

**2006 APS Conference
Physiological Genomics and Proteomics of Lung Disease**

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Univ. of Pittsburgh,
Grad. Sch. Public Hlth.

Acknowledgements

The Conference Organizers and The American Physiological Society gratefully recognize the generous financial support provided through unrestricted educational grants from:

**NIH, National Heart, Lung, and Blood Institute
NIH, National Institute of Diabetes and Digestive and Kidney Diseases
NIH, National Institute of Environmental Health Sciences
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2006 APS Conference
Physiological Genomics and Proteomics of Lung Disease
November 2-5, Marriott Fort Lauderdale North

Thursday, November 2	Friday, November 3	Saturday, November 4	Sunday, November 5
<p>10:00 AM Registration</p> <p>1:30 -1:45 PM Welcome: J. Usha Raj, Harbor-UCLA, Univ. of California, Los Angeles</p> <p>1:45 – 2:45 PM Keynote Address: Allen Cowley, Jr., Med. College of Wisconsin</p> <p>3:00 – 5:00 PM Symposium I: Genomics and Proteomic Approaches to Studying Lung Disease Participants : Augustine Choi, (Chair) Univ. of Pittsburgh Lisa Schwiebert, (Chair) Univ. of Alabama at Birmingham Dean Sheppard, Univ. of California, San Francisco Richard Caprioli, Vanderbilt Univ. Med. Ctr. Constantin Aliferis, Vanderbilt Univ. Med. Ctr. Peter Davies, Univ. of Pennsylvania</p> <p>5:00 -7:00 PM Opening Reception</p>	<p>7:00 AM Registration</p> <p>8:00 -10:00 AM Symposium II: Proteomic and Genomic Approaches to Developing Potential Therapeutic Targets Participants: Bruce Pitt, (Chair) Univ. of Pittsburgh Zea Borok , (Chair) Univ. of Southern California Harry Ischiropoulos, Univ. of Pennsylvania Jeff Whitsett, Cincinnati Children’s Hosp Med. Ctr. Jan Schnitzer, Sidney Kimmel Cancer Ctr. Steve Shapiro, Brigham & Women’s Hosp.</p> <p>10:30 AM-12:30 PM Selected Oral Abstract Presentations Arnold Brody (Chair) North Carolina State Univ. Sadis Matalon (Chair) Univ. of Alabama, Birmingham</p> <p>12:30-2:00 PM FREE TIME</p> <p>2:00-4:00 PM Symposium III: Genomics and Proteomics of Airway and Vascular Disease Participants: Tom Martin, (Chair) Univ. of Washington Barbara Meyrick (Chair) Vanderbilt Univ. Med. Ctr. Patricia Finn, Brigham & Women’s Hospital Jim Loyd, Vanderbilt Univ. Med. Ctr. Scott Weiss, Harvard Med. Sch. David Erle, Univ. of California, San Francisco Jason Moore, Dartmouth-Hitchcock Med. Ctr.</p> <p>4:30-6:30 PM Poster Session: RubinTuder, (Chair) Johns Hopkins Univ. Mark Geraci, (Chair) Univ. of Colorado Hlth. Sci. Ctr.</p>	<p>7:00 AM Registration</p> <p>8:00 -9:00 AM Featured Presentation: David A. Schwartz, NIEHS, NIH</p> <p>9:00 -11:00 AM Symposium IV: Genomics and Proteomics of Environmental Lung Disease Participants: Kenneth Adler, (Chair) North Carolina State Univ. Brooke Mossman, (Chair) Univ. of Vermont Naftali Kaminski, Univ. of Pittsburgh Serpil Erzurum, Cleveland Clinic Foundation John Quackenbush, Dana-Farber Cancer Inst. Tara Sabo-Attwood, Univ. of South Carolina Steve Kleeberger, NIEHS, NIH</p> <p>11:30 AM-1:30 PM Selected Oral Abstract Presentations Robin Steinhorn, (Chair) Northwestern Univ. Peter Jones, (Chair) Univ. of Colorado Hlth. Sci. Ctr.</p> <p>1:30 -6:00 PM FREE TIME</p> <p>6:00-8:00 PM Poster Session: Tom Mariani, (Chair) Harvard Med. Sch. John Torday, (Chair) Univ. of California, Los Angeles</p> <p>8:00 PM Conference Banquet and Awards Presentations</p>	<p>7:00 AM Registration</p> <p>8:00 -10:00 AM Symposium V: Acute Lung Injury and Inflammation Participants: Jahar Bhattacharya, (Chair) Columbia Univ. Ellen Burnham, (Chair) Emory Univ. George Leikauf, Univ. of Cincinnati Kathleen Barnes, Johns Hopkins Univ. Lorraine Ware, Vanderbilt Univ. Med. Ctr. Mark Duncan, Univ. of Colorado Hlth. Sci. Ctr. Mark Wurfel, Univ. of Washington</p> <p>10:00 -11:00 AM Conference Summary Michael Matthay, Univ. of California, San Francisco</p>

Location:

The 2006 APS Conference, Physiological Genomics and Proteomics of Lung Disease will be held November 2-5, 2006 at the Fort Lauderdale Marriott North, 6650 North Andrews Avenue, Fort Lauderdale, FL 33309, telephone (954) 771-0440, FAX: (954) 772-9834.

