodulin. Our results may explain how exocytosis in single nerve terminals is maintained by various forms of endocytosis in various physiological conditions.

RESULTS

Calcium influx initiates slow endocytosis

Similar to trains of action potential-like stimuli[6,14], a 20-ms depolarization (from −80 to +10 mV, unless indicated otherwise) at the calyx induced slow endocytosis with a time constant (τ) of 15.6 ± 2.1 s and an initial endocytosis rate ($rate_{endo}$) of 39.2 ± 7.1 fF s^{-1} ($n = 12$; Fig. 1a) in control conditions, in which the extracellular calcium concentration ($[Ca^{2+}]_o$) was 2 mM and the pipette contained 50 μM BAPTA. Decreasing the $[Ca^{2+}]_o$ from 5.5 to 0.5 mM decreased the calcium

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Curcumin inhibits COPD-like airway inflammation and lung cancer progression in mice

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Recent studies have demonstrated that K-ras mutations in lung epithelial cells elicit inflammation that promotes carcinogenesis in mice (intrinsic inflammation). The finding that patients with chronic obstructive pulmonary disease (COPD), an inflammatory disease of the lung, have an increased risk of lung cancer after controlling for smoking suggests a further link between lung cancer and extrinsic inflammation. Besides exposure to cigarette smoke, it is thought that airway inflammation in COPD is caused by bacterial colonization, particularly with non-typeable *Hemophilus influenzae* (NTHi). Previously, we have shown that NTHi-induced COPD-like airway inflammation promotes lung cancer in an airway conditional K-ras-induced mouse model. To further test the role of inflammation in cancer promotion, we administered the natural anti-inflammatory agent, curcumin, 1% in diet before and during weekly NTHi exposure. This significantly reduced the number of visible lung tumors in the absence of NTHi exposure by 85% and in the presence of NTHi exposures by 53%. Mechanistically, curcumin markedly suppressed NTHi-induced increased levels of the neutrophil chemoattractant keratinocyte-derived chemokine by 80% and neutrophils by 87% in bronchoalveolar lavage fluid. *In vitro* studies of murine K-ras-induced lung adenocarcinoma cell lines (LKR-10 and LKR-13) indicated direct anti-tumoral effects of curcumin by reducing cell viability, colony formation and inducing apoptosis. We conclude that curcumin suppresses the progression of K-ras-induced lung cancer in mice by inhibiting intrinsic and extrinsic inflammation and by direct anti-tumoral effects. These findings suggest that curcumin could be used to protract the premalignant phase and inhibit lung cancer progression in high-risk COPD patients.

Introduction

Lung cancer is the major cause of cancer-related mortality in both men and women worldwide (1,2). Cigarette smoking causes 90% of all lung cancers and is thought to do so primarily by inducing DNA mutations (3). In addition, epidemiologic data indicate that chronic inflammation also plays a role in lung epithelial carcinogenesis (4). Tumor cells produce cytokines and chemokines that attract leukocytes, which provide an inflammatory microenvironment in favor of malignant conversion and tumor development (intrinsic inflammation) (5–7). Lung inflammatory diseases such as chronic obstructive pulmonary disease (COPD) are characterized by leukocyte infiltration of the airways that is regulated by a variety of mediators such as cytokines, chemokines and adhesion molecules (extrinsic inflammation). Multiple studies have found that smokers with COPD have a 1.3- to 4.9-fold increased risk of lung cancer compared with smokers without COPD (4,8,9). In view of the high incidence and mortality of lung cancer (10), the high prevalence and morbidity of COPD (11), and the lack of a recommended screening method for lung cancer, a therapeutic strategy that targets inflammation to prevent progression of COPD and cancer would be of great value.

Despite the fact that smoking causes most cases of COPD, only 25% of smokers develop COPD. This variable susceptibility to COPD most probably reflects genetic variations in the inflammatory response to inhaled smoke and to microorganisms colonizing the injured airways of smokers (12,13). The most common colonizing bacterium is non-typeable (i.e. unencapsulated) *Haemophilus influenzae* (NTHi) (14,15). This organism is found in the lower respiratory tract of ~30% of individuals with COPD at any time, and the acquisition of new serotypes is associated with exacerbations of COPD (14,16–18). We have previously established a COPD-like model of airway inflammation induced by repetitive exposure to an aerosolized lysate of NTHi (19) and shown that this type of inflammation enhances lung carcinogenesis in a K-ras induced mouse model (20).

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a naturally occurring polyphenolic phytochemical isolated from the rhizome of the medicinal plant *Curcuma longa* (turmeric) (21). It is commonly used as a spice, food additive or dietary pigment. It has been shown that curcumin has several pharmacologic effects including anti-inflammatory (22–26), antioxidant (27,28), anti-tumoral (29–34) and wound healing activities (35,36). There are only limited and contradictory data available on the effects of curcumin on lung inflammation and tumor promotion (37–39). In this study, we report that dietary administration of curcumin effectively suppresses NTHi-induced COPD-like airway inflammation and lung cancer progression in mice.

Materials and methods

Animals

Specific pathogen-free 5- to 6-week-old female C57BL/6 mice were purchased from Harlan (Indianapolis, IN). $CCSP^{Cre}$/LSL-K-ras^{G12D} mice (CC-LR) were generated as described previously (20). Briefly, this is a mouse generated by crossing a mouse harboring the LSL-K-ras^{G12D} allele with a mouse containing Cre recombinase inserted into the Clara cell secretory protein (CCSP) locus (20). All mice were housed in specific pathogen-free conditions and handled in accordance with the Institutional Animal Care and Use Committee of M. D. Anderson Cancer Center. Mice were monitored daily for evidence of disease or death.

Curcumin treatment

Female wild type (WT) C57BL/6 and CC-LR mice were fed a powdered diet (5053 Pico Lab Rodent Diet 20, Purina LabDiet, Richmond, IN) mixed with 0.2, 0.5, 1 and 2% wt/wt curcumin from 7 days before NTHi lysate exposure to the end of the study. Curcumin (curcumin 78.1%, demethoxycurcumin 17.7% and bisdemethoxycurcumin 4.2%) was purchased from Sigma–Aldrich (St Louis, MO). Curcumin administration did not alter the weight of mice, and consumption of the diet with curcumin was not noticeably different from the diet without curcumin.

NTHi lysate aerosol exposure

A lysate of NTHi strain 12 was prepared as described previously (19), the protein concentration was adjusted to 2.5 mg/ml in phosphate buffered saline (PBS), and the lysate was frozen in 10 ml aliquots at −80°C. To deliver the lysate to mice by aerosol, a thawed aliquot was placed in an AeroMist CA-209 nebulizer (CIS-US, Bedford, MA) driven by 10 l/min of room air supplemented with 5% CO_2 for 20 min. WT mice were exposed to the lysate starting at 6 weeks of age for 1 week, and CC-LR and LSL-K-ras^{G12D} mice were exposed starting at 6 weeks of age once a week for 8 weeks.

Abbreviations: BALF, bronchoalveolar lavage fluid; CC-LR, $CCSP^{Cre}$/LSL-K-ras^{G12D}mice; CCSP, Clara cell secretory protein; COPD, chronic obstructive pulmonary disease; IL, interleukin; KC, keratinocyte-derived chemokine; NTHi, non-typeable *Hemophilus influenzae*; PBS, phosphate buffered saline; WT, wild type.

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RESPIRATORY RESEARCH

RESEARCH **Open Access**

Host lung gene expression patterns predict infectious etiology in a mouse model of pneumonia

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Abstract

Background: Lower respiratory tract infections continue to exact unacceptable worldwide mortality, often because the infecting pathogen cannot be identified. The respiratory epithelia provide protection from pneumonias through organism-specific generation of antimicrobial products, offering potential insight into the identity of infecting pathogens. This study assesses the capacity of the host gene expression response to infection to predict the presence and identity of lower respiratory pathogens without reliance on culture data.

Methods: Mice were inhalationally challenged with *S. pneumoniae*, *P. aeruginosa*, *A. fumigatus* or saline prior to whole genome gene expression microarray analysis of their pulmonary parenchyma. Characteristic gene expression patterns for each condition were identified, allowing the derivation of prediction rules for each pathogen. After confirming the predictive capacity of gene expression data in blinded challenges, a computerized algorithm was devised to predict the infectious conditions of subsequent subjects.

Results: We observed robust, pathogen-specific gene expression patterns as early as 2 h after infection. Use of an algorithmic decision tree revealed 94.4% diagnostic accuracy when discerning the presence of bacterial infection. The model subsequently differentiated between bacterial pathogens with 71.4% accuracy and between non-bacterial conditions with 70.0% accuracy, both far exceeding the expected diagnostic yield of standard culture-based bronchoscopy with bronchoalveolar lavage.

Conclusions: These data substantiate the specificity of the pulmonary innate immune response and support the feasibility of a gene expression-based clinical tool for pneumonia diagnosis.

Background

Pneumonias result in substantial mortality, causing more premature death and disability worldwide than any other disease [1]. Unfortunately, while patient survival depends upon the rapid identification of infecting pathogens [2], the means for prompt and accurate diagnoses of pulmonary infections remain inadequate.

Despite widespread acceptance as the diagnostic tool of choice for unexplained pulmonary infiltrates [3-5], fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) provides an unambiguous diagnosis in only 25-51% of cases [2,4,6-9]. The diagnostic utility of BAL is predicated on culturing pathogens from lavage effluent, without accounting for ongoing antibiotic therapy, non-pathogenic microbial colonization, or the technical challenge of navigating the bronchoscope into involved airways. Molecular techniques, such as antigen detection and polymerase chain reaction (PCR) testing, enhance BAL sensitivity for a subset of pathogens, but still often fail to explain infiltrates [7].

Often regarded as passive gas exchange barriers, the active responses of the lungs are critical to protection from infections. In the presence of inflammatory stimuli, the respiratory epithelia rapidly recruit inflammatory cells and undergo remarkable structural and functional

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Cancer Prevention Research

Research Article

Interleukin 6, but Not T Helper 2 Cytokines, Promotes Lung Carcinogenesis

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Abstract

Several epidemiologic studies have found that smokers with chronic obstructive pulmonary disease (COPD), an inflammatory disease of the lung, have an increased risk of lung cancer compared with smokers without COPD. We have shown a causal role for COPD-like airway inflammation in lung cancer promotion in the $CCSP^{Cre}$/LSL-$K\text{-}ras^{G12D}$ mouse model (CC-LR). In contrast, existing epidemiologic data do not suggest any definite association between allergic airway inflammation and lung cancer. To test this, CC-LR mice were sensitized to ovalbumin (OVA) and then challenged with an OVA aerosol weekly for 8 weeks. This resulted in eosinophilic lung inflammation associated with increased levels of T helper 2 cytokines and mucous metaplasia of airway epithelium, similar to what is seen in asthmatic patients. However, this type of inflammation did not result in a significant difference in lung surface tumor number (49 ± 9 in OVA vs. 52 ± 5 in control) in contrast to a 3.2-fold increase with COPD-like inflammation. Gene expression analysis of nontypeable *Haemophilus influenzae* (NTHi)-treated lungs showed upregulation of a different profile of inflammatory genes, including interleukin 6 (IL-6), compared with OVA-treated lungs. Therefore, to determine the causal role of cytokines that mediate COPD-like inflammation in lung carcinogenesis, we genetically ablated IL-6 in CC-LR mice. This not only inhibited intrinsic lung cancer development (1.7-fold) but also inhibited the promoting effect of extrinsic COPD-like airway inflammation (2.6-fold). We conclude that there is a clear specificity for the nature of inflammation in lung cancer promotion, and IL-6 has an essential role in lung cancer promotion. *Cancer Prev Res; 4 (1); 51–64. ©2010 AACR.*

Introduction

Worldwide, lung cancer is the leading cause of cancer mortality and is expected to account for 30% of all male and 26% of all female cancer deaths in 2009 (1). Cigarette smoking is the principal cause of lung carcinogenesis and is thought to do so primarily by inducing DNA mutations (2). However, several studies have found that smokers with chronic obstructive pulmonary disease (COPD), an inflammatory disease of the airways and alveoli, have an increased risk of lung cancer (1.3- to 4.9-fold) compared with smokers with comparable cigarette exposure but without COPD (3–5). It has also been shown that increased lung cancer mortality is associated with a history of COPD, even among persons who had never been active smokers (6). These facts suggest a link between airway inflammation and lung cancer.

We have previously established a COPD-like mouse model of airway inflammation induced by repetitive exposure to an aerosolized lysate of nontypeable *Haemophilus influenzae* (NTHi; ref. 7), which is the most common bacterial colonizer of airways in COPD patients (8, 9). We have shown that this type of inflammation enhances lung carcinogenesis in a *K-ras*–induced mouse model (10). The predominant inflammatory cell types in subjects with COPD are neutrophils, macrophages, $CD8^+$ T lymphocytes, and T helper (Th) 1 and Th17 $CD4^+$ lymphocytes (11, 12). The most prominent cytokines are TNF, interleukin (IL)-6, IFN-γ, and IL-8 (11, 12), and this profile of inflammatory cells and cytokines is recapitulated in our mouse model of COPD-like airway inflammation (7). This is in contrast to asthma, in which the predominant inflammatory cell types are eosinophils, mast cells, and Th2-type $CD4^+$ lymphocytes, and the key cytokines are the Th2 cytokines IL-4, IL-5, IL-9, and IL-13, in both animal models and patients (13–16). Of interest, existing epidemiologic data do not suggest an association between allergic inflammation of the airways and lung cancer, and some even suggest a protective role (17–21). In this study, we tested the role of allergic airway inflammation in lung carcinogenesis in mice and found that it neither promotes nor protects against lung cancer in a K-ras mutant mouse model (CC-LR mouse).

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The Journal of Immunology

Synergistic Interactions of TLR2/6 and TLR9 Induce a High Level of Resistance to Lung Infection in Mice

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Infectious pneumonias exact an unacceptable mortality burden worldwide. Efforts to protect populations from pneumonia have focused historically on antibiotic development and vaccine-enhanced adaptive immunity. However, we have reported recently that the lungs' innate defenses can be induced therapeutically by inhalation of a bacterial lysate that protects mice against otherwise lethal pneumonia. In this study, we tested in mice the hypothesis that TLRs are required for this antimicrobial phenomenon and found that resistance could not be induced in the absence of the TLR signaling adaptor protein MyD88. We then attempted to recapitulate the protection afforded by the bacterial lysate by stimulating the lung epithelium with aerosolized synthetic TLR ligands. Although most single or combination treatments yielded no protection, simultaneous treatment with ligands for TLR2/6 and TLR9 conferred robust, synergistic protection against virulent Gram-positive and Gram-negative pathogens. Protection was associated with rapid pathogen killing in the lungs, and pathogen killing could be induced from lung epithelial cells in isolation. Taken together, these data demonstrate the requirement for TLRs in inducible resistance against pneumonia, reveal a remarkable, unanticipated synergistic interaction of TLR2/6 and TLR9, reinforce the emerging evidence supporting the antimicrobial capacity of the lung epithelium, and may provide the basis for a novel clinical therapeutic that can protect patients against pneumonia during periods of peak vulnerability. *The Journal of Immunology*, **2011, 186: 5916–5926.**

Despite decades of antibiotic development and hygiene programs, pneumonia continues to affect hundreds of millions of people annually and remains the leading cause of infectious mortality worldwide (1–4). In the course of normal ventilation, the sterile lower respiratory tract is recurrently exposed to inhaled pathogens, often resulting in serious infections (5–7). Although the lungs have been regarded traditionally as passive gas exchange barriers, it is now apparent that they have robust intrinsic defense mechanisms that prevent the incidence of lower respiratory tract infections from being much higher (6–13).

Epithelial surfaces that are constantly in contact with microbes, such as the colonic mucosa, constitutively generate antimicrobial effectors to modulate their local microbiome (14). Because lung epithelial cells are exposed only intermittently to pathogens and because chronic immune activation would negatively impact gas exchange, their baseline innate immune activity is relatively low. However, when exposed to pathogens, the lung epithelium rapidly responds by enhancing barrier function, recruiting leukocytes, and expressing antimicrobial products (13).

Viewing this epithelial plasticity as an opportunity for intervention, we recently demonstrated that the lungs' antimicrobial defenses are therapeutically inducible. The inhalational pretreatment of mice with an aerosolized lysate of nontypeable *Haemophilus influenzae* (NTHi) protected against otherwise lethal pneumonia from a variety of pathogens (15–18). Supporting an innate immune mechanism underlying this inducible resistance was the observation of protection against pathogens noncognate with the stimulus, protecting against all of the tested bacterial, fungal, and viral organisms (15, 17, 18). Furthermore, induction of resistance occurred too rapidly for an adaptive immune response (onset within 2 h of treatment, maximal by 24 h) and did not rely upon innate immune leukocytes (neutrophils, macrophages, or mast cells) (15, 17).

In contrast to the highly refined epitope sensing of T and B cell-expressed adaptive immune receptors, innate immune signaling depends upon recognition of conserved pathogen-associated molecular patterns (PAMPs) by host pattern recognition receptors (PRRs). TLRs remain the best characterized of the PRRs (19, 20). They are highly conserved transmembrane proteins, consisting of an ectodomain with multiple leucine-rich repeats for pattern recognition, a membrane-spanning α helix, and a Toll/IL-1 receptor (TIR) domain for intracellular signaling. At least 13 mammalian TLRs have been identified, each specifically localizing to either the plasma membrane or the endosomal membranes and each detecting unique complements of PAMPs (20–22). Upon PAMP recognition, signal transduction occurs via TLR-specific recruitment of cytosolic TIR adaptor protein combinations. The TIR

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Abbreviations used in this article: BAL, bronchoalveolar lavage; CYC, cyclophosphamide; [DC_{max}], ligand concentration resulting in maximal dendritic cell cytokine expression; IRF, IFN regulatory factor; MLPA, monophosphoryl lipid A; mTEC, mouse tracheal epithelial cell; NTHi, nontypeable *Haemophilus influenzae*; ODN, oligodeoxynucleotide; Pam2CSK4, *S*-[2,3-bis(palmitoyloxy)-propyl]-(*R*)-cysteinyl-(lysyl)3-lysine; Pam3CSK4, *N*-palmitoyl-*S*-[2,3-bis(palmitoyloxy)-propyl]-(*R*)-cysteinyl-(lysyl)3-lysine; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; TIR, Toll/IL-1 receptor; TRIF, Toll/IL-1 receptor domain-containing adaptor inducing IFN-β; TSA, tryptic soy agar.

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REVIEW

Nontypeable *Haemophilus influenzae* in chronic obstructive pulmonary disease and lung cancer

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Abstract: Chronic obstructive pulmonary disease (COPD) is predicted to become the third leading cause of death in the world by 2020. It is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases, most commonly cigarette smoke. Among smokers with COPD, even following withdrawal of cigarette smoke, inflammation persists and lung function continues to deteriorate. One possible explanation is that bacterial colonization of smoke-damaged airways, most commonly with nontypeable *Haemophilus influenzae* (NTHi), perpetuates airway injury and inflammation. Furthermore, COPD has also been identified as an independent risk factor for lung cancer irrespective of concomitant cigarette smoke exposure. In this article, we review the role of NTHi in airway inflammation that may lead to COPD progression and lung cancer promotion.