Onsite Registration Hours:

Thursday, November 210:00 AM—6:30 PM
 Friday, November 37:00 AM—6:30 PM
 Saturday, November 47:00 AM—1:30 PM
 Saturday, November 46:00—7:30 PM
 Sunday, November 57:00—10:00 AM

On-Site Registration Fees:

APS Member.....\$340
 Retired Member\$225
 Nonmember.....\$390
 Postdoctoral.....\$275
 Student\$225

The registration fee includes entry into all scientific sessions, opening reception and banquet.

Payment Information:

Registrants may pay by institutional or personal check, traveler's check, MasterCard, VISA or American Express. Checks must be payable to "The American Physiological Society" and drawn on a United States bank payable in US dollars.

Student Registration

Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student fee. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. Nonmember Students who register onsite must provide a valid university student ID card. APS Student members should present their current APS membership card indicating their student category status.

Postdoctoral Registration

Any person who has received a Ph.D. degree in physiology or related field, within four years of this meeting, as attested to by the department head is eligible to register at the postdoctoral fee.

A statement signed by the department head must accompany the registration form and remittance when registering.

Press

Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

Continuing Medical Education (CME)

The Federation of American Societies for Experimental Biology is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

Category I Continuing Medical Education (CME) credits will be offered at this meeting. CME application forms will be available in the Onsite Meeting Registration Counter. For the purposes of Continuing Medical Education credits toward the American Medical Association Physician's Recognition Award, the 2006 APS Intersociety Meeting: Comparative Physiology 2006: Integrating Diversity is jointly sponsored by the Federation of American Societies for Experimental Biology. There is a \$45 application fee, payable upon submission of the form. Please include your payment with the completed CME form. For more information, contact the FASEB Office of Scientific Meetings and Conferences at 301-634-7010.

Program Objective

Although our understanding of lung pathology has grown rapidly in recent decades, the underlying mechanisms of many diseases remain obscure. Genomic research offers a new opportunity for determining how diseases occur, by taking advantage of experiments of nature and a growing array of sophisticated research tools to identify the molecular abnormalities underlying disease processes. This conference will examine the current utilization of these powerful tools of genomics and proteomics in the comprehension of lung disease and explore the potential application of newer methodologies in an attempt to understand their pathogenesis and find means to prevent and cure them.

Target Audience

This meeting is intended for all students, young investigators and professionals involved in teaching, research and clinical fields related to genomics and proteomics.

DAILY SCHEDULE

THURSDAY, NOVEMBER 2, 2006

- 1.0 WELCOME ANNOUNCEMENT**
Thur., 1:30 - 1:45 PM, Salon D.
- 1.1** Introduction. **J. Usha Raj.** Harbor-UCLA Med. Ctr., Univ. of California, Los Angeles.
- Keynote
- 2.0 GENOMIC APPROACHES TO COMPLEX DISEASES**
Thur., 1:45 - 2:45 PM, Salon D.
- Speaker: **Allen Cowley, Jr.** Med. Col. of Wisconsin.
- Symposium
- 3.0 GENOMICS AND PROTEOMIC APPROACHES TO STUDYING LUNG DISEASE**
Thur., 3:00 - 5:00 PM, Salon D.
- Chair: **Augustine Choi**, Univ. of Pittsburgh.
Lisa Schwiebert, Univ. of Alabama at Birmingham.
- 3:00 PM **3.1** Introduction. **Augustine Choi.** Univ. of Pittsburgh.
- 3:05 PM **3.2** Integration of Functional Genomics and Mouse Mutagenesis to Identify Pathways of Lung Development and Disease. **Dean Sheppard.** Univ. of California, San Francisco.
- 3:35 PM **3.3** In situ Molecular Protein Profiles and Images of Cancer Tissue Using Mass Spectrometry. **Richard Caprioli.** Vanderbilt Univ. Med. Ctr.
- 4:05 PM **3.4** Pathway Induction and High-fidelity Simulation for Molecular Signature and Biomarker Discovery in Lung Cancer using Microarray Gene Expression Data. **Constantin Aliferis.** Vanderbilt Univ. Med. Ctr.
- 4:35 PM **3.5** Multiscale Studies of Endothelial Phenotype. **Peter Davies.** Univ. of Pennsylvania.

Plan to Attend the Welcome and Opening Reception

*Thursday, November 2
5:00 – 7:00 PM*

Pool deck (weather permitting)

FRIDAY, NOVEMBER 3, 2006

- Symposium
- 4.0 PROTEOMIC AND GENOMIC APPROACHES TO DEVELOPING POTENTIAL THERAPEUTIC TARGETS**
Fri., 8:00 - 10:00 AM, Salon D.
- Chair: **Bruce R. Pitt**, Univ. of Pittsburgh.
Zea Borok, Univ. of Southern California.
- 8:00 AM **4.1** Introduction. **Bruce Pitt.** Univ. of Pittsburgh.
- 8:05 AM **4.2** Application of Proteomic Approaches to Reveal New Functional Roles of Nitric Oxide. **Harry Ischiropoulos.** Univ. of Pennsylvania.
- 8:35 AM **4.3** Transcriptional Regulation of Respiratory Epithelial Differentiation. **Jeffrey Whitsett.** Cincinnati Children's Hosp. Med. Ctr.
- 9:05 AM **4.4** Proteomic Mapping of the Endothelial Surface in Lung for Tissue-specific Therapy. **Jan Schnitzer.** Sidney Kimmel Cancer Ctr., San Diego.
- 9:35 AM **4.5** Genomic and Proteomic Approach to Finding New Therapeutic Approaches for COPD. **Steven Shapiro.** Brigham & Women's Hosp.
- Oral Presentations
- 5.0 SELECTED ORAL PRESENTATIONS**
Fri., 10:30 AM - 12:30 PM, Salon D.
- Chair: **Arnold R. Brody**, North Carolina State Univ.
Sandis Matalon, Univ. of Alabama at Birmingham.
- 10:30 AM **5.1** Introduction. **Arnold R. Brody.** North Carolina State Univ.
- 10:35 AM **5.2** Mycoplasma Pneumoniae Decreases Airway Epithelial Vascular Endothelial Growth Factor Gene Expression in Mice: Microarray and Laser Capture Microdissection Approaches. **Hong Wei Chu.** Natl. Jewish Med. and Res. Ctr. (15.5).
- 10:50 AM **5.3** Laser Capture Microdissection and Microarray Analysis Reveal MMP19 as a Potential New Regulator of Fibrosis. **Elizabeta Naumovski.** Univ. of Pittsburgh. (13.2).

- 11:05 AM **5.4** Differential Gene Expression in Primary Human Airway Epithelium Exposed to Isomers of Albuterol. **Rebecca Persinger**. *Sepracor, Inc.* (15.4).
- 11:20 AM **5.5** Gene Expression and Cytokine Profiling of Lung Tissue in a Mouse Model of LPS Tolerance. **Judie Howrylak**. *Univ. of Pittsburgh.* (7.3).
- 11:35 AM **5.6** Peroxynitrite-Induced Nitration and Cross-Linking of Human Surfactant Protein D (SP-D). **Sandis Matalon**, *Univ. of Alabama at Birmingham.* (8.5).
- 11:50 AM **5.7** Cigarette Smoke Sensitive Lung Transcriptome is Modulated by Tocopherol Transfer Protein. **K. Gohil**. *Univ. of California, Davis.* (14.3).
- 12:05 PM **5.8** Evidence for Attempted Regional Elastic Fiber Repair in Severe Emphysema. **Jason Woods**. *Washington Univ.* (14.4).
- 12:20 PM **5.9** Molecular Markers for Quantitative and Discrete COPD Phenotypes. **Thomas Mariani**. *Brigham & Women's Hosp.* (14.7).
- Symposium
6.0 **GENOMICS AND PROTEOMICS OF AIRWAY AND VASCULAR DISEASE**
Fri., 2:00 - 4:00 PM, Salon D.
- Chair: **Tom R. Martin**, *Univ. of Washington.*
Barbara Meyrick, *Vanderbilt Univ. Med. Ctr.*
- 2:00 PM **6.1** Introduction. **Tom R. Martin**. *Univ. of Washington.*
- 2:05 PM **6.2** Proteomic Analysis of Asthma. **Patricia Finn**. *Brigham & Women's Hosp.*
- 2:30 PM **6.3** Genetics of Pulmonary Hypertension. **James Loyd**. *Vanderbilt Univ. Med. Ctr.*
- 2:55 PM **6.4** Genomics of Asthma. **Scott Weiss**. *Harvard Med. Sch.*
- 3:20 PM **6.5** Asthma Genomics: Models and Mechanisms. **David Erle**. *Univ. of California, San Francisco.*
- 3:45 PM **6.6** Exploiting Expert Knowledge for Genome-wide Genetic Analysis. **Jason Moore**. *Dartmouth-Hitchcock Med. Ctr., Lebanon, NH.*

*Don't forget....
Pick up your Banquet Ticket by
11:00 AM on FRIDAY
This banquet is free but you MUST have a
ticket for entry*

Poster Session

7.0 ACUTE LUNG INJURY AND REJECTION

Royal Palm Room.