Keywords: COPD, NTHi, inflammation

Introduction

The pooled global prevalence of chronic obstructive pulmonary disease (COPD) in adults aged 40 years or older is ~10%, and it is a leading cause of morbidity and mortality in the US.[1–5] COPD is believed to be caused by inflammation induced by inhaled smoke and particulates, and possibly by infecting pathogens as well, leading to the structural changes in airways and alveoli that result in irreversible airflow limitation (Figure 1). Despite the fact that smoking causes most cases of COPD, only 25% of smokers develop COPD.[6,7] Conversely, epidemiologic data indicate that approximately 1 of 6 patients with COPD has never smoked.[8] This variable susceptibility to COPD most likely reflects genetic variations in the inflammatory and structural responses to inhaled smoke and to microorganisms colonizing the airways of smokers.[9–11] The most common colonizing bacterium is nontypeable (ie, unencapsulated) *Haemophilus influenzae* (NTHi).[12,13] This organism is found in the lower respiratory tract of ~30% of individuals with COPD at any time.[12,14–17] In addition to colonization during clinically stable periods, acquisition of new strains of NTHi is an important cause of lower respiratory tract infection, resulting in exacerbations of COPD.[13,18–20] Together, these findings suggest that persistent or repetitive exposure of the airway to NTHi products may contribute to airway inflammation in COPD.[18] Furthermore, several studies have found that smokers with COPD have an increased risk of lung cancer (3- to 10-fold) compared with smokers with comparable cigarette exposure but without COPD.[21,22] In this review, we will discuss the existing data regarding the roles that NTHi plays in COPD development and lung cancer promotion.

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online data

Munc18b is an essential gene in mice whose expression is limiting for secretion by airway epithelial and mast cells

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Airway mucin secretion and MC (mast cell) degranulation must be tightly controlled for homoeostasis of the lungs and immune system respectively. We found the exocytic protein Munc18b to be highly expressed in mouse airway epithelial cells and MCs, and localized to the apical pole of airway secretory cells. To address its functions, we created a mouse with a severely hypomorphic Munc18b allele such that protein expression in heterozygotes was reduced by ~50%. Homozygous mutant mice were not viable, but heterozygotes showed a ~50% reduction in stimulated release of mucin from epithelial cells and granule contents from MCs. The defect in MCs affected only regulated secretion and not constitutive or transporter-mediated secretion. The severity of passive cutaneous anaphylaxis was also reduced by ~50%, showing that reduction of Munc18b expression results in an attenuation of physiological responses dependent on MC degranulation. The Munc18b promoter is controlled by INR (initiator), Sp1 (specificity protein 1), Ets, CRE (cAMP-response element), GRE (glucocorticoid-response element), GATA and E-box elements in airway epithelial cells; however, protein levels did not change during mucous metaplasia induced by allergic inflammation. Taken together, the results of the present study identify Munc18b as an essential gene that is a limiting component of the exocytic machinery of epithelial cells and MCs.

Key words: exocytosis, mast cell, mucin, mucus, Munc18, secretion.

INTRODUCTION

SNARE [SNAP (soluble *N*-ethylmaleimide-sensitive factor-attachment protein) receptor] proteins and SM (Sec1/Munc18) proteins collaborate to mediate vesicular transport at every step of the exocytic and endocytic pathways of eukaryotic cells [1–5]. SNARE proteins form a four-helix bundle that is responsible for the specific pairing of donor and target membranes and for inducing membrane fusion. SM proteins provide a scaffold for the assembly of SNARE bundles, and in regulated exocytosis they can also hold the SNARE protein Syntaxin in a closed conformation to prevent its unregulated interaction with other SNARE proteins. Together, SM and SNARE proteins comprise the core vesicle trafficking machinery, whereas other trafficking proteins help to position the SM and SNARE proteins for productive interactions or regulate the timing of their function.

There are seven SM proteins in mammals, and three of these function in regulated exocytosis: Munc18a, b and c. Munc18a is expressed in neurons [6–8], and its deletion in mice causes a complete failure of neurotransmitter release, death at birth and a gradual cell-autonomous loss of neuronal viability [9]. Munc18c is expressed ubiquitously [10], and in polarized epithelial cells is localized to the basolateral membrane [11,12]. Munc18c knockout mice die either *in utero* or shortly after birth, and heterozygous mice have impaired glucose tolerance due to partial defects in both insulin release from pancreatic islet cells and translocation of glucose transporters to the plasma membrane of adipocytes and skeletal muscle cells [13–15]. Munc18b is expressed in epithelial cells and leucocytes [10,16–20], but Munc18b-knockout mice have not been reported. In epithelial cells, Munc18b is localized to the apical membrane [11,21] and appears to participate in apical exocytosis based upon *in vitro* overexpression experiments [22–24]. In MCs (mast cells), Munc18b overexpression or knockdown results in impaired degranulation [25,26]. We targeted the murine Munc18b gene to study its role in airway epithelial and leucocyte secretion.

Secretory epithelial cells of the conducting airways synthesize mucin glycoprotein polymers and secrete them into the airway lumen [27,28]. After secretion, mucins become hydrated to form a viscoelastic mucus gel that is swept from distal to proximal airways by the beating of ciliated epithelial cells intercalated among the secretory cells. Mucociliary clearance removes inhaled particles and pathogens from the lungs by transporting them to the pharynx to be swallowed. Insufficient mucus leaves the lungs vulnerable to injury, but excessive mucus obstructs the airway lumen and contributes to the pathophysiology of common lung

Abbreviations used: AB-PAS, Alcian Blue/periodic acid/Schiff reagent; bHLH, basic helix–loop–helix; CCSP, Clara cell secretory protein; Clca3, chloride channel, calcium-activated, family member 3; CRE, cAMP-response element; DNP, 2,4-dinitrophenol; FBS, fetal bovine serum; FcεRIα, high-affinity IgE receptor, α subunit; FRT, flippase recognition target; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GRE, glucocorticoid-response element; HA, haemagglutinin; HSA, human serum albumin; HRP, horseradish peroxidase; IL-3, interleukin-3; INR, initiator; ISH, *in situ* hybridization; MC, mast cell; mBMMC, mouse bone-marrow-derived MC; mClca3, mouse Clca3; MFI, mean fluorescent intensity; mtCC, mouse transformed Clara cell; NK, natural killer; OCT, optimal cutting temperature compound; PAFS, periodic acid/fluorescent Schiff reagent; PBST, PBS containing 0.05% Tween 20; PGD_2, prostaglandin D_2; PGK, phosphoglucokinase; SCF, stem cell factor; SM, Sec1/Munc18; SNAP, soluble *N*-ethylmaleimide-sensitive factor-attachment protein; SNARE, SNAP receptor; Stxbp2, syntaxin-binding protein 2; TK, thymidine kinase; TNFα, tumour necrosis factor α; WT, wild-type; YFP, yellow fluorescent protein.

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ARTICLES

Lung epithelial cells are essential effectors of inducible resistance to pneumonia

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Infectious pneumonias are the leading cause of death worldwide, particularly among immunocompromised patients. Therapeutic stimulation of the lungs' intrinsic defenses with a unique combination of inhaled Toll-like receptor (TLR) agonists broadly protects mice against otherwise lethal pneumonias. As the survival benefit persists despite cytotoxic chemotherapy-related neutropenia, the cells required for protection were investigated. The inducibility of resistance was tested in mice with deficiencies of leukocyte lineages due to genetic deletions and in wild-type mice with leukocyte populations significantly reduced by antibodies or toxins. Surprisingly, these serial reductions in leukocyte lineages did not appreciably impair inducible resistance, but targeted disruption of TLR signaling in the lung epithelium resulted in complete abrogation of the protective effect. Isolated lung epithelial cells were also induced to kill pathogens in the absence of leukocytes. Proteomic and gene expression analyses of isolated epithelial cells and whole lungs revealed highly congruent antimicrobial responses. Taken together, these data indicate that lung epithelial cells are necessary and sufficient effectors of inducible resistance. These findings challenge conventional paradigms about the role of epithelia in antimicrobial defense and offer a novel potential intervention to protect patients with impaired leukocyte-mediated immunity from fatal pneumonias.

INTRODUCTION

Lower respiratory tract infections constitute a tremendous worldwide public health threat, affecting hundreds of millions of people annually.[1–4] Patients with hematological malignancies, those with advanced HIV disease, and certain transplant recipients face extreme pneumonia risks due to impaired leukocyte-mediated immunity.[5–7] In fact, in the transfusion era, pneumonia is the primary cause of death among patients with leukemia.[8,9]

We have previously reported that the lungs' mucosal defenses can be stimulated to protect mice against otherwise lethal pneumonias.[10–14] This phenomenon, known as inducible resistance, results from inhaled treatments targeting pattern recognition receptors in the lungs and yields broad protection against bacterial, viral, and fungal pathogens.[10,13,15–17] Most recently, we showed that robust pneumonia protection could be induced by a single inhaled treatment consisting of a unique, synergistic combination of Toll-like receptor (TLR) agonists: a diacylated lipopeptide ligand for TLR2/6, Pam2CSK4, and a class C unmethylated 2′-deoxyribo cytidine-phosphate-guanosine ligand for TLR9, oligonucleotide (ODN) M362 (hereafter, 'Pam2-ODN').[15,17]

Notably, although we have shown that onset of protection temporally correlates with treatment-induced neutrophil influx into the lungs, we have also demonstrated persistent protection, despite profound depletion of neutrophils and macrophages.[10,11,15] As these leukocytes are widely considered the primary mediators of mucosal immunity in the lungs, these findings raised the question of which cells are principally required for inducible resistance.

Although the airway and alveolar epithelia are often regarded as passive airflow conduits and inert gas exchange barriers, it is evident that they possess intrinsic antimicrobial capacity that contributes to pathogen clearance under physiological

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LETTER

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Muc5b is required for airway defence

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Respiratory surfaces are exposed to billions of particulates and pathogens daily. A protective mucus barrier traps and eliminates them through mucociliary clearance (MCC)[1,2]. However, excessive mucus contributes to transient respiratory infections and to the pathogenesis of numerous respiratory diseases[1]. *MUC5AC* and *MUC5B* are evolutionarily conserved genes that encode structurally related mucin glycoproteins, the principal macromolecules in airway mucus[1,3]. Genetic variants are linked to diverse lung diseases[4–6], but specific roles for MUC5AC and MUC5B in MCC, and the lasting effects of their inhibition, are unknown. Here we show that mouse Muc5b (but not Muc5ac) is required for MCC, for controlling infections in the airways and middle ear, and for maintaining immune homeostasis in mouse lungs, whereas Muc5ac is dispensable. Muc5b deficiency caused materials to accumulate in upper and lower airways. This defect led to chronic infection by multiple bacterial species, including *Staphylococcus aureus*, and to inflammation that failed to resolve normally[7]. Apoptotic macrophages accumulated, phagocytosis was impaired, and interleukin-23 (IL-23) production was reduced in *Muc5b*$^{-/-}$ mice. By contrast, in mice that transgenically overexpress *Muc5b*, macrophage functions improved. Existing dogma defines mucous phenotypes in asthma and chronic obstructive pulmonary disease (COPD) as driven by increased MUC5AC, with MUC5B levels either unaffected or increased in expectorated sputum[1,8]. However, in many patients, MUC5B production at airway surfaces decreases by as much as 90%[9–11]. By distinguishing a specific role for Muc5b in MCC, and by determining its impact on bacterial infections and inflammation in mice, our results provide a refined framework for designing targeted therapies to control mucin secretion and restore MCC.

Mucosal surfaces are central interfaces between organisms and their external environments. Mucus-coated barriers defend against pathogens[12] and re-distributed commensal organisms[13]. Gastrointestinal mucins prevent *Helicobacter pylori* growth[12], colitis[14] and colorectal carcinogenesis[15]. To test whether secreted airway mucins serve correspondingly important roles, we examined MCC and responses to bacterial infections in *Muc5ac*$^{-/-}$ mice (ref. 16), *Muc5b*$^{-/-}$ mice, and lung specific-Muc5b-overexpressing transgenic (Tg(*Scgb1a1-Muc5b*)) mice (Extended Data Fig. 1a–d). We identified unique mechanisms by which Muc5b mediates effective respiratory mucosal defence (Extended Data Fig. 2). In *Muc5b*$^{-/-}$ upper airways, olfactory gland glycoconjugates were absent, but nasopharyngeal surfaces were unaffected (Fig. 1a, b). Muc5ac and Muc5b were lacking in respective knockout airways, but tracheobronchial glycoconjugates increased in *Muc5b*$^{-/-}$ mice owing to induced Muc5ac (Fig. 1c, d and Extended Data Figs 1f and 3). Despite retaining mucous phenotypes in many airway tissues, growth and survival were impaired in *Muc5b*$^{-/-}$ animals (Fig. 1e, f), whereas *Muc5ac*$^{-/-}$ and *Scgb1a1-Muc5b* mice survived normally (Fig. 1f). Acute MCC was normal in *Muc5ac*$^{-/-}$ and *Scgb1a1-Muc5b* mice, but severely reduced in *Muc5b*$^{-/-}$ mice (Fig. 1g, h and Extended Data Fig. 1e), even though functional ciliated cells were present (Fig. 1d, i). Mucus transport was impaired in *Muc5b*$^{-/-}$ tracheal epithelial cells *in vitro*, confirming that defective clearance reflected altered mucociliary interactions specifically (Fig. 1j–l and Supplementary Videos 1 and 2). Collectively, these data identify non-redundant protective requirements for Muc5b in survival and MCC.

Impaired MCC in *Muc5b*$^{-/-}$ mice was accompanied by abnormal breathing (Fig. 2a, b) and hypoxaemia (Fig. 2c). We assessed lung function in mechanically ventilated mice. Baseline airflow and responses to the bronchoconstricting agent methacholine were normal in the lower airways (Fig. 2d–f). Circumventing the upper airways restored ventilation in spontaneously breathing tracheostomized animals (Fig. 2g and Supplementary Videos 3 and 4). Thus, upper respiratory obstruction impeded airflow in *Muc5b*$^{-/-}$ mice. Micro-computed tomography (micro-CT) confirmed this with radiological evidence of upper airway obstruction (Fig. 2h), and middle-ear effusion consistent with otitis media (Extended Data Fig. 4a, b). The latter was unexpected given associations between increased MUC5B and human otitis media[17]. In *Muc5b*$^{-/-}$ mice, but not *Muc5ac*$^{-/-}$ mice (data not shown), hair fragments encased in mucus-like material were consistently found in posterior nasopharynxes (Fig. 2i, j and Extended Data Fig. 4c) and middle ears (Fig. 3a and Extended Data Fig. 4d). Bacteria and inflammation in *Muc5b*$^{-/-}$ middle-ear lavage samples confirmed infectious otitis media (Fig. 3b, c).

In *Muc5b*$^{-/-}$ lower airways, aspirated materials and inflammatory infiltrates were also common (Fig. 3d, e). Culturable bacteria in the lungs increased 2.9–21.6-fold over time, and 7.6–75-fold further in spontaneously moribund mice who also had increased bacteria in spleen cultures (Fig. 3f, g), suggesting that disseminated infections contributed to mortality. To test this hypothesis, mice were placed on antibiotic-supplemented diets. Antibiotics reversed spontaneous lethality and reduced lung bacterial burden, but did not restore normal ventilation in *Muc5b*$^{-/-}$ mice (Fig. 3h–j). Thus, the cause of death in *Muc5b*$^{-/-}$ mice was infectious, and not directly due to airflow limitation. These data identify a natural course in which *Muc5b* deficiency causes upper airway obstruction, spontaneous infection of connecting auditory tubes and the lungs, and fatal bacteraemia.

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nature COMMUNICATIONS

ARTICLE

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The polymeric mucin Muc5ac is required for allergic airway hyperreactivity

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In asthma, airflow obstruction is thought to result primarily from inflammation-triggered airway smooth muscle (ASM) contraction. However, anti-inflammatory and smooth muscle-relaxing treatments are often temporary or ineffective. Overproduction of the mucin MUC5AC is an additional disease feature that, while strongly associated pathologically, is poorly understood functionally. Here we show that Muc5ac is a central effector of allergic inflammation that is required for airway hyperreactivity (AHR) to methacholine (MCh). In mice bred on two well-characterized strain backgrounds (C57BL/6 and BALB/c) and exposed to two separate allergic stimuli (ovalbumin and Aspergillus extract), genetic removal of *Muc5ac* abolishes AHR. Residual MCh responses are identical to unchallenged controls, and although inflammation remains intact, heterogeneous mucous occlusion decreases by 74%. Thus, whereas inflammatory effects on ASM alone are insufficient for AHR, Muc5ac-mediated plugging is an essential mechanism. Inhibiting MUC5AC may be effective for treating asthma and other lung diseases where it is also overproduced.

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Original Paper

OPEN ACCESS

SNAP23 is selectively expressed in airway secretory cells and mediates baseline and stimulated mucin secretion

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Synopsis

Airway mucin secretion is important pathophysiologically and as a model of polarized epithelial regulated exocytosis. We find the trafficking protein, SNAP23 (23-kDa paralogue of synaptosome-associated protein of 25 kDa), selectively expressed in secretory cells compared with ciliated and basal cells of airway epithelium by immunohistochemistry and FACS, suggesting that SNAP23 functions in regulated but not constitutive epithelial secretion. Heterozygous SNAP23 deletant mutant mice show spontaneous accumulation of intracellular mucin, indicating a defect in baseline secretion. However mucins are released from perfused tracheas of mutant and wild-type (WT) mice at the same rate, suggesting that increased intracellular stores balance reduced release efficiency to yield a fully compensated baseline steady state. In contrast, acute stimulated release of intracellular mucin from mutant mice is impaired whether measured by a static imaging assay 5 min after exposure to the secretagogue ATP or by kinetic analysis of mucins released from perfused tracheas during the first 10 min of ATP exposure. Together, these data indicate that increased intracellular stores cannot fully compensate for the defect in release efficiency during intense stimulation. The lungs of mutant mice develop normally and clear bacteria and instilled polystyrene beads comparable to WT mice, consistent with these functions depending on baseline secretion that is fully compensated.

Key words: 23-kDa paralogue of synaptosome-associated protein of 25 kDa (SNAP23), exocytosis, mucin, mucus, secretion.

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INTRODUCTION

Airway mucus entraps inhaled particles and pathogens, clearing them from the lungs when ciliary action propels mucus to the pharynx to be swallowed [1,2]. The absence of airway mucus in mice results in death from microbial infection [3]. However, mucus that is excessive in amount or density cannot be cleared by ciliary action, blocking airflow and paradoxically providing a protected niche for microbial colonization. Thus, the production,

Abbreviations: CCSP, club cell secretory protein/secretoglobin 1A1; DMEM, Dulbecco's modified Eagle's medium; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HRP, horseradish peroxidase; Het, heterozygous gene deletant; KO, homozygous gene deletant; MCh, methacholine; Muc5ac and Muc5b, secreted mucin glycoproteins encoded by distinct genes; Munc13, mammalian orthologues of the C. elegans unc-13 protein uncovered in a screen for uncoordinated mutants; Munc18, mammalian orthologues of the C. elegans unc-18 protein; OPT, optical projection tomography; PAFS, periodic acid fluorescent Schiff's reagent; PBST, PBS containing 0.05% Tween-20; PFA, paraformaldehyde; SM protein, a protein of the extended Sec1/Munc18 family, comprised in mammals of three Munc18 proteins, Sec1, Vps33a, Vps33b and Vps45; SNAP23, 23-kDa paralogue of synaptosome-associated protein of 25 kDa; SNARE, soluble NSF attachment protein receptor; Syt, synaptotagmin; t-SNARE, target membrane SNARE protein; VAMP, vesicle-associated membrane protein; v-SNARE, vesicle membrane SNARE protein; WT, wild-type.