Posters on display: 7:00 AM - 8:00 PM.
Authors in attendance: 4:30 - 6:30 PM.

Chair: **Rubin Tudor**, *Johns Hopkins Univ.*
Mark W. Geraci, *Univ. of Colorado Hlth. Sci. Ctr.*

Board #

- 1 **7.1** Proteomic Analysis of Tyrosine Phosphorylated L Proteins after High Airway Pressure Injury in Mice. **J. Parker R. Frost, D. Weber, and L. Pannell**. *Univ. of South Alabama.*
- 2 **7.2** Diagnosis of Acute Lung Rejection Based on Gene Expression in Bronchoalveolar Lavage Cells. **J. Lande, J. Patil, N. Li, T. Berryman, R. King, and M. Hertz**. *Univ. of Minnesota.*
- 3 **7.3** Gene Expression and Cytokine Profiling of Lung Tissue in a Mouse Model of LPS Tolerance. **J. Howrylak, W. Wu, N. Kaminski, and A. M.K. Choi**. *Univ. of Pittsburgh Sch. of Med.*
- 4 **7.4** Carboxyl Methylation and Endothelial Unfolded Protein Response. **S. Rounds, Q. Lu, E. Harrington, and M. Jankowich**. *Brown Med. Sch./Providence VAMC.*
- 5 **7.5** Measurement of an Extensive Panel of Chemokines in Bronchoalveolar Lavage Fluid Following Mechanical Ventilation. **C. Douillet, T. Navratil, S. Beidler, A. Stitzel, P. Riesenman, and P. Rich**. *Univ. of North Carolina, Chapel Hill and Inspire, Durham, NC.*
- 6 **7.6** Proteomic Biomarkers of Chronic Lung Allograft Rejection. **C. Wendt, T. Cervenka, M. Haddican, Y. Zhang, and G. Nelsestuen**. *Univ. of Minnesota.*

DAILY SCHEDULE

- Board #
7 **7.7** Peripheral Blood Gene Expression Profiles as a Surrogate Biomarker in Chronic Lung Graft Rejection. **S. Studer, T. Richards, Y.Zhang, W. Wu, and N. Kaminski.** *Univ. of Pittsburgh.*
- 8 **7.8** Alterations in the Proteome of Pulmonary Alveolar Type II Cells in the Rat after Liver Ischemia. **J. Hirsch, C. Niemann, K. Hansen, S. Choi, A. Sapru, A. Burlingame, and M. Matthay.** *Univ. of California, San Francisco.*
- 9 **7.9** Abstract withdrawn by author.
- 10 **7.10** Computational Approaches and Lung Transcriptional Networks. **K. Harrod and M.J. Martinez.** *Lovelace Respiratory Res. Inst., Albuquerque.*
- 11 **7.11** Chronic Alcohol Ingestion Renders the Lung Epithelium Susceptible to Acute Injury by Alterations in Granulocyte/Macrophage Colony-stimulating Factor Signaling and Alveolar Epithelial Permeability. **D. Guidot, P. Joshi, J. Roman, L. Brown, and M. Koval.** *Emory Univ.*
- 12 **7.12** Inflammatory Responses in Inbred Mice with Different Susceptibility Phenotypes to Influenza A Virus Infection. **R. Trammel and L. Toth.** *Southern Illinois Univ. Sch. of Med.*
- 13 **7.13** Microarray Analysis of Gene Expression in Lung of Influenza-infected C57BL/6J and BALB/cByJ Mice. **L. Toth and M. Ding.** *Southern Illinois Univ. Sch. of Med.*

Poster Session

8.0 **OXIDATIVE STRESS**

Royal Palm Room.

Posters on display: 7:00 AM - 8:00 PM.

Authors in attendance: 4:30 - 6:30 PM.

Chair:

Rubin Tudor, *Johns Hopkins Univ.*
Mark W. Geraci, *Univ. of Colorado Hlth. Sci. Ctr.*

Board

- 15 **8.1** Oxidative Stress, Protein Ubiquitination, and Metabolic Alterations in Diaphragm Muscle Induced by Diabetes: Evidence from Gene Expression Profiling. **E. van**