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RESEARCH ARTICLE

The development and plasticity of alveolar type 1 cells

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ABSTRACT

Alveolar type 1 (AT1) cells cover >95% of the gas exchange surface and are extremely thin to facilitate passive gas diffusion. The development of these highly specialized cells and its coordination with the formation of the honeycomb-like alveolar structure are poorly understood. Using new marker-based stereology and single-cell imaging methods, we show that AT1 cells in the mouse lung form expansive thin cellular extensions via a non-proliferative two-step process while retaining cellular plasticity. In the flattening step, AT1 cells undergo molecular specification and remodel cell junctions while remaining connected to their epithelial neighbors. In the folding step, AT1 cells increase in size by more than 10-fold and undergo cellular morphogenesis that matches capillary and secondary septa formation, resulting in a single AT1 cell spanning multiple alveoli. Furthermore, AT1 cells are an unexpected source of VEGFA and their normal development is required for alveolar angiogenesis. Notably, a majority of AT1 cells proliferate upon ectopic SOX2 expression and undergo stage-dependent cell fate reprogramming. These results provide evidence that AT1 cells have both structural and signaling roles in alveolar maturation and can exit their terminally differentiated non-proliferative state. Our findings suggest that AT1 cells might be a new target in the pathogenesis and treatment of lung diseases associated with premature birth.

KEY WORDS: Lung development, Cell plasticity, Alveolar angiogenesis

INTRODUCTION

The mammalian lung consists of a tree-like airway compartment and a honeycomb-like gas exchange compartment. The two major epithelial cell types covering the gas exchange compartment are alveolar type 1 (AT1) and type 2 (AT2) cells, which are in close contact with underlying capillaries and fibroblasts (Williams, 2003; Herzog et al., 2008; Weibel, 2015). AT1 cells are flat and cover more than 95% of the gas exchange surface, whereas AT2 cells are cuboidal and synthesize surfactants to prevent the alveoli from collapsing (Crapo et al., 1982; Williams, 2003). Classical electron microscopy studies show that AT1 cells are extremely thin (<0.1 µm), presumably to facilitate passive gas diffusion, and have a complex morphology that can be traced over multiple alveoli (Weibel, 1971, 2015). Whereas AT2 cells have recently been shown to self-renew and give rise to AT1 cells during homeostasis and injury repair (Barkauskas et al., 2013; Desai et al., 2014), AT1 cells are generally considered terminally differentiated *in vivo*, although they exhibit some plasticity in culture (Danto et al., 1995; Williams, 2003; Gonzalez et al., 2005, 2009). One recent study suggests that, although infrequent, AT1 cells may convert to AT2 cells and proliferate upon pneumonectomy or oncogenic KRAS expression (Jain et al., 2015).

During development, recent studies suggest that a population of bipotential progenitors expressing markers of both AT1 and AT2 cells differentiate into mature AT1 or AT2 cells by upregulating additional markers of the corresponding cell fate and downregulating markers of the alternative cell fate (Desai et al., 2014; Treutlein et al., 2014). However, it is unknown how alveolar cell number, morphology and fate are regulated during subsequent lung maturation. In particular, how do AT1 cells adopt their convoluted morphology in coordination with the formation of the honeycomb-like alveolar structure? When and to what extent are the fates of AT1 and AT2 cells specified?

In this study, we focus on the poorly understood AT1 cells during the perinatal period. We develop a new marker-based stereology method to follow the change in cell number and alveolar surface area, and use single-cell three-dimensional (3D) imaging and three AT1 cell genetic drivers to follow changes in cell morphology and cell fate plasticity. We show that AT1 cells develop via a non-proliferative two-step growth process of cell flattening and cell folding, but retain cellular plasticity. Furthermore, AT1 cells, but not AT2 cells, express *Vegfa*, and disruption of AT1 cell development leads to reduced alveolar angiogenesis. These findings pave the way for future investigation of the role of AT1 cells in alveolar maturation and of AT1 cell plasticity *in vivo*.

RESULTS

AT1 cell growth fuels postnatal alveolar growth

To understand AT1 cell development, we first set out to determine the number of AT1 cells during postnatal lung growth in mice. AT1 cells have been commonly identified based on morphology using electron microscopy (Stone et al., 1992; Weibel, 2015), which limits the analysis to small regions and makes it technically challenging to obtain the total cell number as it requires the dissector method (Hsia et al., 2010) or an assumption of uniform nuclear shapes (Kauffman et al., 1974; Weibel, 2015). This prompted us to develop a new marker-based stereology method that combines stereological sampling principles with 3D imaging of molecular markers (Fig. S1A). Our method has several advantages. First, we confirmed that, unlike membrane-localized AT1 markers, HOPX stains both the nucleus and cytosol of AT1 cells (Barkauskas et al., 2013), thus allowing nucleus-based cell counting. HOPX expression was AT1 specific throughout postnatal development, as alveolar epithelial cells were marked in a mutually exclusive manner by nuclear HOPX and LAMP3 [an AT2 cell marker (Chang et al., 2013; Desai et al., 2014)] or by nuclear HOPX and cuboidal E-cadherin (E-CAD;

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Microenvironment and Immunology

Cancer Research

IL6 Blockade Reprograms the Lung Tumor Microenvironment to Limit the Development and Progression of K-ras–Mutant Lung Cancer

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Abstract

Activating mutations of K-ras are the most common oncogenic alterations found in lung cancer. Unfortunately, attempts to target K-ras–mutant lung tumors have thus far failed, clearly indicating the need for new approaches in patients with this molecular profile. We have previously shown NF-κB activation, release of IL6, and activation of its responsive transcription factor STAT3 in K-ras–mutant lung tumors, which was further amplified by the tumor-enhancing effect of chronic obstructive pulmonary disease (COPD)-type airway inflammation. These findings suggest an essential role for this inflammatory pathway in K-ras–mutant lung tumorigenesis and its enhancement by COPD. Therefore, here we blocked IL6 using a monoclonal anti-IL6 antibody in a K-ras–mutant mouse model of lung cancer in the absence or presence of COPD-type airway inflammation. IL6 blockade significantly inhibited lung cancer promotion, tumor cell–intrinsic STAT3 activation, tumor cell proliferation, and angiogenesis markers. Moreover, IL6 inhibition reduced expression of protumor type 2 molecules (arginase 1, Fizz 1, Mgl, and IDO), number of M2-type macrophages and granulocytic myeloid-derived suppressor cells, and protumor T-regulatory/Th17 cell responses. This was accompanied by increased expression of antitumor type 1 molecule (Nos2), and antitumor Th1/CD8 T-cell responses. Our study demonstrates that IL6 blockade not only has direct intrinsic inhibitory effect on tumor cells, but also reeducates the lung microenvironment toward an antitumor phenotype by altering the relative proportion between protumor and antitumor immune cells. This information introduces IL6 as a potential druggable target for prevention and treatment of K-ras–mutant lung tumors. *Cancer Res; 76(11); 3189–99. ©2016 AACR.*

Introduction

Lung cancer is the leading cause of cancer mortality worldwide due to its high incidence and low cure rate (1), and cigarette smoke is by far the most common cause of it (2). Activating mutations of K-ras, found in approximately 30% of lung cancer patients, are one of the most prevalent genetic alterations associated with tobacco exposure (1). Unfortunately, pharmacologic attempts to develop targeted therapies to interfere with K-ras activity have shown limited success to date; therefore, alternative strategies are needed to inhibit this oncogenic signaling pathway and bring clinical benefits to lung cancer patients with mutant K-ras. In addition, several studies have found that smokers with chronic obstructive pulmonary disease (COPD) have an increased risk of lung cancer (3- to 10-fold) compared to smokers with comparable cigarette exposure but without COPD (3–5). COPD is a chronic inflammatory disease of the lung, which is present in 40% to 70% of lung cancer patients (6). Importantly, among smokers with COPD, even following withdrawal of cigarette smoke, inflammation persists and lung function continues to deteriorate as does the increased risk of lung cancer (7, 8). Furthermore, because of the persistent lung cancer risk among former smokers, and increased diagnosis of early-stage lung cancer with the recommended screening method (low-dose CT scan; ref. 9), strategies targeting pathways that stop the progression of COPD and early-stage lung cancer to advanced lung cancer would also be valuable.

We and other groups have demonstrated that K-ras–mutant lung tumors have intrinsic inflammatory characteristics, with activation of the NF-κB pathway, increased levels of the

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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ORIGINAL RESEARCH

Tumor necrosis factor links chronic obstructive pulmonary disease and K-ras mutant lung cancer through induction of an immunosuppressive pro-tumor microenvironment

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ABSTRACT

Tumor necrosis factor (TNF) is known as an important regulator of tumor microenvironment and inflammation. TNF levels are markedly elevated in the bronchoalveolar lavage fluid (BALF) of patients with chronic obstructive pulmonary disease (COPD), which is an independent risk factor for lung cancer. We have previously shown that COPD-like airway inflammation promotes lung cancer in a K-ras mutant mouse model (CC-LR mouse). This was associated with a significant increase of neutrophils in BALF, accompanied by a marked increase in TNF level, suggesting a link between COPD, TNF, and lung cancer promotion. Therefore, we first overexpressed TNF in the airway epithelium of CC-LR mice, which promoted lung cancer by ~2-fold. This was associated with increased numbers of Ki67 and CD31 positive cells in lung tumors of CC-LR/TNF-Tg mice. We also found a robust increase in NF-κB activation, and numbers of neutrophils and myeloid-derived suppressor cells (MDSCs) in lung. Accordingly, we depleted MDSCs in CC-LR/TNF-Tg mice, which lead to significant tumor suppression emphasizing on the role of TNF-induced MDSCs in K-ras induced lung tumorigenesis. Finally, we targeted TNF expression by crossing CC-LR mice with TNF knock-out mice (CC-LR/TNF-KO), which resulted in a significant decrease in lung tumor burden in the absence or presence of COPD-like airway inflammation. Interestingly, there were less MDSCs and lower Ki67 and CD31 expression in the lung of the CC-LR/TNF-KO mice. We conclude that TNF links COPD to lung cancer promotion by induction of an immunosuppressive MDSC response, and subsequent amplification of proliferation and angiogenesis in tumors.

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KEYWORDS
COPD; K-ras; lung cancer; MDSC; NF-κB; TNF

Introduction

Cancer-related inflammation is an essential process in malignant progression, which became one of the cancer hallmarks with enabling effects.[1,2] It is known now that both intrinsic (induced by a genetic event) and extrinsic (e.g., infection-induced) inflammation could promote cancer by inducing tumor cells to produce various cytokines and chemokines and subsequently attracting leukocytes to further amplify inflammation in the tumor microenvironment.[1,3] Among the wide range of cytokines produced by tumor and stromal cells, TNF has been reported as an important regulator in the tumor microenvironment.[4] The role of TNF in tumor immunity is complex and remains controversial. Research studies using high doses of exogenous TNF or gene modified tumor cell lines secreting TNF demonstrate an antitumor activity for TNF.[5-7] However, TNF has also shown tumor-promoting effects in various tumor models. In murine models of colitis, it has been linked to colon and liver tumorigenesis through NF-κB activation and induction of inflammation-associated cytokines and anti-apoptotic proteins.[8-10] TNF is also involved in the induction, maturation, differentiation and recruitment of myeloid derived suppressor cells (MDSCs) that may result in a pro-tumor immunosuppressive inflammatory condition.[11-14]

Lung cancer is the leading cause of cancer death worldwide both because of a high incidence and a low cure rate.[15] Accumulating evidence has shown a role for inflammation in the pathogenesis of lung cancer, especially when induced by cigarette smoke, that develops in patients with chronic obstructive pulmonary disease (COPD).[16] COPD is characterized by chronic lung inflammation[17,18] and is considered an independent risk factor for lung cancer.[19,20] We have previously established a COPD-like mouse model of airway inflammation[21] and shown that this type of airway inflammation,[22] but not asthma-like airway inflammation,[23] promotes lung cancer in a

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Supplemental data for this article can be accessed on the publisher's website.

TEC – MD Anderson Publicaciones (34; 7 en revisión)

Regular Article

blood

MYELOID NEOPLASIA

Inducible epithelial resistance protects mice against leukemia-associated pneumonia

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Key Points

- Survival of acute myelogenous leukemia is frequently limited by pneumonia, due to disease- and therapy-associated immune defects.
- Inducible epithelial resistance protects neutropenic, leukemic mice against lethal pneumonia without impacting AML cell proliferation.

Despite widespread infection prevention efforts, pneumonia remains the leading cause of death among patients with acute leukemia, due to complex disease- and treatment-dependent immune defects. We have reported that a single inhaled treatment with a synergistic combination of Toll-like receptor 2/6 (TLR 2/6) and TLR9 agonists (Pam2-ODN) induces protective mucosal defenses in mice against a broad range of pathogens. As Pam2-ODN–induced protection persists despite depletion of several leukocyte populations, we tested whether it could prevent pneumonia in a mouse model of acute myeloid leukemia (AML) remission induction therapy. Pam2-ODN prevented death due to pneumonia caused by *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Aspergillus fumigatus* when mice were heavily engrafted with leukemia cells, had severe chemotherapy-induced neutropenia or both. Pam2-ODN also extended survival of pneumonia in NSG mice engrafted with primary human AML cells. Protection was associated with rapid pathogen killing in the lungs at the time of infection and with reduced pathogen burdens at distant sites at the end of observation. Pathogen killing was inducible directly from isolated lung epithelial cells and was not abrogated by the presence of leukemia cells or cytotoxic agents. Pam2-ODN had no discernible effect on replication rate, total tumor population, or killing by chemotherapy of mouse or human leukemia cells, either in vitro or in vivo. Taken together, we report that therapeutic stimulation of lung epithelial defenses robustly protects against otherwise lethal pneumonias despite the profound immune dysfunction associated with acute leukemia and its treatment. These findings may suggest an opportunity to protect this population during periods of peak vulnerability. (*Blood*. 2016;128(7):982-992)

Introduction

Among both healthy and immunosuppressed people worldwide, pneumonia is a leading cause of premature death and disability,[1-4] and nosocomial pneumonias cause more deaths than any other hospital infection.[5] Patients with acute myelogenous leukemia (AML) or high-risk myelodysplastic syndrome (MDS) face a particular pneumonia risk, as both disease and treatment impair immune function.[6-11] In the transfusion era, autopsy studies reveal that pneumonia is the most frequent cause of death among leukemia patients,[12,13] and recent studies find that the presence of pneumonia is the leading hazard for death during leukemia remission induction therapy.[14] Moreover, these figures fail to capture leukemia-related deaths caused by withholding essential myeloablative therapies due to concerns about immunosuppression in patients with suspected lung infections. Further, current unacceptably high rates of pneumonia persist despite widespread use of environmental hygiene protocols and prophylactic antibiotics. Thus, although enhanced control of pneumonia would substantially enhance the chance of long-term survival for patients with acute leukemias, novel strategies are necessary to achieve success in this regard.

One theoretically appealing approach to improve pneumonia-related outcomes in patients with leukemia is to preferentially augment those host defense elements that are relatively less impaired by the disease process. Leukemia patients frequently present with complex leukocyte defects, often arising from both multilineage cytopenias and functional impairments of chemotaxis, diapedesis, and pathogen killing.[15-17] Thus, therapeutic manipulation of lung parenchymal cells might present an opportunity to protect patients against pneumonia without reliance on the cells most negatively impacted by disease and treatment.

To this end, we reported that therapeutic ligation of pattern recognition receptors in the lungs can stimulate protective antimicrobial responses directly from lung epithelial cells, a phenomenon termed inducible resistance.[18-22] Given the relative tolerance of lung epithelial cells to immunosuppressive therapies[23,24] and our observation that inducible resistance persists despite leukocyte depletion,[25-27] we hypothesized that this strategy could protect against pneumonia in the

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Fungal Pneumonia in Patients with Hematologic Malignancy and Hematopoietic Stem Cell Transplantation

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KEYWORDS

- Fungal pneumonia • Neutropenia • Hematologic malignancy • Stem cell transplant
- Immunocompromised host pneumonia • Galactomannan

KEY POINTS

- Fungal pneumonias cause significant morbidity and mortality in patients with hematologic malignancies (HM) and recipients of hematopoietic stem cell transplantations (HSCT).
- Neutropenia, cytotoxic chemotherapy, graft-versus-host disease, genetic polymorphisms, and other immune derangements increase the risk of developing life-threatening fungal pneumonias.
- Chest imaging is often nonspecific but may aid in diagnoses. Bronchoscopy with bronchoalveolar lavage is recommended in patients at high risk of fungal pneumonia with new infiltrates on chest imaging, unexplained respiratory symptoms, or persistent fever.
- Immunoassays for fungal cell wall components, such as galactomannan and (1,3)-β-D-glucan, may aid the early diagnosis of invasive fungal infections in patients with HM/HSCT.
- Investigations into novel preventive strategies and host-directed therapies are ongoing.

INTRODUCTION

Immunocompetent hosts are estimated to inhale hundreds of fungal conidia daily, but most fungal pathogens are cleared without development of clinical infection.[1,2] By contrast, many patients with hematologic malignancies (HM) or those who have received hematopoietic stem cell transplantation (HSCT) have impaired antifungal defenses, and account for a disproportionate number of fungal pneumonias in North America and Europe.[3,4] Despite the use of mold-active prophylaxis since the 1990s, invasive fungal infections (IFI) remain a leading cause of morbidity in patients with HM/HSCT, with unacceptably high attributable mortality.[3,5–7] This review describes the immune deficits that enhance susceptibility to fungal pneumonia in patients with HM/HSCT, as well as the diagnosis

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REVIEW

Lung epithelial cells: therapeutically inducible effectors of antimicrobial defense

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Lung epithelial cells are increasingly recognized to be active effectors of microbial defense, contributing to both innate and adaptive immune function in the lower respiratory tract. As immune sentinels, lung epithelial cells detect diverse pathogens through an ample repertoire of membrane-bound, endosomal, and cytosolic pattern-recognition receptors (PRRs). The highly plastic epithelial barrier responds to detected threats via modulation of paracellular flux, intercellular communications, mucin production, and periciliary fluid composition. Epithelial PRR stimulation also induces production of cytokines that recruit and sculpt leukocyte-mediated responses, and promotes epithelial generation of antimicrobial effector molecules that are directly microbicidal. The epithelium can alternately enhance tolerance to pathogens, preventing tissue damage through PRR-induced inhibitory signals, opsonization of pathogen-associated molecular patterns, and attenuation of injurious leukocyte responses. The inducibility of these protective responses has prompted attempts to therapeutically harness epithelial defense mechanisms to protect against pneumonias. Recent reports describe successful strategies for manipulation of epithelial defenses to protect against a wide range of respiratory pathogens. The lung epithelium is capable of both significant antimicrobial responses that reduce pathogen burdens and tolerance mechanisms that attenuate immunopathology. This manuscript reviews inducible lung epithelial defense mechanisms that offer opportunities for therapeutic manipulation to protect vulnerable populations against pneumonia.