Lunteren, and M. Moyer. *Cleveland VA and Case Western Reserve Univ.*

Board

- 16 **8.2** Cadmium and Mercury Cause an Oxidative Stress-induced Endothelial Dysfunction in Bovine Pulmonary Monolayers. **M. Wolf.** *Univ. of South Carolina Sch. of Med.*
- 17 **8.3** Lung-Genome Responses to Ozone in Alpha-tocopherol Transfer Protein Deficient Mice. **V. Vasu, S. Oommen, Y. Lim, H.H. Aung, G. Valacchi, B. Hobson, B.C. Schock, S.W. Leonard, M.G. Traber, K. Gohil, and C.E. Cross.** *Univ. of California, Davis, Queens Univ., Belfast, Ireland, and Oregon State Univ.*
- 18 **8.4** Up-regulation of the Expression of FcγRIIIb on the Surface of Human Blood Neutrophils by Anti-IL-8:IL-8 Complexes. **R. Fudala, D. Stankowska, and A. Kurdowska.** *Univ. of Texas Hlth. Ctr. at Tyler.*
- 19 **8.5** Peroxynitrite-Induced Nitration and Cross-Linking of Human Sufactact Protein D. **S. Matalon, K. Shrestha, M. Kirk, E. Postlethwait, and E.C. Crouch.** *Univ. of Alabama at Birmingham and Washington Sch. of Med.*
- 20 **8.6** Quantitative Proteomic Profiles of BALF in Wild Type and SP-A KO Mice after Exposure to Ozone. **R. Haque, T. Umstead, W. Freeman, D. Phelps, and J. Floros.** *Penn State Col. of Med.*

This meeting has been made possible through the generous support from:

NIH, National Heart, Lung, and Blood Institute

NIH, National Institute of Environmental Health Sciences

Sepracor, Inc.

Poster Session

9.0 **ENDOTHELIAL CELLULAR PERMEABILITY AND PULMONARY EDEMA**

Royal Palm Room.

DAILY SCHEDULE

- Posters on display: 7:00 AM - 8:00 PM.
 Authors in attendance: 4:30 - 6:30 PM.
- Chair: **Rubin Tuder**, *Johns Hopkins Univ.*
Mark W. Geraci, *Univ. of Colorado Hlth. Sci. Ctr.*
- Board #
 21 **9.1** Endothelial-derived Nitric Oxide Inhibits Alveolar Fluid Reabsorption. **S. Kaestle, C. Reich, J. Weimann, and W. Kuebler**. *Charité- Univ. Med. Berlin, Germany.*
- 22 **9.2** NO-regulated Feedback Loop Protects Lung Microvascular Barrier in Hydrostatic Stress. **J. Yin, S. Kaestle, H. Kuppe, and W. Kuebler**. *Charité- Univ. Med. Berlin, Germany and German Heart Ctr. Berlin, Germany.*
- 23 **9.3** Actin Rearrangement Causes Lung Endothelial Dysfunction in Congestive Heart Failure. **J. Hoffmann, A. Kerem, S. Kaestle, and W. Kuebler**. *Charité- Univ. Med. Berlin, Germany.*
- 24 **9.4** The Impact of Cytokine Treatment on Gene Expression in Lung Endothelial Cells. **M. Alameddine and C. Lin**. *Univ. of Sao Paulo, Sch. of Med., Brazil.*

SATURDAY, NOVEMBER 4, 2006

Featured Presentation

- 10.0 ENVIRONMENTAL GENOMICS AND HUMAN HEALTH**
 Sat., 8:00 - 9:00 AM, Salon D.

Speaker: **David Schwartz**. *NIEHS, NIH.*

Symposium

- 11.0 GENOMICS AND PROTEOMICS OF ENVIRONMENTAL LUNG DISEASE**
 Sat., 9:00 - 11:00 AM, Salon D.

Chair: **Kenneth B. Adler**, *North Carolina State Univ.*
Brooke T. Mossman, *Univ. of Vermont.*

- 9:00 AM **11.1** Introduction. **Kenneth Adler**. *North Carolina State Univ.*

- 9:05 AM **11.2** Genomic Approach to Determining Susceptibility to Lung Disease. **Naftali Kaminski**. *Univ. of Pittsburgh.*
- 9:30 AM **11.3** Proteomics of Oxidant Lung Injury. **Serpil Erzurum**. *Cleveland Clinic Fdn.*
- 9:55 AM **11.4** Gene Expression: Transcript Abundance and Disease. **John Quackenbush**. *Dana-Farber Cancer Inst., Boston.*
- 10:20 AM **11.5** Gene Profiling of Epithelial Cell Remodeling after Inhalation of Asbestos. **Tara Sabo-Attwood**. *Univ. of South Carolina.*
- 10:45 AM **11.6** Gene-environment Interactions in Lung Disease. **Steven Kleeberger**. *NIEHS, NIH.*

Oral Presentations

- 12.0 SELECTED ORAL PRESENTATIONS**
 Sat., 11:30 AM - 1:30 PM, Salon D.