INTRODUCTION

The lung epithelium has long been perceived as a passive conduit for bulk airflow or an inert barrier to gas exchange, seldom encountering microbes and irrelevant to host–pathogen interactions. However, modern molecular techniques have revealed the complexity of the lower respiratory tract microbiome[1] and accumulating evidence demonstrate that lung epithelial cells function as important mediators of host defense.[2] Lung epithelial cells express an expansive complement of pattern-recognition receptors (PRRs) with oligospecificity for conserved microbial and host motifs. PRR activation by pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) initiates signaling cascades that can promote pathogen exclusion or expulsion, recruit and activate leukocyte-mediated defenses, directly kill microbes, and restore host homeostasis. These varied mechanisms provide manifold theoretical opportunities for intervention, and recent studies confirm that epithelial defenses can be therapeutically manipulated to protect the host, even in the setting of immunosuppression or leukodepletion.[3] This review addresses important lung epithelial pathogen detection and response mechanisms that may be therapeutically manipulated to prevent and treat lower respiratory tract infections in healthy and immunocompromised populations.

INDUCIBLE BARRIER DEFENSES

Cellular junctions and cytoskeletal elements

The histological complexity of the lung epithelium portends the specialized functions of its component cells. The pseudostratified airway epithelium is predominantly comprised of ciliated cells and secretory cells, interspersed with regenerative basal cells and neuroendocrine cells (**Figure 1a,b**). The vast majority of the alveolar epithelial surface area is contributed by exceptionally thin, broad type I pneumocytes that are optimized for gas exchange, while the considerably more numerous type II pneumocytes are principally responsible for

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Full length article

Combined aerosolized Toll-like receptor ligands are an effective therapeutic agent against influenza pneumonia when co-administered with oseltamivir

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ABSTRACT

Influenza pneumonia remains a common and debilitating viral infection despite vaccination programs and antiviral agents developed for prophylaxis and treatment. The neuraminidase inhibitor oseltamivir is frequently prescribed for established influenza A virus infections, but the emergence of neuraminidase inhibitor resistant viruses, a brief therapeutic window and competing diagnoses complicate its use. PUL-042 is a clinical stage, aerosol drug comprised of synthetic ligands for Toll-like receptor (TLR) 2/6 and TLR 9. This host-targeted, innate immune stimulant broadly protects against bacterial, fungal and viral pneumonias, including those caused by influenza, when given prophylactically to animals. This study evaluated the therapeutic antiviral effects of PUL-042 against established influenza A pneumonia, when given alone or in combination with oseltamivir. Mice were treated with PUL-042 aerosol, oseltamivir or both at varying time points before or after challenge with influenza pneumonia. Treating established, otherwise lethal influenza A pneumonia (> 1 LD_{100}) with multiple inhaled doses of PUL-042 aerosol plus oral oseltamivir resulted in greater mouse survival than treatment with either drug alone. Single agent PUL-042 also protected mice against established infections following challenges with lower viral inocula (approximately 1 LD_{20}). Aerosolized oseltamivir further enhanced survival when co-delivered with PUL-042 aerosol. The prophylactic and therapeutic benefits of PUL-042 were similar against multiple strains of influenza virus. In vitro influenza challenge of human IIBEC3kt lung epithelial cells revealed PUL-042-induced protection against infection that was comparable to that observed in vivo. These studies offer new insights into means to protect susceptible populations against influenza A pneumonia.

1. Introduction

Influenza viruses remain common causes of serious infection worldwide, despite large scale vaccination programs. In the United States, 20,000–40,000 cases of seasonal influenza occur annually, with attributable mortality as high as 7.9% (Russell et al., 2016). This translates to estimated hospitalization costs of $10.4 billion and lost earnings of $16.3 billion dollars per year (Molinari et al., 2007). Influenza causes disproportionate morbidity in certain populations, with individuals at the extremes of age (< 2 years, > 50 years) and those with comorbid or immunocompromising conditions the most susceptible to influenza pneumonia (Louie et al., 2009; Jain et al., 2009; Pochling et al., 2006).

The available treatments for influenza infections are adamantane derivatives (rimantadine and amantadine) or neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir) (Fiore et al., 2011). Neuraminidase inhibitors are first line agents, due to their efficacy against influenza A and B viruses and the high prevalence of adamantane resistant influenza. Unfortunately, viruses resistant to neuraminidase inhibitors such as oseltamivir have been increasingly reported from both seasonal and pandemic H1N1 influenza isolates (Baz et al., 2009; Gubareva et al., 2001; Stephenson et al., 2009). Current guidelines recommend treating with neuraminidase inhibitors within 48 h of symptom development in the general population. However, resistance has been shown to emerge as early as 48 h after initiation of treatment (Inoue et al., 2010), and transmission of resistant strains has been documented (Hatakeyama et al., 2007; Le et al., 2010).

We have previously reported that lung epithelial cells can be

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JBC ARTICLE

Munc13 proteins control regulated exocytosis in mast cells

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Mast cells (MCs) are involved in host defenses against pathogens and inflammation. Stimulated MCs release substances stored in their granules via regulated exocytosis. In other cell types, Munc13 (mammalian homolog of *Caenorhabditis elegans* uncoordinated gene 13) proteins play essential roles in regulated exocytosis. Here, we found that MCs express Munc13-2 and -4, and we studied their roles using global and conditional knock-out (KO) mice. In a model of systemic anaphylaxis, we found no difference between WT and Munc13-2 KO mice, but global and MC-specific Munc13-4 KO mice developed less hypothermia. This protection correlated with lower plasma histamine levels and with histological evidence of defective MC degranulation but not with changes in MC development, distribution, numbers, or morphology. *In vitro* assays revealed that the defective response in Munc13-4-deficient MCs was limited to regulated exocytosis, leaving other MC secretory effector responses intact. Single cell capacitance measurements in MCs from mouse mutants differing in Munc13-4 expression levels in their MCs revealed that as levels of Munc13-4 decrease, the rate of exocytosis declines first, and then the total amount of exocytosis decreases. A requirement for Munc13-2 in MC exocytosis was revealed only in the absence of Munc13-4. Electrophysiology and EM studies uncovered that the number of multigranular compound events (*i.e.* granule-to-granule homotypic fusion) was severely reduced in the absence of Munc13-4. We conclude that although Munc13-2 plays a minor role, Munc13-4 is essential for regulated exocytosis in MCs, and that this MC effector response is required for a full anaphylactic response.

During exocytosis, the membrane of a secretory vesicle fuses with the plasma membrane, allowing the release of vesicular contents into the extracellular space and the incorporation of vesicle membrane components into the plasma membrane (1). Exocytosis can be constitutive or regulated (2). In constitutive exocytosis, newly formed products are secreted as they are synthesized, and the amount of secreted product is controlled by the rate of expression of the vesicular cargo. In contrast, in regulated exocytosis the formed products are stored in secretory vesicles (*e.g.* MC[2] granules) and released upon stimulation using diacylglycerol (DAG) and Ca^{2+} as second messengers (3, 4). The amount of secreted product is controlled by the rate and number of vesicles to plasma membrane fusion events. Regulated exocytosis can adopt various forms. In single-vesicle exocytosis, individual secretory granules fuse with the plasma membrane. In sequential compound exocytosis, these primary fused vesicles become targets for secondary fusion events with vesicles lying deeper in the cell. In multigranular compound exocytosis, secretory vesicles fuse homotypically with each other inside the cell before fusing heterotypically with the plasma membrane (5). Some cells (*e.g.* MCs) use all three forms of regulated exocytosis (6).

Regulated exocytosis involves the generation of secretory vesicles and their transport toward the plasma membrane. Then, tethering and docking establish physical proximity between the vesicle and plasma membrane. The final event involves the fusion of both membranes (1), which requires the assembly of complexes between the SNARE (soluble *N*-ethylmaleimide-sensitive factor-activated protein receptor) domains of proteins on the vesicular (vesicle-associated membrane protein (VAMP)) and target membranes (syntaxin (Stx) and synaptosome-associated protein 25 (SNAP25)) (7) and is highly regulated by complexin and synaptotagmin (8). The physical proximity established by docking is not sufficient to drive fusion,

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[2] The abbreviations used are: MC, mast cell; B6, C57BL6/J mouse line; BMMC, bone marrow-derived MC; C_{mv}, capacitance; ΔC_{mv}, capacitance gain; ΔT, change in body core temperature; DAG, diacylglycerol; DKO, Munc13-2/Munc13-4 double KO; DNP, 2,4-dinitrophenol; F, farad; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; HSA, human serum albumin; LTC_4, leukotriene C_4; Neo, neomycin phosphotransferase; PCMC, peritoneal cell-derived MC; PGD_2, prostaglandin D_2; PGK, phosphoglucokinase promoter; PI, PMA/ionomycin; PMA, phorbol 12-myristate 13-acetate; qPCR, quantitative PCR; RACE, rapid amplification of cDNA ends; RIM, Rab3-interacting molecule; S, siemen; SCF, stem cell factor; SNARE, soluble *N*-ethylmaleimide-sensitive factor activated protein receptor; Stx, syntaxin; S_v, surface density; TK, herpes simplex virus thymidine kinase; VAMP, vesicle-associated membrane protein; V_v, volume density.

JBC ARTICLE

Munc18-2, but not Munc18-1 or Munc18-3, controls compound and single-vesicle–regulated exocytosis in mast cells

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Mast cells (MCs) play pivotal roles in many inflammatory conditions including infections, anaphylaxis, and asthma. MCs store immunoregulatory compounds in their large cytoplasmic granules and, upon stimulation, secrete them via regulated exocytosis. Exocytosis in many cells requires the participation of Munc18 proteins (also known as syntaxin-binding proteins), and we found that mature MCs express all three mammalian isoforms: Munc18-1, -2, and -3. To study their functions in MC effector responses and test the role of MC degranulation in anaphylaxis, we used conditional knockout (cKO) mice in which each Munc18 protein was deleted exclusively in MCs. Using recordings of plasma membrane capacitance for high-resolution analysis of exocytosis in individual MCs, we observed an almost complete absence of exocytosis in Munc18-2–deficient MCs but intact exocytosis in MCs lacking Munc18-1 or Munc18-3. Stereological analysis of EM images of stimulated MCs revealed that the deletion of Munc18-2 also abolishes the homotypic membrane fusion required for compound exocytosis. We confirmed the severe defect in regulated exocytosis in the absence of Munc18-2 by measuring the secretion of mediators stored in MC granules. Munc18-2 cKO mice had normal morphology, development, and distribution of their MCs, indicating that Munc18-2 is not essential for the migration, retention, and maturation of MC-committed progenitors. Despite that, we found that Munc18-2 cKO mice were significantly protected from anaphylaxis. In conclusion, MC-regulated exocytosis is required for the anaphylactic response, and Munc18-2 is the sole Munc18 isoform that mediates membrane fusion during MC degranulation.

Mast cells (MCs)[3] derive from hematopoietic progenitors, circulate in immature form, and migrate into different tissues where they complete their differentiation. The widespread distribution of MCs throughout the body favors fast immune and inflammatory responses, including anaphylaxis (1, 2). To accomplish this, MCs release a variety of mediators through several mechanisms, including regulated exocytosis. During exocytosis, the membrane of a secretory vesicle fuses with the plasma membrane, releasing its cargo into the extracellular space and translocating proteins associated with or integral to its membrane to the plasma membrane. Regulated exocytosis in MCs (MC degranulation) is characterized by the almost immediate release of mediators that are premade and stored in large secretory vesicles (MC granules), such as histamine, MC proteases, and other enzymes. This requires MC activation by stimuli that usually employ Ca^{2+} and diacylglycerol as second messengers (3, 4). Degranulation in MCs uses both single-vesicle and compound exocytosis (5, 6). In single-vesicle exocytosis, the membrane of a single MC granule fuses with the plasma membrane. In multivesicular compound exocytosis, granules fuse with each other before fusing with the plasma membrane. In sequential compound exocytosis, granules fuse with granules already fused with the plasma membrane. Both forms of compound exocytosis allow the rapid discharge of granules located deep within the cell (5, 7).

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[3] The abbreviations used are: MC, mast cell; A, cell profile area; cKO, conditional KO; DNP, 2,4-dinitrophenol; fF, femtofarad; Flp, Flp recombinase; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; HSA, human serum albumin; Neo, neomycin phosphotranferase; PCMC, peritoneal cell-derived MC; PGK, phosphoglucokinase promoter; PI, PMA plus ionomycin; PMA, phorbol 12-myristate 13-acetate; RT-qPCR, reverse transcriptase-quantitative PCR; S, siemens; SM, Sec1/Munc18 family of proteins; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors; Stx, Syntaxin; S_V, surface density; VAMP, vesicle-associated membrane protein; V_V, volume density.

TEC – MD Anderson Publicaciones (34; 7 en revisión)

ARTICLE

Platelet Munc13-4 regulates hemostasis, thrombosis and airway inflammation

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ABSTRACT

Platelet degranulation is crucial for hemostasis and may participate in inflammation. Exocytosis in platelets is mediated by SNARE proteins and should be controlled by Munc13 proteins. We found that platelets express Munc13-2 and -4. We assessed platelet granule exocytosis in Munc13-2 and -4 global and conditional knockout (KO) mice, and observed that deletion of Munc13-4 ablates dense granule release and indirectly impairs alpha granule exocytosis. We found no exocytic role for Munc13-2 in platelets, not even in the absence of Munc13-4. *In vitro*, Munc13-4-deficient platelets exhibited defective aggregation at low doses of collagen. In a flow chamber assay, we observed that Munc13-4 acted as a rate-limiting factor in the formation of thrombi. *In vivo*, we observed a dose-dependency between Munc13-4 expression in platelets and both venous bleeding time and time to arterial thrombosis. Finally, in a model of allergic airway inflammation, we found that platelet-specific Munc13-4 KO mice had a reduction in airway hyper-responsiveness and eosinophilic inflammation. Taken together, our results indicate that Munc13-4-dependent platelet dense granule release plays essential roles in hemostasis, thrombosis and allergic inflammation.

Introduction

A key effector response from platelets is exocytosis of their alpha, dense and lysosomal granules. Alpha granules are the most abundant, and contain soluble molecules and receptors that propagate platelet activation and aggregation.[1] Dense granules are secreted at a faster rate and store ADP, an autocrine agonist for platelet activation.[2] Lysosomal granules contain membrane-associated proteins and acid-hydrolases, and may contribute to thrombus remodeling.[3]

During exocytosis, the membrane of a platelet granule fuses with the plasma membrane. This requires the formation of a SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex by proteins localized on both membranes.[4] Prior to fusion, granules are brought close to the plasma membrane by tethering and docking processes, but this proximity is not sufficient to drive fusion, it also requires priming.[5] Fundamental to priming is the interaction of Munc (mammalian homolog of *C. elegans* uncoordinated gene) 13 with Munc18, which allows Syntaxin to interact with the other exocytic SNARE proteins.[6] Among the four paralogs of Munc13, only Munc13-4 has been studied in mouse platelets.[7] Different groups have agreed that deletion of Munc13-4 inhibits primarily dense granule release,[8,9] which may affect alpha granule exocytosis[9,10] or integrin $\alpha_{IIb}\beta_3$ activation[11] depending on experimental variables.[12] In addition, global deficiency of Munc13-4 affects hemostasis, probably due to defective platelet exocytosis,[10,11,13] a difficult conclusion to reach because Munc13-4 is also expressed in other tissues important for hemostasis (e.g. endothelial cells).[14] In humans, mutations in the gene encoding Munc13-4 cause familial hemophagocytic lymphohistiocytosis type 3 (FHL3), an autosomal recessive disorder characterized by defective secretion in cytotoxic T lymphocytes and natural killer cells, multisystemic inflam-

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Research Article

Cancer Immunology Research

IL22 Promotes *Kras*-Mutant Lung Cancer by Induction of a Protumor Immune Response and Protection of Stemness Properties

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Abstract

Somatic *KRAS* mutations are the most common oncogenic variants in lung cancer and are associated with poor prognosis. Using a *Kras*-induced lung cancer mouse model, CC-LR, we previously showed a role for inflammation in lung tumorigenesis through activation of the NF-κB pathway, along with induction of interleukin 6 (IL6) and an IL17-producing $CD4^+$ T-helper cell response. IL22 is an effector molecule secreted by $CD4^+$ and γδ T cells that we previously found to be expressed in CC-LR mice. IL22 mostly signals through the STAT3 pathway and is thought to act exclusively on nonhematopoietic cells with basal IL22 receptor (IL22R) expression on epithelial cells. Here, we found that higher expression of *IL22R1* in patients with *KRAS*-mutant lung adenocarcinoma was an independent indicator of poor recurrence-free survival. We then showed that genetic ablation of *Il22* in CC-LR mice (CC-LR/IL22KO mice) caused a significant reduction in tumor number and size. This was accompanied by significantly lower tumor cell proliferation, angiogenesis, and STAT3 activation. *Il22* ablation was also associated with significant reduction in lung-infiltrating inflammatory cells and expression of protumor inflammatory cytokines. Conversely, this was accompanied with increased antitumor Th1 and cytotoxic $CD8^+$ T-cell responses, while suppressing the protumor immunosuppressive T regulatory cell response. In CC-LR/IL22KO mice, we found significantly reduced expression of core stemness genes and the number of prototypical SPC^+CCSP^+ stem cells. Thus, we conclude that IL22 promotes *Kras*-mutant lung tumorigenesis by driving a protumor inflammatory microenvironment with proliferative, angiogenic, and stemness contextual cues in epithelial/tumor cells. *Cancer Immunol Res; 6(7); 788–97. ©2018 AACR.*

Introduction

Tumor-promoting inflammation is a cancer hallmark that depends on cytokines and their downstream pathways (1, 2). The roles of interleukin 22 (IL22)- and IL22-expressing cells have been reported in a variety of human diseases (3, 4), yet, our knowledge of their functions in cancer, particularly in lung oncogenesis, is lacking. IL22 is a unique member of the IL10 family of cytokines, and the functional outcome of its expression depends on the presence and relative amounts of other cytokines (5, 6). IL22 seems to act exclusively on nonhematopoietic cells expressing a heterodimer transmembrane complex of IL22R1 and IL10R2 (7). IL22R1 is almost entirely expressed on cells of nonhematopoietic origin. The main signaling pathway downstream of IL22R1 is the STAT3 cascade, which mediates most IL22-induced effects (7). IL22 is known to be produced mostly by $CD4^+$ T helper (Th) lymphocytes (Th17, Th22, and Th1), natural killer T cells (NKT), innate lymphoid cells (ILC), γδ T cells, and less commonly $CD8^+$ lymphocytes (6–8).

Previous studies have demonstrated high IL22 and $IL22^+$ cells in primary lung tumors and sera of patients with lung cancer (9, 10). It is also known that high expression of IL22R1 in non–small cell lung cancer (NSCLC) is an independent indicator of poor overall survival (11). These suggest a role for IL22 signaling in the pathogenesis of lung cancer. *KRAS* mutations are the most common oncogenic mutation in NSCLCs and are associated with chemoresistance and poor prognosis (12). Our group and others have shown that activation of oncogenic K-ras in the lung (mouse and human) is associated with intrinsic inflammatory responses

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Note: Supplementary data for this article are available at Cancer Immunology Research Online (http://cancerimmunolres.aacrjournals.org/).

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TEC – MD Anderson Publicaciones (34; 7 en revisión)

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ORIGINAL ARTICLE

Risk factors for pleural effusion recurrence in patients with malignancy

Horiana B. Grosu,[1] Sofia Molina,[1,2] Roberto Casal,[3] Juhee Song,[4] Liang Li,[4] Javier Diaz-Mendoza,[5] Chakravarthy Reddy,[6] Lonny Yarmus,[7] Dante Schiavo,[8] Michael Simoff,[5] Jared Johnstun,[6] Abu-Awwad Raid,[3] David Feller-Kopman,[7] Hans Lee,[7] Sarina Sahetya,[7] Finbar Foley,[8] Fabien Maldonado,[9] Xin Tian,[1] Laila Noor,[1] Russell Miller,[1] Lakshmi Mudambi,[1] Timothy Saettele,[1] Macarena Vial-Rodriguez,[1] Gerogie A. Eapen[1] and David E. Ost[1]

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ABSTRACT

Background and objective: **The main purpose of treatment in patients with malignant pleural effusion (MPE) is symptom palliation. Currently, patients undergo repeat thoracenteses prior to receiving a definitive procedure as clinicians are not aware of the risk factors associated with fluid recurrence. The primary objective of this study was to identify risk factors associated with recurrent symptomatic MPE.**

Methods: **Retrospective multicentre cohort study of patients who underwent first thoracentesis was performed. The primary outcome was time to fluid recurrence requiring intervention in patients with evidence of metastatic disease. We used a cause-specific hazard model to identify risk factors associated with fluid recurrence. We also developed a predictive model, utilizing Fine-Gray subdistribution hazard model, and externally validated the model.**

Results: **A total of 988 patients with diagnosed metastatic disease were included. Cumulative incidence of recurrence was high with 30% of patients recurring by day 15. On multivariate analysis, size of the effusion on chest X-ray (up to the top of the cardiac silhouette (hazard ratio (HR): 1.84, 95% CI: 1.21–2.80, $P = 0.004$) and above the cardiac silhouette (HR: 2.22, 95% CI: 1.43–3.46, $P = 0.0004$)), larger amount of pleural fluid drained (HR: 1.06, 95% CI: 1.04–1.07, $P < 0.0001$) and higher pleural fluid LDH (HR: 1.008, 95% CI: 1.004–1.011, $P < 0.0001$) were associated with increased hazard of recurrence. Negative cytology (HR: 0.52, 95% CI: 0.43–0.64, $P < 0.0001$) was associated with decreased hazard of recurrence. The model had low prediction accuracy.**

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SUMMARY AT A GLANCE

Factors such as larger pleural effusion size, amount of pleural fluid drained, LDH and pleural fluid cytology were found to be risk factors for pleural fluid recurrence. Knowing what risk factors are associated with recurrence of pleural effusion would allow physicians to identify patients who are more likely to recur.

Conclusion: **Pleural effusion size, amount of pleural fluid drained, LDH and pleural fluid cytology were found to be risk factors for recurrence.**

Key words: effusion recurrence, pleural effusion, thoracentesis, malignant pleural effusion.

Abbreviations: CHF, congestive heart failure; CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; MPE, malignant pleural effusion; PET, positron emission tomography; ROC, receiver operating characteristics.

INTRODUCTION

Malignant pleural effusion (MPE) is associated with a median survival of 3–6 months and can cause significant dyspnoea resulting in poor quality of life.[1,2] The main purpose of treatment in patients with MPE is symptom palliation. There are a number of treatment alternatives available. Some, such as thoracentesis, achieve only temporary relief while others such as placement of an indwelling pleural catheter (IPC), chest tube with chemical pleurodesis and pleuroscopy with chemical pleurodesis are more definitive solutions.

Current recommendations on the management of pleural effusion in patients with malignancy propose

STEM CELLS AND REGENERATION RESEARCH ARTICLE

β-Catenin maintains lung epithelial progenitors after lung specification

Edwin J. Ostrin[1,2], Danielle R. Little[3], Kamryn N. Gerner-Mauro[1], Elizabeth A. Sumner[3], Ricardo Ríos-Corzo[4], Elizabeth Ambrosio[5], Samantha E. Holt[6], Nicolas Forcioli-Conti[7], Haruhiko Akiyama[8], Sam M. Hanash[6], Shioko Kimura[9], Sarah X. L. Huang[7] and Jichao Chen[1,*]

ABSTRACT

The entire lung epithelium arises from SRY box 9 (SOX9)-expressing progenitors that form the respiratory tree and differentiate into airway and alveolar cells. Despite progress in understanding their initial specification within the embryonic foregut, how these progenitors are subsequently maintained is less clear. Using inducible, progenitor-specific genetic mosaic mouse models, we showed that β-catenin (CTNNB1) maintains lung progenitors by promoting a hierarchical lung progenitor gene signature, suppressing gastrointestinal (GI) genes, and regulating NK2 homeobox 1 (NKX2.1) and SRY box 2 (SOX2) in a developmental stage-dependent manner. At the early, but not later, stage post-lung specification, CTNNB1 cell-autonomously maintained normal NKX2.1 expression levels and suppressed ectopic SOX2 expression. Genetic epistasis analyses revealed that CTNNB1 is required for fibroblast growth factor (Fgf)/Kirsten rat sarcoma viral oncogene homolog (*Kras*)-mediated promotion of the progenitors. *In silico* screening of Eurexpress and translating ribosome affinity purification (TRAP)-RNAseq identified a progenitor gene signature, a subset of which depends on CTNNB1. Wnt signaling also maintained NKX2.1 expression and suppressed GI genes in cultured human lung progenitors derived from embryonic stem cells.

KEY WORDS: Lung development, Epithelial progenitors, Beta-catenin, Mouse

INTRODUCTION

The epithelium in a mature lung consists of proximal air-conducting airways and distal gas exchange alveoli. Despite such spatial and functional distinction, both the airway and alveolar epithelia arise from common embryonic epithelial progenitors that express SRY box 9 (SOX9) and inhibitor of DNA binding 2 (ID2) in mice (Rawlins et al., 2009; Alanis et al., 2014; Yang and Chen, 2014; Volckaert and De Langhe, 2015). These SOX9-expressing progenitors (hereafter 'SOX9 progenitors') constitute the lung buds as early as their specification and emergence from the lateral side of the embryonic foregut, around embryonic day (E) 9.5, and before they extend and branch away from the foregut (Yang and Chen, 2014). The SOX9 progenitors undergo branching morphogenesis to build the respiratory tree until at least E18 and leave behind daughter cells that differentiate into airway and alveolar cells at early and late stages of lung development, respectively (Rawlins et al., 2009; Alanis et al., 2014). Thus, formation of a functional lung epithelium depends on the precise control of the morphogenesis and differentiation of these SOX9 progenitors, calling for a better understanding of the associated molecular mechanisms.

Although mouse genetics experiments have shown that CTNNB1-mediated canonical Wnt signaling is required for the foregut specification of NK2 homeobox 1 (NKX2.1)-expressing lung cells, which include SOX9 progenitors (Goss et al., 2009; Harris-Johnson et al., 2009), how these progenitors are subsequently maintained is less understood (Warburton et al., 2005; Cardoso and Lu, 2006; Morrisey and Hogan, 2010; Volckaert and De Langhe, 2015). Epithelial deletion of fibroblast growth factor receptor 2 (*Fgfr2*) leads to a complete loss of SOX9 progenitors and no branch formation beyond the two primary lung buds (De Moerlooze et al., 2000; Abler et al., 2009; Chang et al., 2013). Overexpression of an Fgf ligand-encoding gene, *Fgf10*, or epithelial expression of a hyperactive mutant of *Kras*, a small GTPase downstream of receptor tyrosine kinases, including FGFR2, leads to excessive SOX9 progenitors and overgrown branches that interfere with normal branching (Chang et al., 2013; Volckaert et al., 2013). Besides Fgf signaling, although multiple other signaling pathways are required for lung branching and patterning (Morrisey and Hogan, 2010), their specific role in the SOX9 progenitors is unclear, in part because prior studies have not specifically targeted and analyzed these cells. For example, epithelial *Ctnnb1* deletion using a *Sftpc-rtTA* driver led to a higher proportion of proximal airways versus distal alveoli (Mucenski et al., 2003; Shu et al., 2005), which might result from inefficient expansion of the SOX9 progenitors and/or their excessive differentiation into SOX2-expressing cells. Moreover, the relationship between CTNNB1-mediated Wnt signaling and Fgf signaling in SOX9 progenitors is still unclear (Shu et al., 2005; Wang et al., 2012; Volckaert et al., 2013). In addition, it is unknown to what extent the molecular program of the SOX9 progenitors depends on CTNNB1.

SOX9 is not only a progenitor marker, but is also required for normal progenitor branching (Chang et al., 2013; Rockich et al., 2013). However, the epithelial *Sox9* mutant lung still branches and expresses many genes that have the same expression pattern as SOX9 (Chang et al., 2013), suggesting the presence of additional upstream regulators of the progenitor program. In the current study, after screening multiple signaling pathways, we focused on the CTNNB1-

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RESEARCH ARTICLE

Inducible Lung Epithelial Resistance Requires Multisource Reactive Oxygen Species Generation To Protect against Viral Infections

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ABSTRACT Viral pneumonias cause profound worldwide morbidity, necessitating novel strategies to prevent and treat these potentially lethal infections. Stimulation of intrinsic lung defenses via inhalation of synergistically acting Toll-like receptor (TLR) agonists protects mice broadly against pneumonia, including otherwise-lethal viral infections, providing a potential opportunity to mitigate infectious threats. As intact lung epithelial TLR signaling is required for the inducible resistance and as these cells are the principal targets of many respiratory viruses, the capacity of lung epithelial cells to be therapeutically manipulated to function as autonomous antiviral effectors was investigated. Our work revealed that mouse and human lung epithelial cells could be stimulated to generate robust antiviral responses that both reduce viral burden and enhance survival of isolated cells and intact animals. The antiviral protection required concurrent induction of epithelial reactive oxygen species (ROS) from both mitochondrial and dual oxidase sources, although neither type I interferon enrichment nor type I interferon signaling was required for the inducible protection. Taken together, these findings establish the sufficiency of lung epithelial cells to generate therapeutically inducible antiviral responses, reveal novel antiviral roles for ROS, provide mechanistic insights into inducible resistance, and may provide an opportunity to protect patients from viral pneumonia during periods of peak vulnerability.

IMPORTANCE Viruses are the most commonly identified causes of pneumonia and inflict unacceptable morbidity, despite currently available therapies. While lung epithelial cells are principal targets of respiratory viruses, they have also been recently shown to contribute importantly to therapeutically inducible antimicrobial responses. This work finds that lung cells can be stimulated to protect themselves against viral challenges, even in the absence of leukocytes, both reducing viral burden and improving survival. Further, it was found that the protection occurs via unexpected induction of reactive oxygen species (ROS) from spatially segregated sources without reliance on type I interferon signaling. Coordinated multisource ROS generation has not previously been described against viruses, nor has ROS generation been reported for epithelial cells against any pathogen. Thus, these findings extend the potential clinical applications for the strategy of inducible resistance to protect vulnerable people against viral infections and also provide new insights into the

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Sensitivity of Initial Thoracentesis for Malignant Pleural Effusion Stratified by Tumor Type in Patients with Strong Evidence of Metastatic Disease

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Keywords
Pleural effusion · Thoracentesis · Malignant pleural effusion

Abstract

Background: Thoracentesis with cytological examination of pleural fluid is the initial test of choice for evaluation of pleural effusions in patients with suspected malignant pleural effusion (MPE). There is limited data on the sensitivity of thoracentesis stratified by tumor type. A better understanding of stratified sensitivities is of clinical interest, and may guide early and appropriate referral for pleural biopsy. ***Objective:*** The primary objective was sensitivity of thoracentesis with pleural fluid cytology stratified by tumor type. ***Methods:*** This is a retrospective cohort study of consecutive patients with a solid tumor malignancy with proven or strong suspicion for metastatic disease with new pleural effusions that underwent an initial thoracentesis. Only patients with metastatic disease were included. ***Results:*** Of the 725 patients examined, 63% had pleural fluid cytology positive for malignancy. Sensitivity of thoracentesis varied from a low of 0.38 (95% CI 0.13–0.68) in head and neck malignancy, 0.38 (95% CI 0.15–0.65) in sarcoma, and 0.53 (95% CI 0.34–0.72) in renal cancer to a high of 93 (95% CI 88–97) in breast cancer, and 100 (95% CI 0.82–100) in pancreatic cancer. Factors associated with an increased risk of MPE included larger amount of fluid drained ($p = 0.014$) and higher pleural fluid protein ($p = 0.002$). The only factor associated with decreased risk of MPE if first cytology was negative for malignancy was the presence of contralateral effusion ($p = 0.005$). ***Conclusions:*** Sensitivity of thoracentesis for solid tumors varies significantly depending on the type of tumor and is lowest in those with sarcomas, head and neck malignancies, and renal cell cancers.

Introduction

Approximately, 1.5 million new pleural effusions are diagnosed each year in the United States [1]. Thoracentesis with chemical, hematologic, and cytologic analysis of pleural fluid is the most common procedure performed for the diagnosis of pleural effusion not readily explained by clinical history [2]. In patients with cancer, pleural effusion may represent metastatic disease and making a diagnosis is paramount [3]. A definitive diagnosis of malignant pleural effusion (MPE) can be made by thoracentesis

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Otras agencias		0
Aplicaciones no fundadas		6
Aplicaciones para patentes		2

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02	**Alejandra Gómez**	Pamplona	NS/Neurology	Barcelona	Interventional	Vancouver General	Y
03	**Daniel Moreira**	Cincinnati	Pediatrics	Colorado	Oncology Peds	St. Jude's, International Oncology	Y
04	**Miguelina de la Garza**	Methodist	Pathology	Methodist	Derm / Cytology	San Antonio	N
05	**Guillermo Valdez**	Yale	Psychiatry	Yale	Adolescent Psych	New York	N
06	**John Manllo**	Henry Ford	IM	J Hopkins	Nephrology	McAllen	N
07	**César Ochoa**	Henry Ford	IM	Mayo Clinic	Oncology	Florida	N
08	**Alfredo Dávalos**	Baylor	Neurology	Columbia	Electrophysiology	Henry Ford Health	Y
09	**Francisco Guzmán**	Harvard	Surgery	U Ohio	Surgery		
10	**David Moreno**	UT Dallas	Pediatrics	Baylor	Pulmonary Peds		
11	**Elsa Rodarte**	UT Houston	Neurology				
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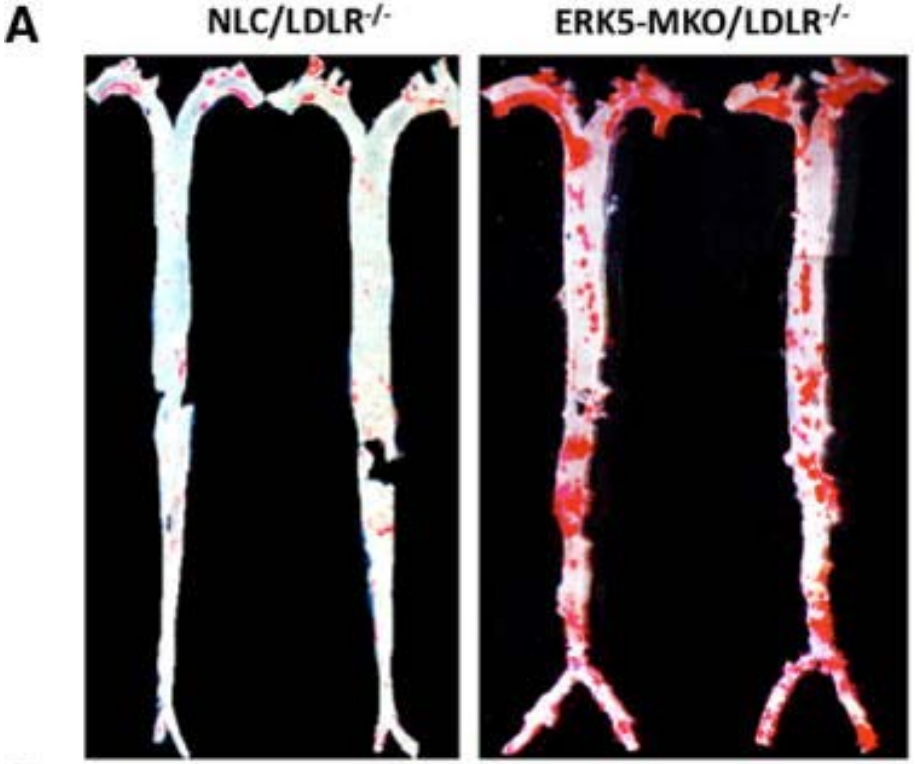
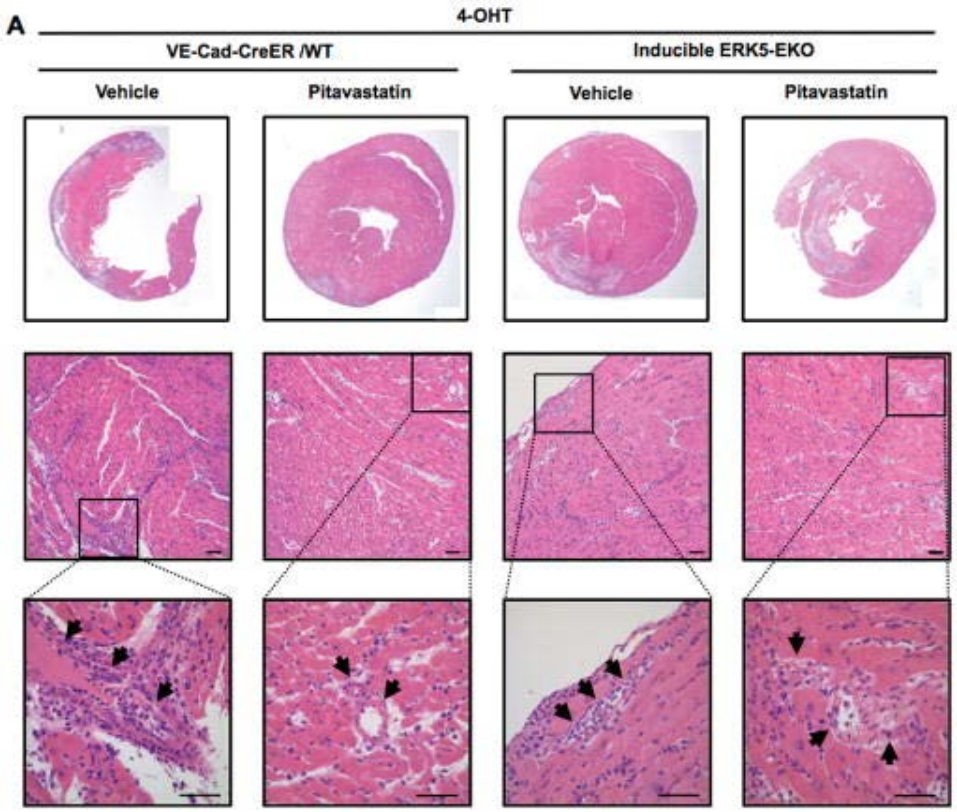
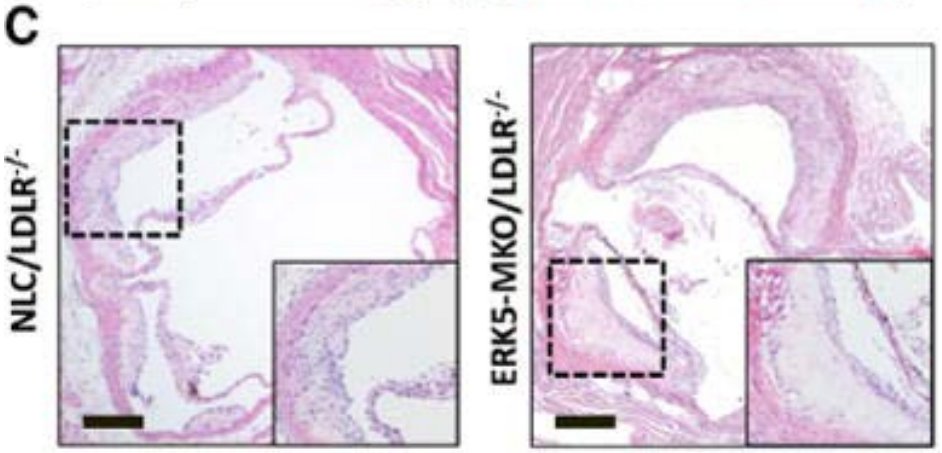
Jun-ichi Abe
Cardiology