Chair: **Robin H. Steinhorn**, *Northwestern Univ.*
Peter L. Jones, *Univ. of Pennsylvania.*

- 11:30 AM **12.1** Introduction **Robin Steinhorn** *Northwestern Univ.*
- 11:35 AM **12.2** Pharmac-interactomics of Chaperone-mediated Rescue of $\Delta F508$ CFTR from ERAD. **O. Singh, D. Minkovsky, and P. Zeitlin**. *Johns Hopkins Sch. of Med. (18.1).*
- 11:50 AM **12.3** A Comparative Proteomic Analysis of Bronchoalveolar Lavage Fluid in Rats with Aging using 2-DIGE and MALDI-ToF/ToF. **T. Umstead, W. Freeman, and D. Phelps**. *Penn. State Col. of Med. (18.4).*
- 12:05 PM **12.4** Alterations in the Proteome of Pulmonary Alveolar Type II Cells in the Rat after Liver Ischemia. **J. Hirsch, C. Niemann, K. Hansen, S. Choi, A. Sapru, A. Burlingame, and M. Matthay**. *Univ. of California, San Francisco. (7.8).*
- 12:20 PM **12.5** Quantitative Proteomic Profiles of BALF in Wild Type and SP-A KO Mice after Exposure to Ozone. **R. Haque, T. Umstead, W. Freeman, D. Phelps, and J. Floros**. *Penn. State Col. of Med. (8.6).*

DAILY SCHEDULE

12:35 PM **12.6** Proteomic Biomarkers of Chronic Lung Allograft Rejection. **C. Wendt, T. Cervenka, M. Haddican, Y. Zhang, and G. Nelsestuen.** *Univ. of Minnesota.* (7.6).

12:50 PM **12.7** Diagnosis of Acute Lung Rejection Based On Gene Expression in Bronchoalveolar Lavage Cells. **J. Lande, J. Patil, N. Li, T. Berryman, R. King, and M. Hertz.** *Univ. of Minnesota.*

1:05 PM **12.8** Peripheral Blood Gene Expression Profiles as a Surrogate Biomarker in Chronic Lung Graft Rejection. **S. Studer, T. Richards, Y. Zhang, W. Wu, and N. Kaminski.** *Univ. of Pittsburgh.* (7.7).

1:20 PM **12.9** Molecular Profiling of Lung Homeostasis and Fibrosis. **J. Torday and V. Rehan.** *Los Angeles Biomedical Res. Inst. at Harbor, UCLA.* (13.3).

Poster Session

13.0 PULMONARY FIBROSIS Royal Palm Room.

Posters on display: 7:00 AM - 8:00 PM.
Authors in attendance: 6:00 - 8:00 PM.

Chair: **Thomas J. Mariani, Brigham & Women's Hosp.**
John S. Torday, Los Angeles Biomedical Res. Inst. at Harbor, UCLA.

Board #

25 **13.1** Comparative Expression of SPLUNC1, SPLUNC2 and LPLUNC1 in Normal and Diseased Lung. **L. Bingle and C. Bingle.** *Univ. of Sheffield Med. Sch.*

26 **13.2** Laser Capture Microdissection and Microarray Analysis Reveal MMP19 as a Potential New Regulator of Fibrosis. **E. Naumovski, A. Pardo, K. Gibson, T. Richards, Y. Zhang, V. Ruiz, R. Ramirez, C. Lopez Otin, M. Selman, and N. Kaminski.** *Univ. of Pittsburgh, Fac. de Ciencias, Mexico City, Mexico, Natl. de Enfermedades Resp., Mexico City, Mexico, and Fac. de Med., Oviedo, Spain.*

27 **13.3** Molecular Profiling of Lung Homeostasis and Fibrosis. **J. Torday, and V. Rehan.** *Los Angeles Biomedical Res. Inst. at Harbor, UCLA.*

28 **13.4** WISP-1, a Novel Mediator and Therapeutic Target in Pulmonary Fibrosis. **M. Königshoff, J. Wilhelm, K. Kitowska, O. Amarie, M. Obert, W. Seeger, F. Rose, L. Fink, A. Günther, and O. Eickelberg.** *Univ. of Giessen Lung Ctr., Germany.*

Poster Session

14.0 CHRONIC OBSTRUCTION PULMONARY DISEASE Royal Palm Room.

Posters on display: 7:00 AM - 8:00 PM.
Authors in attendance: 6:00 - 8:00 PM.

Chair: **Thomas J. Mariani, Brigham & Women's Hosp.**
John S. Torday, Los Angeles Biomedical Res. Inst. at Harbor, UCLA.

Board #

29 **14.1** Abstract withdrawn by author.

30 **14.2** Early Transcriptomic Predictors of Cigarette Smoke Carcinogenesis. **K. Gohil, S. Oommen, I. Espiritu, V. Vasu, H. H. Aung, D. Uyeminami, K.E. Pinkerton, C.E. Cross, and H. Witschi.** *Univ. of California, Davis.*

31 **14.3** Cigarette Smoke Sensitive Lung Transcriptome is Modulated by Tocopherol Transfer Protein. **K. Gohil, S. Oommen, V. Vasu, H.H. Aung, and C.E. Cross.** *Univ. of California, Davis.*

32 **14.4** Evidence for Attempted Regional Elastic Fiber Repair in Severe Emphysema. **J. Woods, K. Castillo, A. Patterson, J. Cooper, J. Hogg, and R. Pierce.** *Washington Univ., Univ. of Pennsylvania, and St Paul's Hospital, Vancouver.*

33 **14.5** Abstract withdrawn by author.

34 **14.6** Defective FGF Signaling Induces Aberrant Elastogenesis in Mice with Postnatal Airspace Enlargement. **T. Mariani, S. Bhattacharya, S. Tyagi, B. Starcher, and S. Srisuma.** *Brigham & Women's Hosp. and Univ. of Texas Hlth. Ctr.*

35 **14.7** Molecular Markers for Quantitative and Discrete COPD Phenotypes. **T. Mariani, S. Bhattacharya, S. Srisuma, D. DeMeo, E. Silverman, S. Shapiro, R. Bueno, and J. Reilly.** *Brigham & Women's Hosp.*

Poster Session

15.0

ASTHMA

Royal Palm Room.

Posters on display: 7:00 AM - 8:00 PM.

Authors in attendance: 6:00 - 8:00 PM.

Chair:

Thomas J. Mariani, *Brigham & Women's Hosp.*

John S. Torday, *Los Angeles Biomedical Res. Inst. at Harbor, UCLA.*

Board #

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15.1 Genetic Effect of CysLTR2 Polymorphisms on its mRNA Synthesis and Stabilization. **C. Park, J. Shin, S. M. Park, H. S. Chang, J. S. Park, A. Jang, T. Rhim, I. Y. Chung, B. L. Park, and H. D. Shin.** *Soonchunhyang Univ. Hosp., Gyeonggi-do, Rep. of Korea, Hanyang Univ., Seoul, Rep. of Korea, and SNP Genetics, Inc, Rep. of Korea.*

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15.2 Proteomic Analysis of Human BALF: Comparison of Bronchial Asthma with Normal Control. **T. Rhim, K. H. Kim, S. Park, A. Jang, Y. Paik, and C. Park.** *Soonchunhyang Univ. Hosp., Gyeonggi-do, Rep. of Korea, and Yonsei Proteome Ctr., Seoul, Rep. of Korea.*

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15.3 β_2 -Adrenergic Receptor Genotypic Variants in African Americans with Asthma. **J. Ford, M. LeNoir, M. E. Snider, K. Schaefer, and R. Baumgartner.** *Johns Hopkins Sch. Of Med., Bay Area Pediatrics, and Sepracor Inc., Malborough, MA.*

39

15.4 Differential Gene Expression in Primary Human Airway Epithelium Exposed to Isomers of Albuterol. **R. Persinger, M. Chang, M. E. Snider, and R. Wu.** *Sepracor, Inc., Malborough, MA and Univ. of California, Davis.*

40

15.5 Mycoplasma Pneumoniae Decreases Airway Epithelial Vascular Endothelial Growth Factor Gene Expression in Mice: Microarray and Laser Capture Microdissection Approaches. **H. W. Chu, J. Rino, T. Moss, Q. Wu, and R. Martin.** *Natl. Jewish Med. and Res. Ctr., Denver.*

Poster Session

16.0

LUNG DEVELOPMENT

Royal Palm Room.

Posters on display: 7:00 AM - 8:00 PM.

Authors in attendance: 6:00 - 8:00 PM.

Chair:

Thomas J. Mariani, *Brigham & Women's Hosp.*

John S. Torday, *Los Angeles Biomedical Res. Inst. at Harbor, UCLA.*

Board #

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16.1 CFTR-dependent Lung Organogenesis: Molecular Mechanism. **J.C. Cohen, and J. Larson.** *Stony Brook Hlth. Sci. Ctr.*

42

16.2 *In Vitro* Increase on Tracheal Smooth Muscle Contractility After Recovery from Moderate Hyperoxia. **T. Gamboa, G. Fernandes, I. Ribeiro da Silva, M. Correia, and A. Rendas.** *Fac. of Med. Sci. of Lisbon, Portugal.*

43

16.3 Dexamethasone-mediated Regulation of beta-carotene 15,15'-monooxygenase Gene Expression in Human Pulmonary Epithelial Cells. **X. Gong, M. Yang, W. Gong, S. Zaripheh, and L. Rubin.** *Cleveland Clinic Fdn.*

Poster Session

17.0

PULMONARY HYPERTENSION

Royal Palm Room.

Posters on display: 7:00 AM - 8:00 PM.

Authors in attendance: 6:00 - 8:00 PM.