Signal transduction in atherosclerosis
Chemotherapy-induced cardiomyopathy

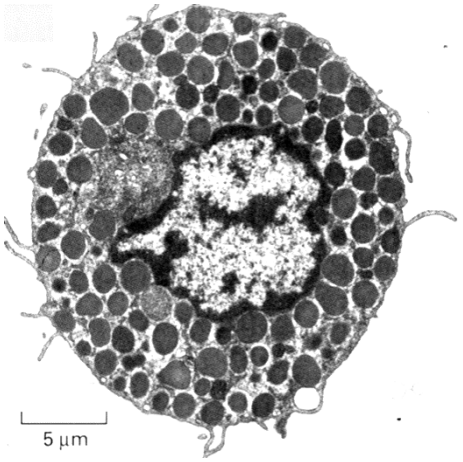


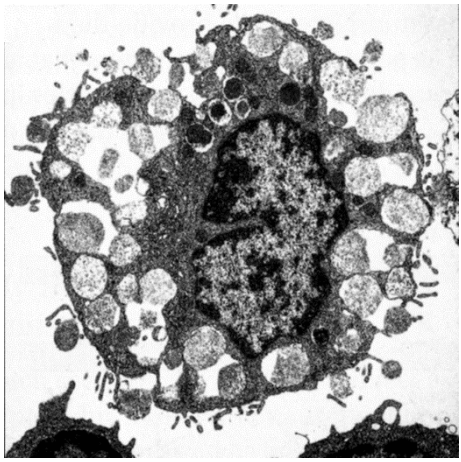


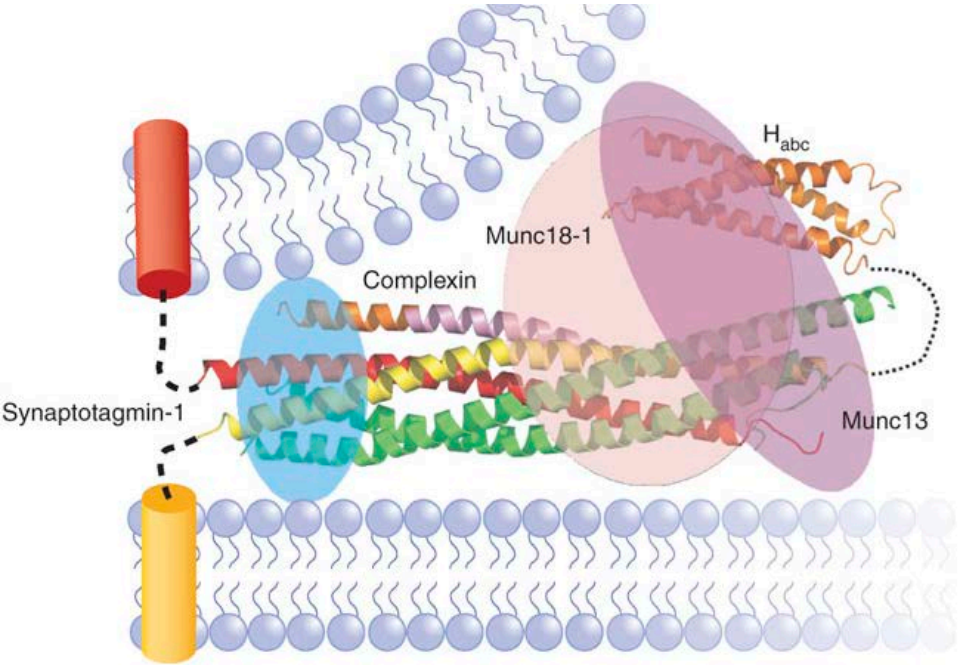


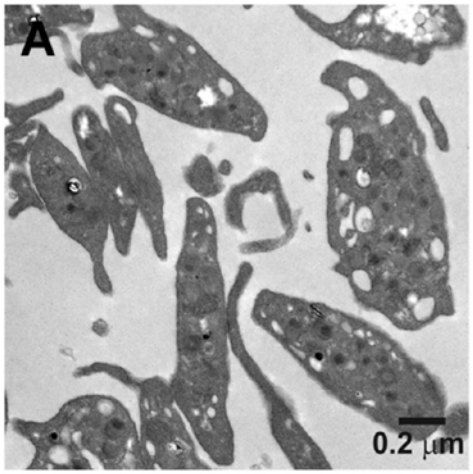
Roberto Adachi
Pulmonary Medicine

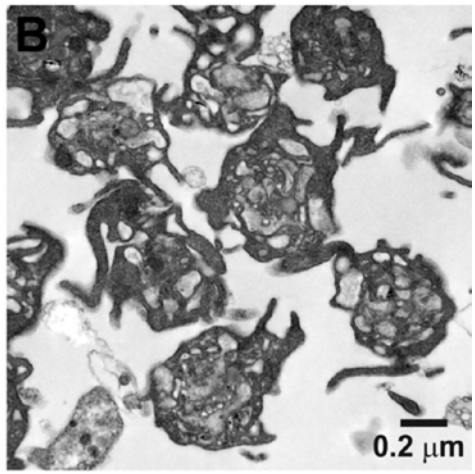
Regulated exocytosis
Mast cell degranulation
Platelet degranulation











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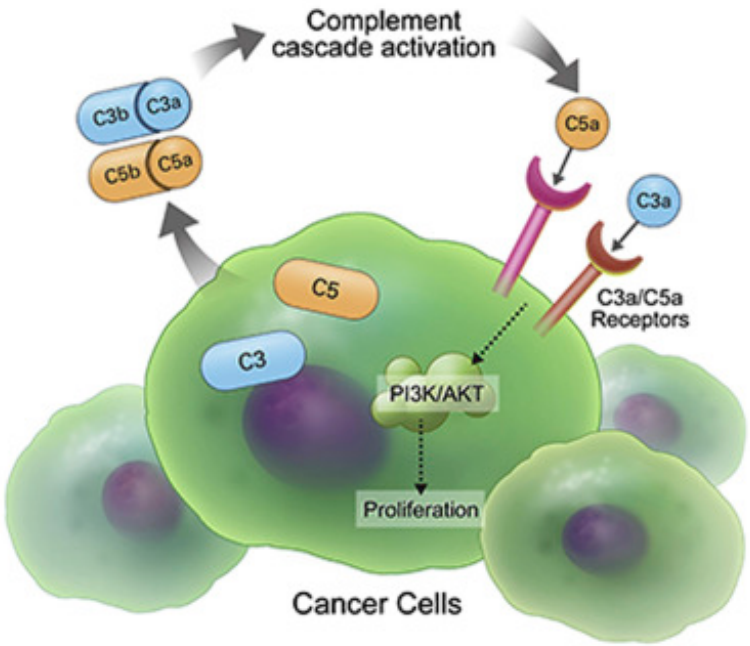
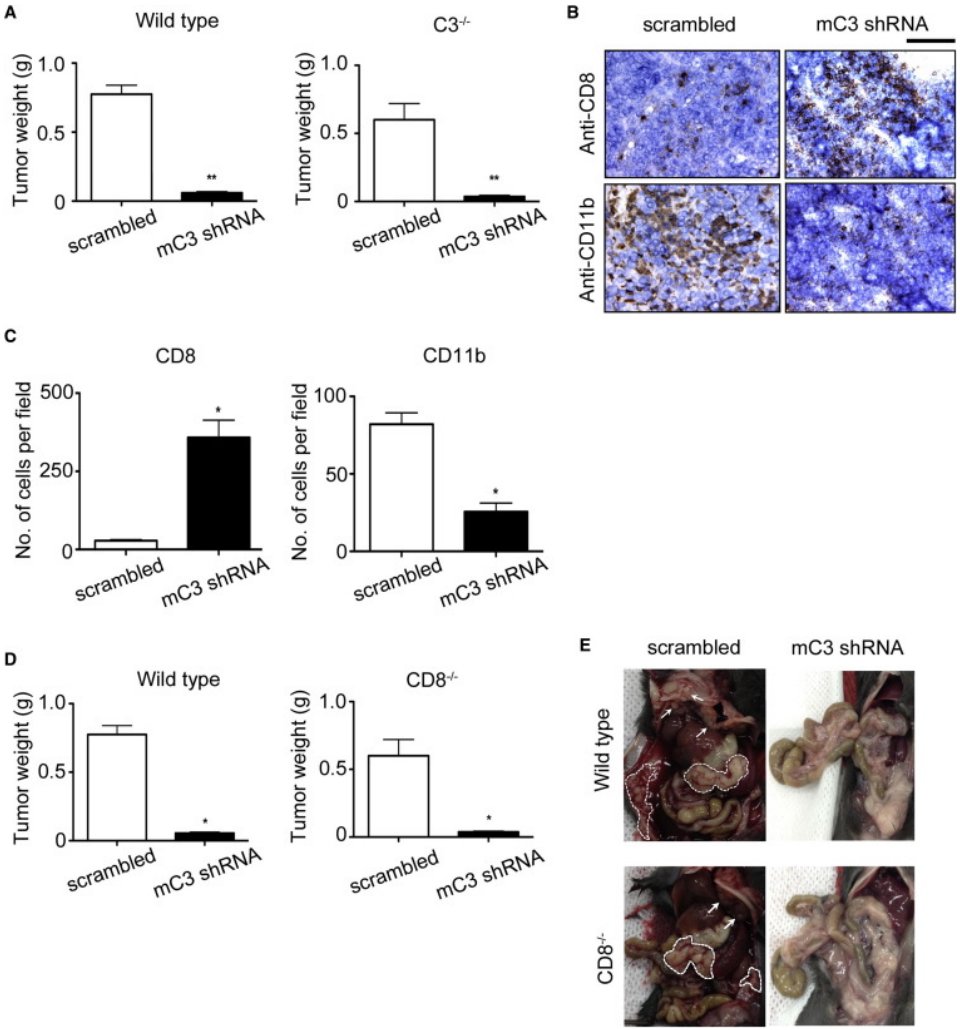
Hematology

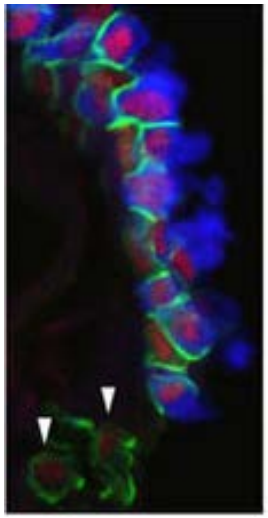
Platelets and the complement system

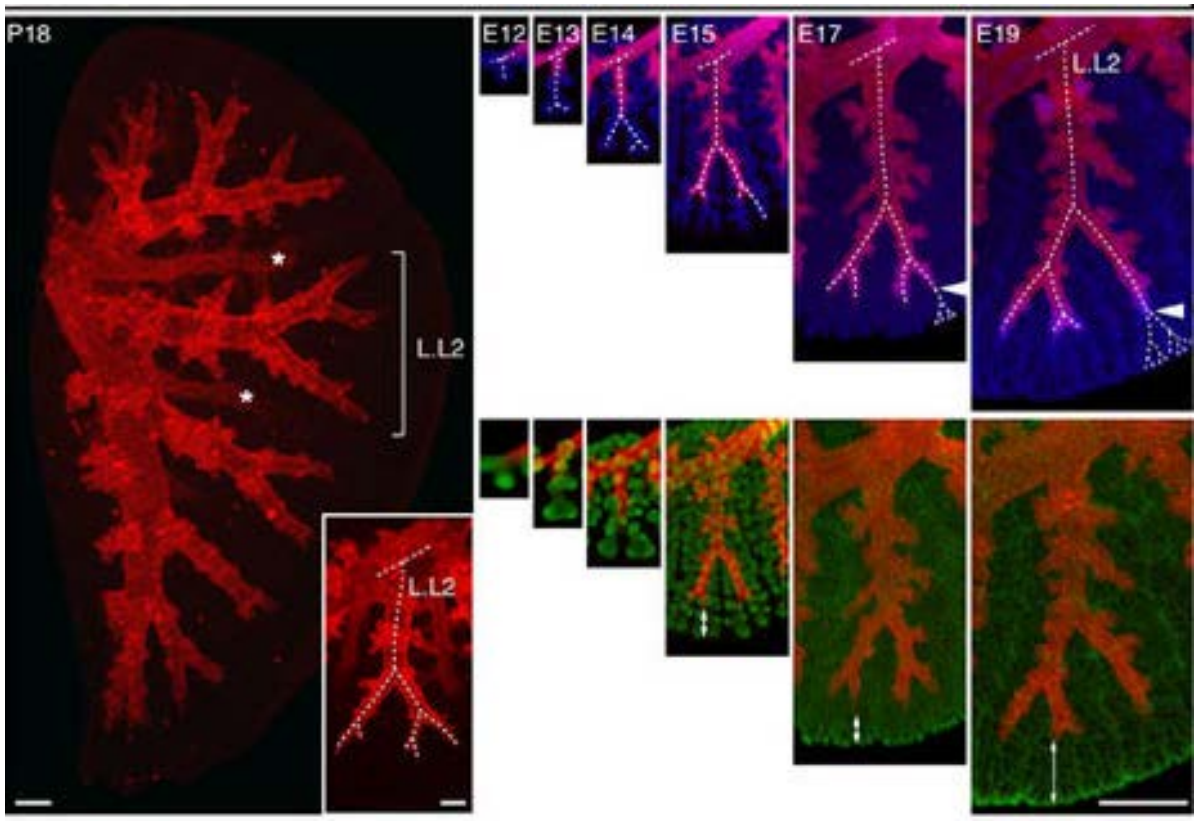


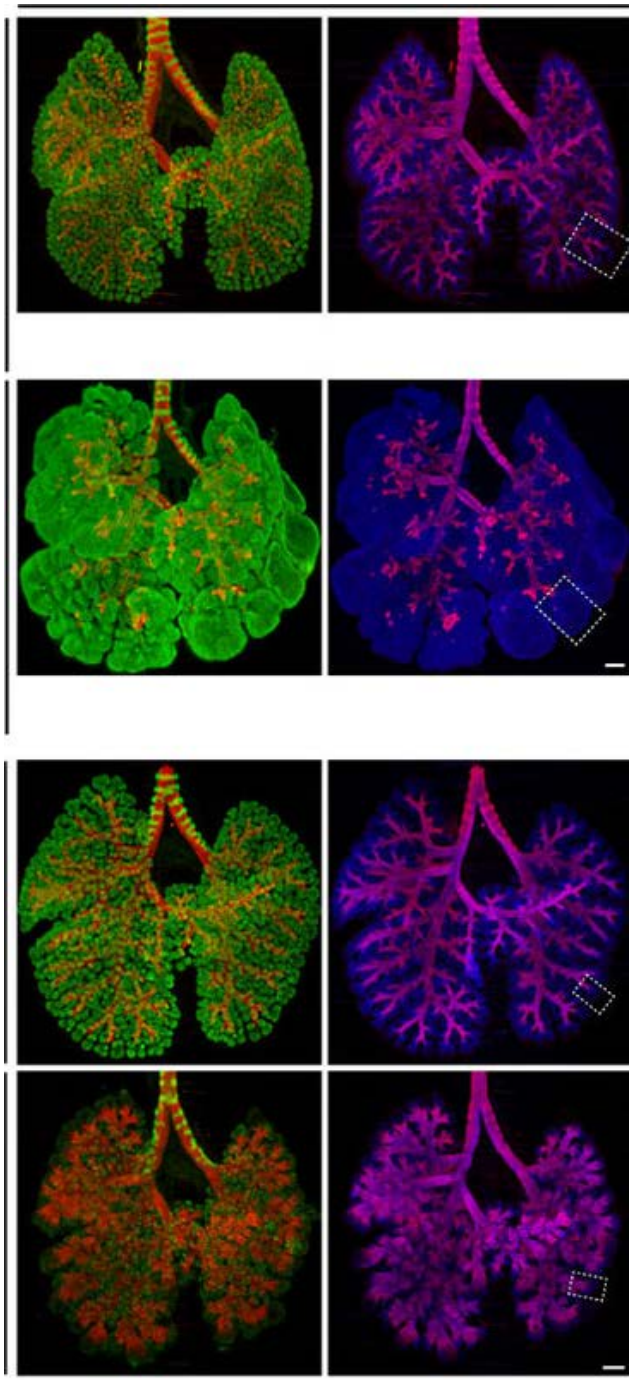


Ji-chao Chen
Pulmonary Medicine

Lung development

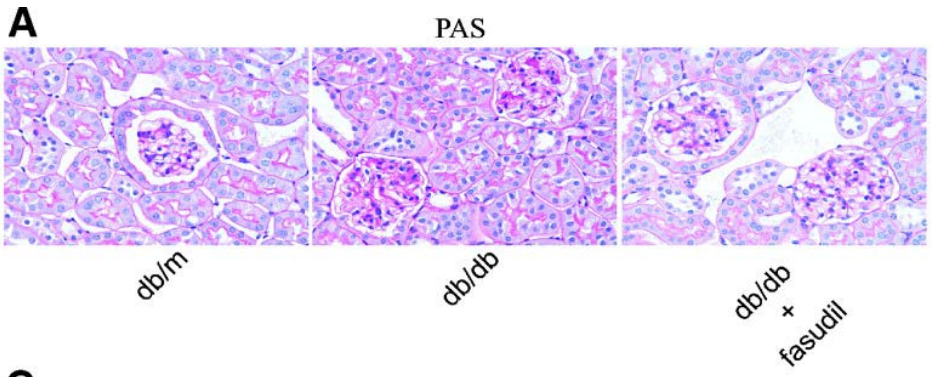


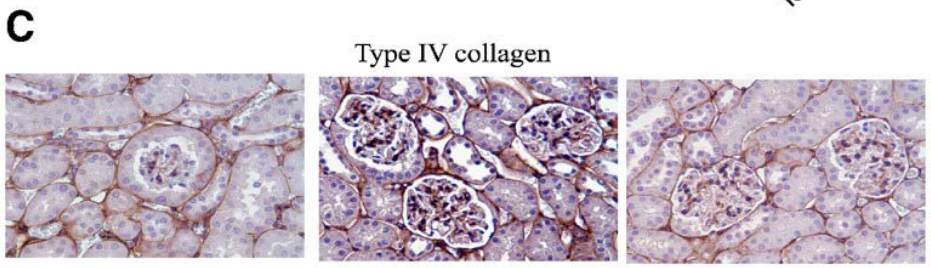


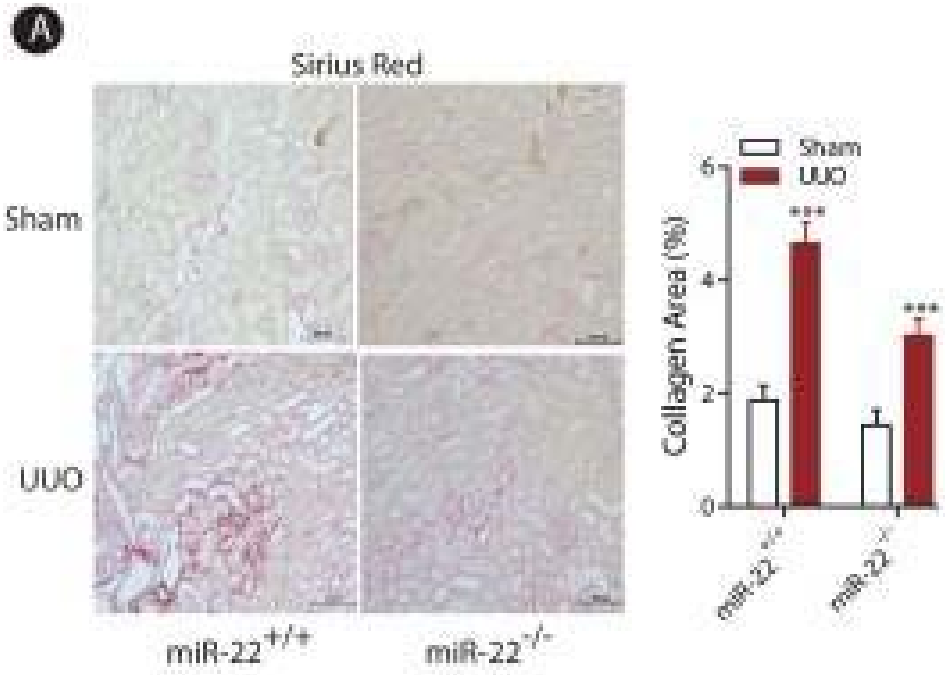


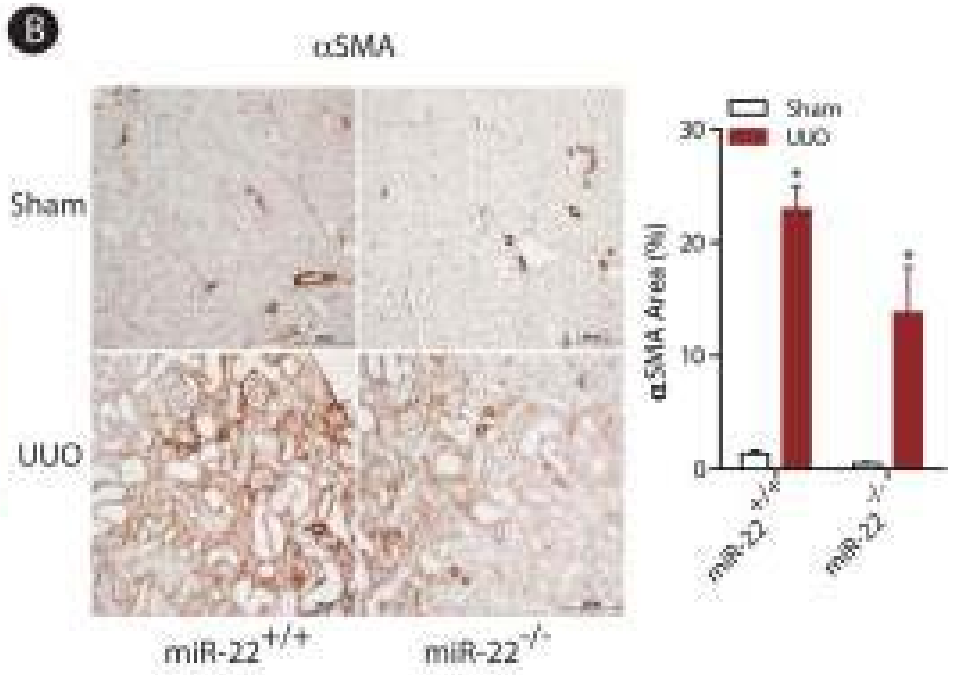
Farhad Danesh
Nephrology

Mitochondrial dysfunction and microRNAs in diabetic nephropathy



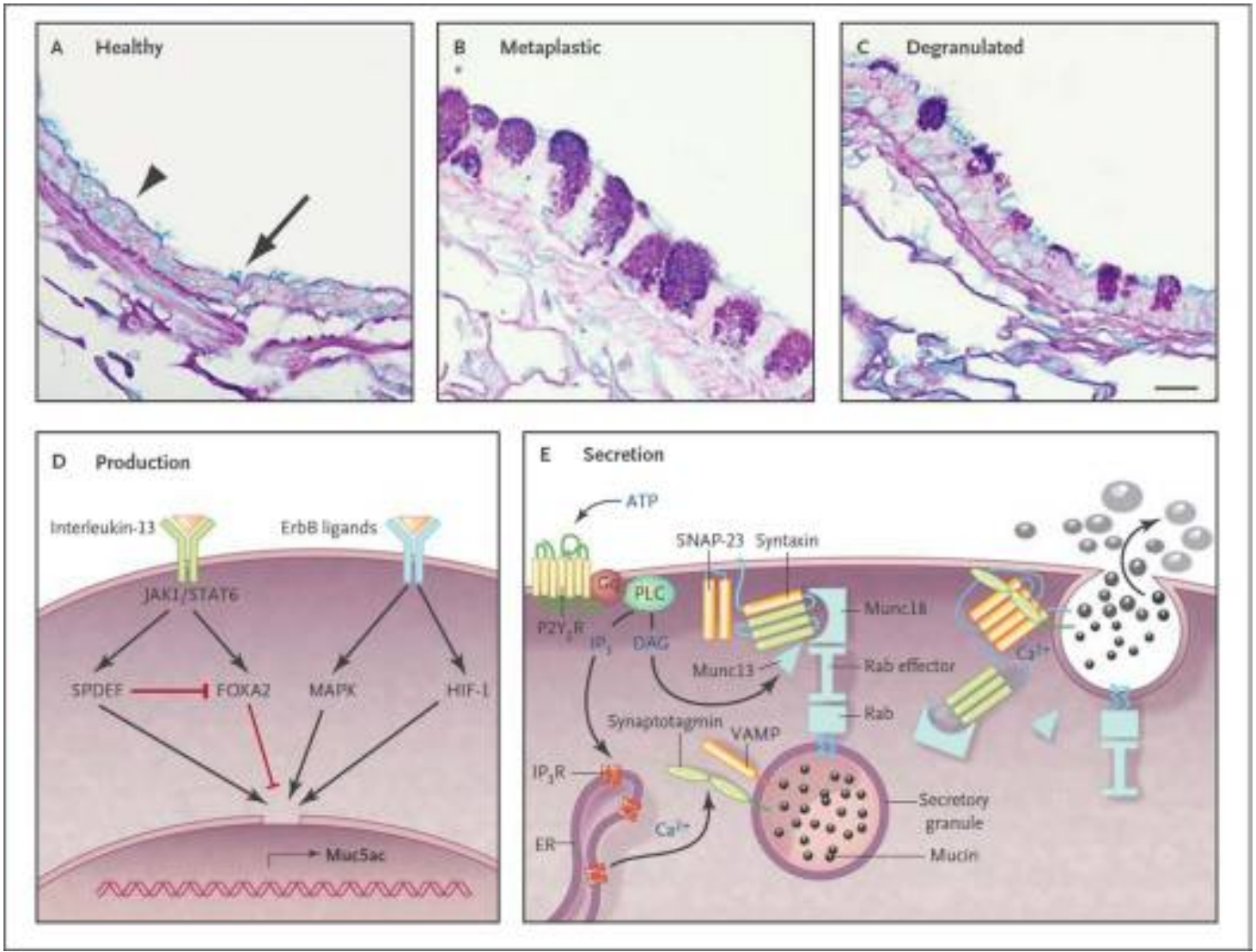






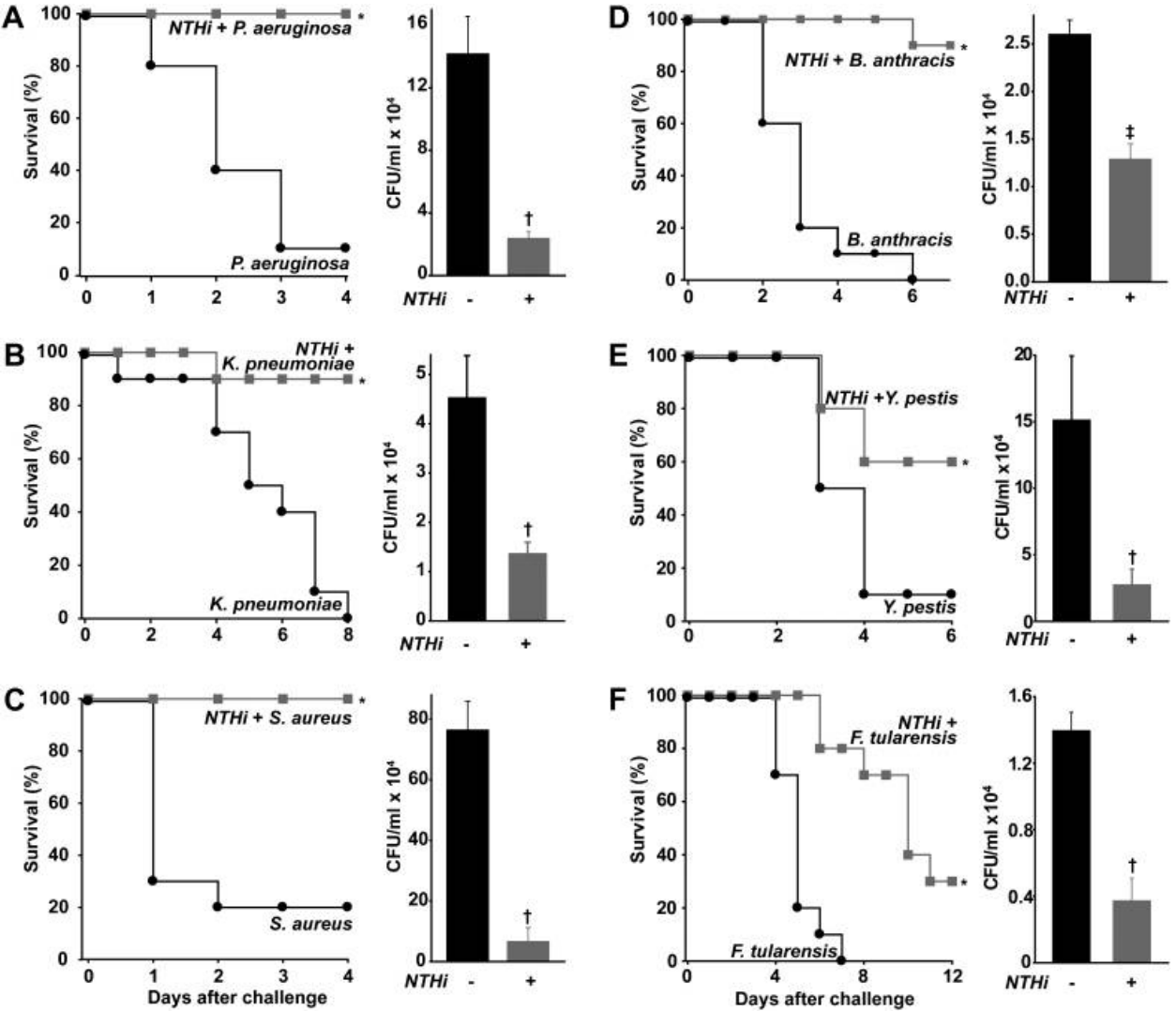
Burton Dickey, Michael Tuvim
Pulmonary Medicine

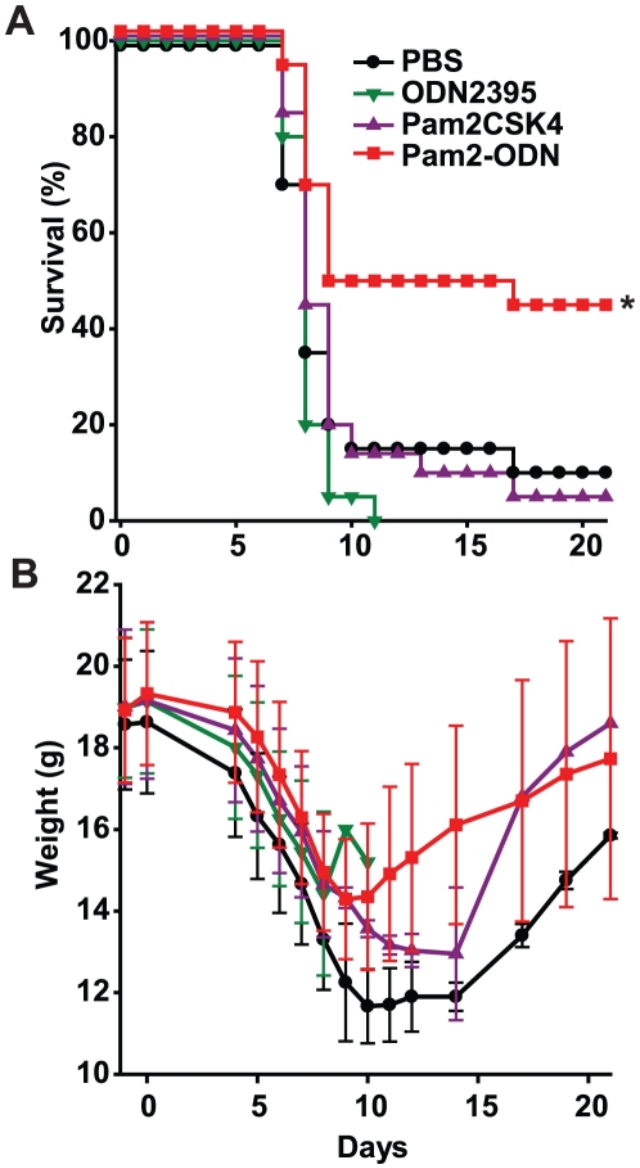
Exocytosis of mucins into the airways



Scott Evans
Pulmonary Medicine

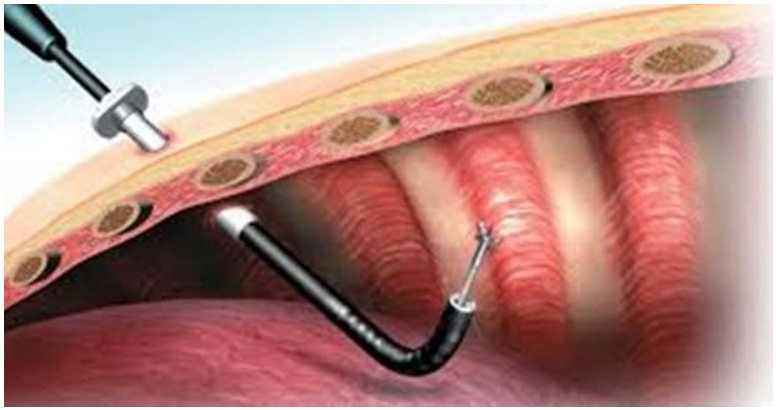
Innate immune responses of airway epithelium

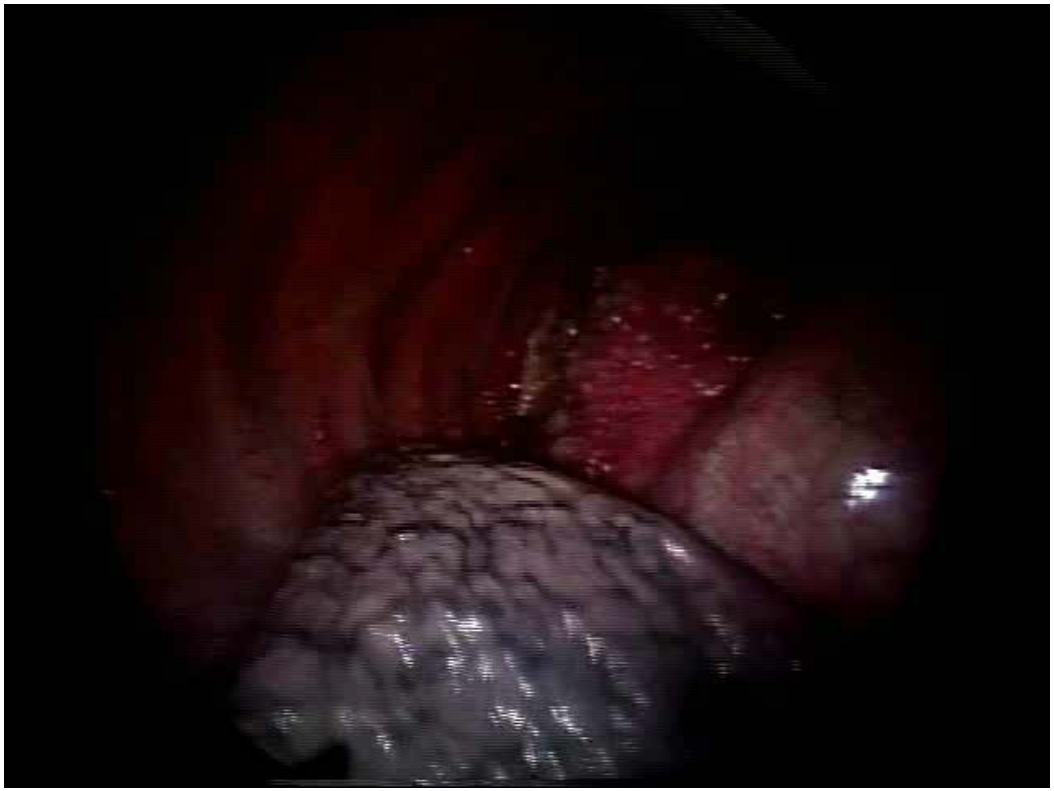




Horiana Grosu
Pulmonary Medicine

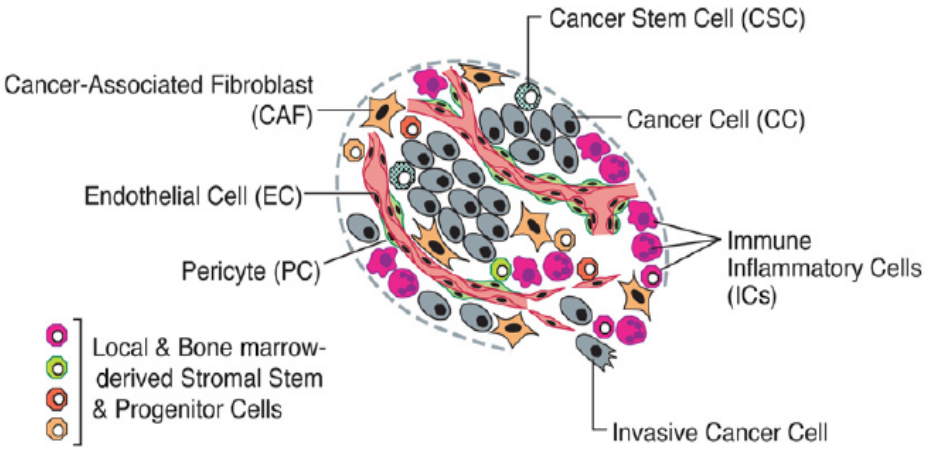
Clinical validation of Interventional Pleural techniques

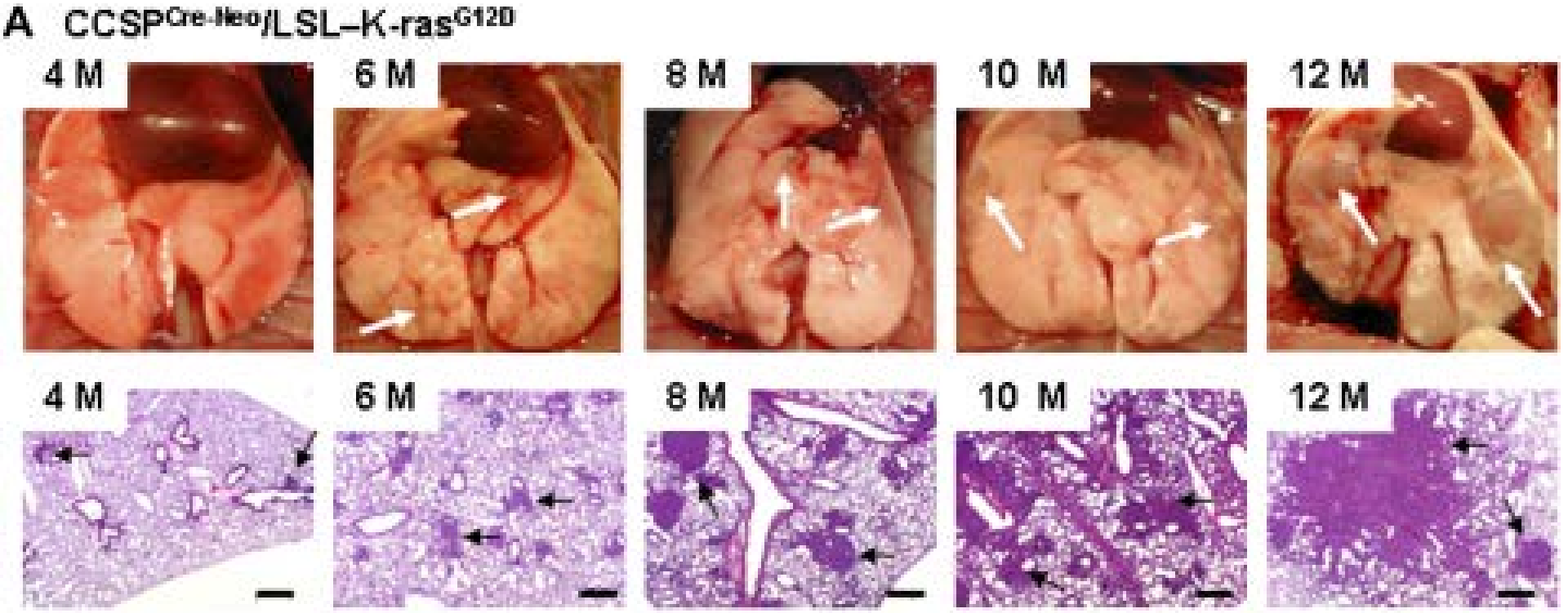




Seyed Moghaddam (“Peyman”)
Pulmonary Medicine

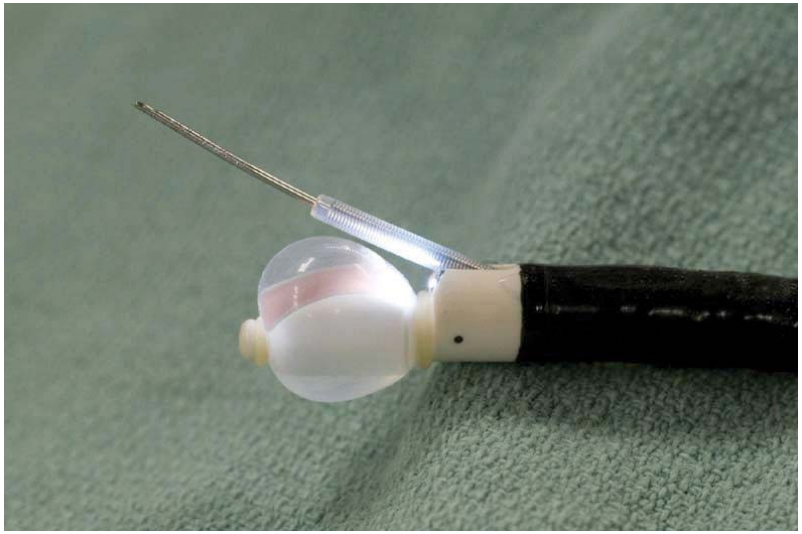
Inflammation-induced Carcinogenesis

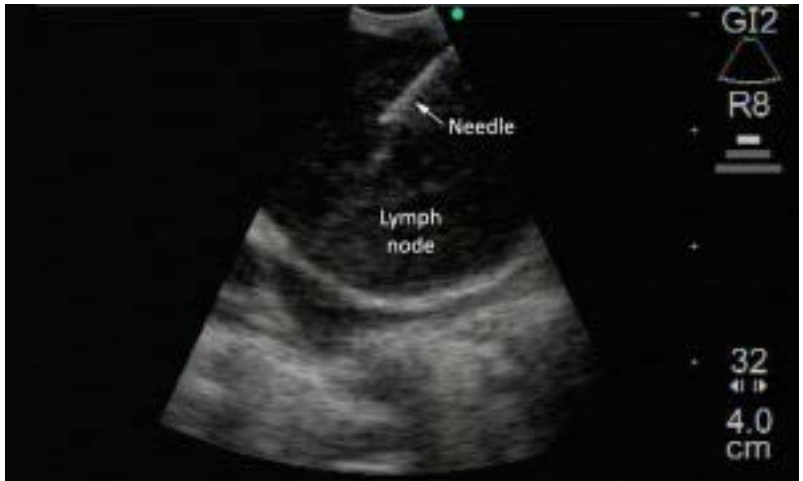


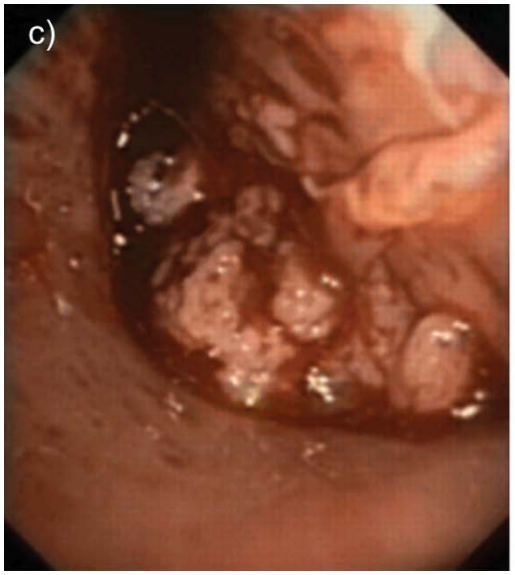


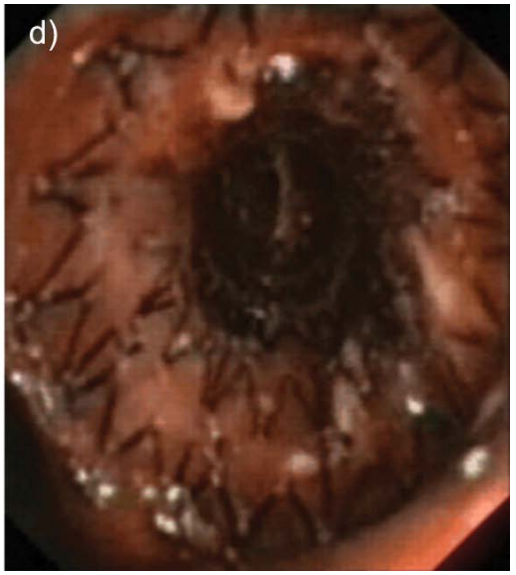
David Ost
Pulmonary Medicine

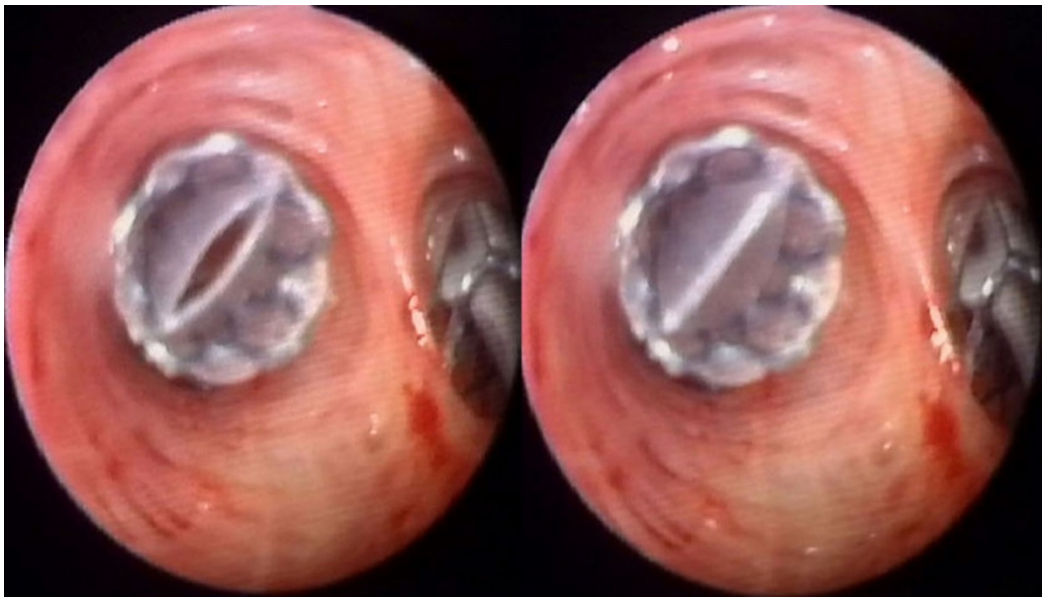
Clinical validation of Interventional Airway techniques





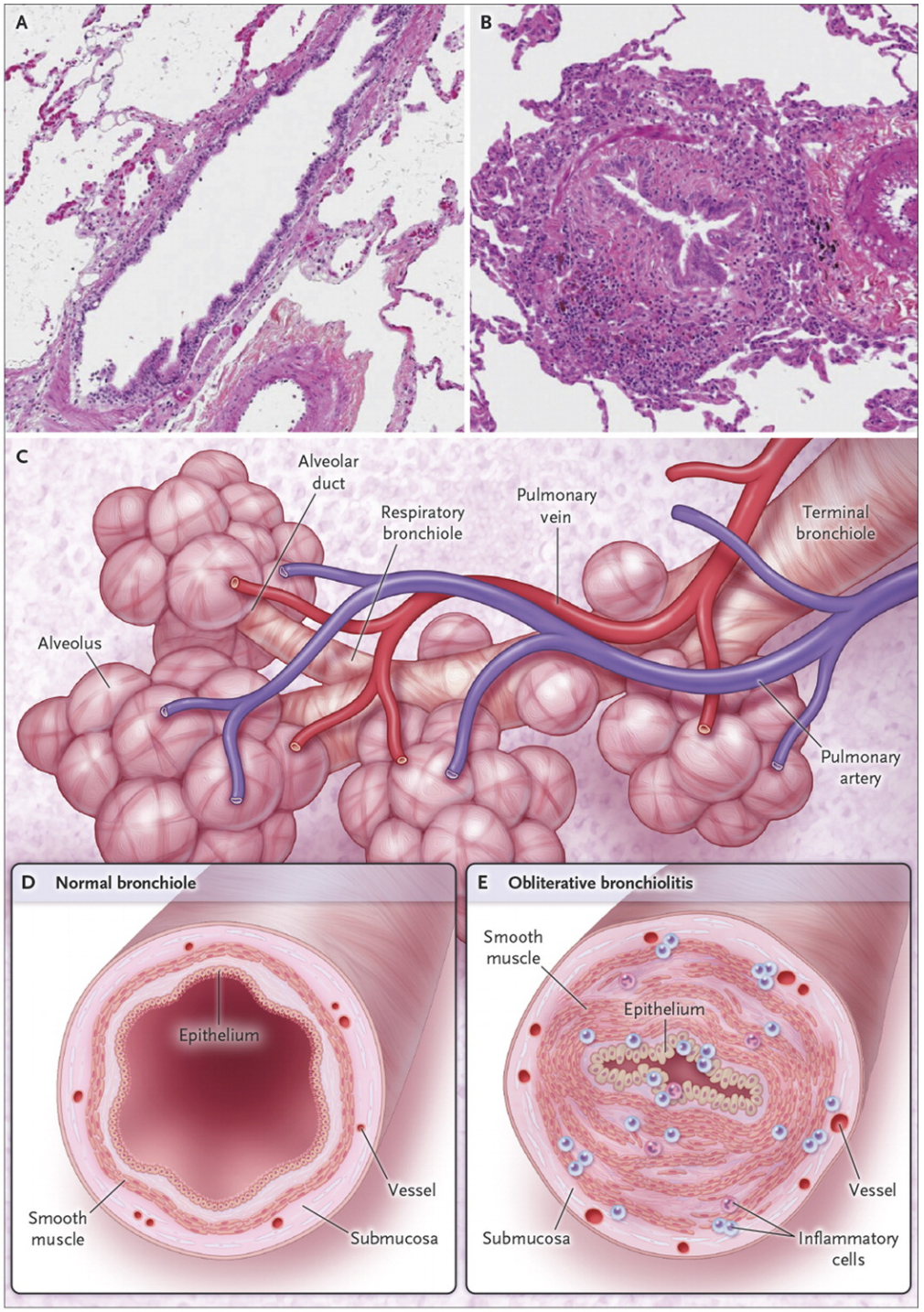






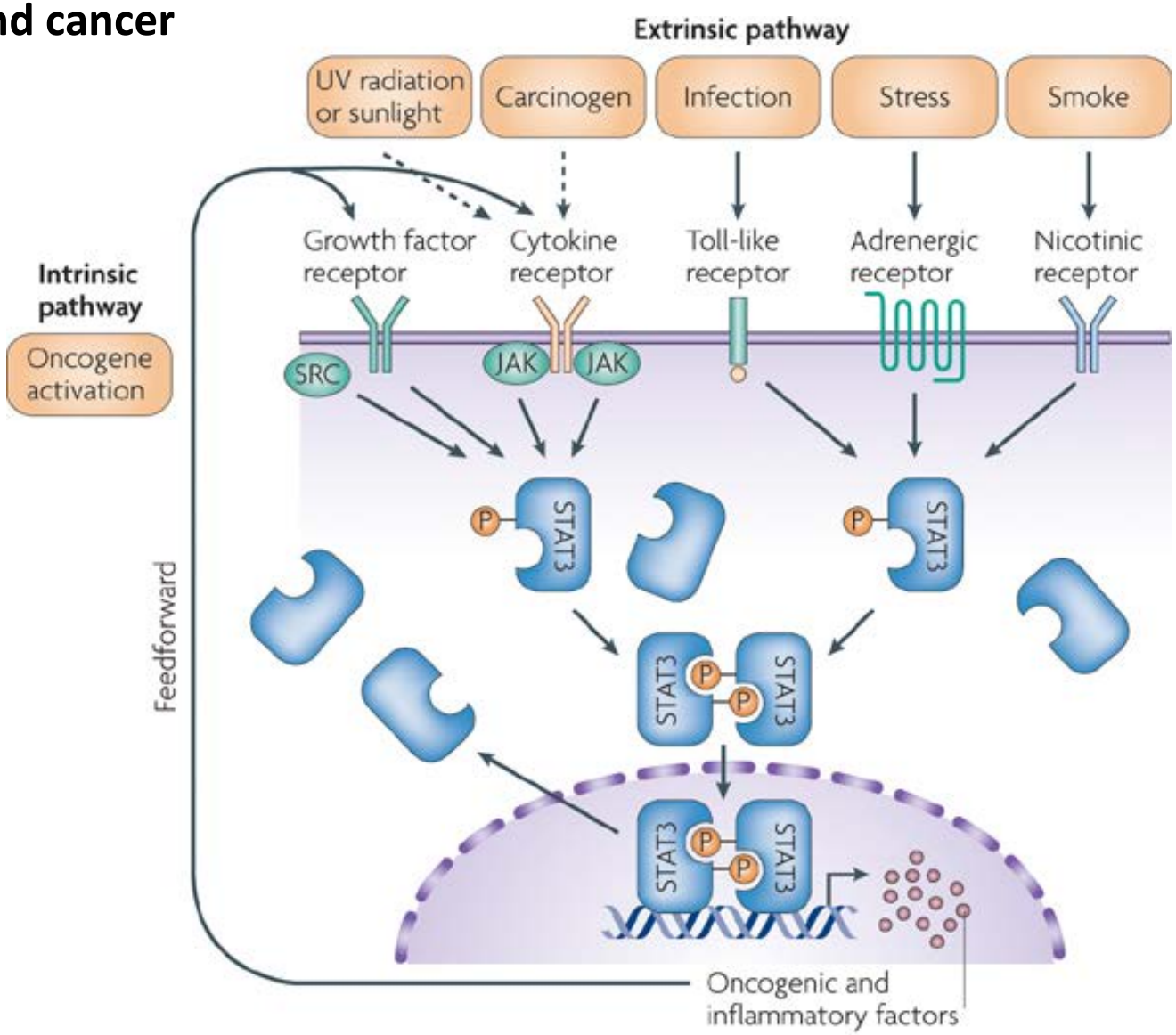
Ajay Sheshadri
Pulmonary Medicine

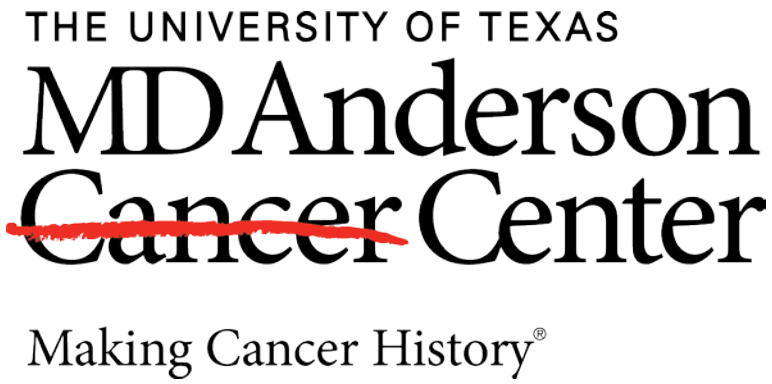
Bronchiolitis obliterans in stem cell transplant patients



David Tweardy
Infectious Diseases

STAT3 in inflammation and cancer

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