Chair:

Thomas J. Mariani, *Brigham & Women's Hosp.*

John S. Torday, *Los Angeles Biomedical Res. Inst. at Harbor, UCLA.*

Board #

44

17.1 Perinatal Hypoxia Induces Long-term Effects on Pulmonary Artery Smooth Muscle Cells Potassium Channels. **M. Marino, J.F Tolsa, A.C Peyter, G. Diaceri, and J.L Beny.** *Univ. of Geneva, Switzerland, and Univ. Hosp. CHUV, Lausanne, Switzerland.*

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17.2 The Interleukin 13 Receptor System: A Novel Pathogenetic Mechanism in Pulmonary Arterial Hypertension. **O. Eickelberg, M. Hecker, G. Kwapiszewska, A. Zakrzewicz, L. Marsh, R. Schermuly, and W. Seeger.** *Univ. of Giessen Lung Ctr., Germany.*

DAILY SCHEDULE

Poster Session

18.0 GENERAL CATEGORY

Royal Palm Room.

Posters on display: 7:00 AM – 8:00 PM.
Authors in attendance: 6:00 - 8:00 PM.

Chair: **Thomas J. Mariani**, *Brigham & Women's Hosp.*
John S. Torday, *Los Angeles Biomedical Res. Inst. at Harbor, UCLA.*

Board #
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18.1 Pharmacology-interactomics of Chaperone-mediated Rescue of $\Delta F508$ CFTR from ERAD. **O. Singh, D. Minkovsky, and P. Zeitlin.** *The Johns Hopkins Sch. of Med.*

47 **18.2** Microarray Analysis of MDM4 Function in a Mesothelial Cell Line Using shRNA Technology. **M. Schelvan, A. Erbe, C. Schwanke, S. T. Heivly, and M. Pershouse.** *Univ. of Montana.*

48 **18.3** GM-CSF Regulates Macrophages and Innate Immune Responses in Tuberculosis. **Z. Chroneos, and J. Szeliga.** *Univ. of Texas Hlth. Ctr. at Tyler.*

49 **18.4** A Comparative Proteomic Analysis of Bronchoalveolar Lavage Fluid in Rats with Aging using 2-DIGE and MALDI-ToF/ToF. **T. Umstead, W. Freeman, and D. Phelps.** *Penn State Col. of Med.*

50 **18.5** Gene Alteration Analysis of Asbestos Exposed Murine Lungs. **A. Smartt, M. Brezinsky, M. Pershouse, and E. A. Putnam.** *Univ. of Montana, Missoula.*

51 **18.6** The Role of Neurofibromatosis Type 2 in the Development of Malignant Mesothelioma. **A. Erbe, M.B. Schelvan, C. Schwanke, S.T. Heivly, and M. Pershouse.** *Univ. of Montana, Missoula.*

SUNDAY, NOVEMBER 5, 2006

Symposium

19.0 ACUTE LUNG INJURY AND INFLAMMATION

Sun., 8:00 - 10:00 AM, Salon D.

Chair: **Jahar Bhattacharya**, *Columbia Univ.*
Ellen Burnham, *Univ. of Colorado at Denver.*

8:00 AM **19.1** Introduction. **Jahar Bhattacharya.** *Columbia Univ.*

8:05 AM **19.2** Acute Lung Injury: Functional Genomics and Genetic Susceptibility. **George Leikauf.** *Univ. of Cincinnati.*

8:30 AM **19.3** Genetic Determinants of Sepsis Induced Lung Injury. **Kathleen Barnes.** *Johns Hopkins Sch. of Med.*

8:55 AM **19.4** Proteomic Analysis of Plasma in ARDS. **Lorraine Ware.** *Vanderbilt Univ. Med. Ctr.*

9:20 AM **19.5** Proteomics Analysis of Acute Lung Injury. **Mark Duncan.** *Univ. of Colorado Hlth. Sci. Ctr.*

9:45 AM **19.6** Using Genetic Variation in the Human Toll-like Receptor Pathway to Understand the Role of Innate Immune Inflammation in Critical Illness. **Mark Wurfel.** *Univ. of Washington.*

20.0 CONFERENCE SUMMARY

Sun., 10:00 - 11:00 AM, Salon D.

20.1 Summary. **Michael Matthay.** *Univ. of California, San Francisco.*

**2006 APS Conference
Physiological Genomics and Proteomics of Lung Disease**

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2006 APS Conference: Physiological Genomics and Proteomics of Lung Disease

ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

3.0: GENOMICS AND PROTEOMIC APPROACHES TO STUDYING LUNG DISEASE

3.4

PATHWAY INDUCTION AND HIGH-FIDELITY SIMULATION FOR MOLECULAR SIGNATURE AND BIOMARKER DISCOVERY IN LUNG CANCER USING MICROARRAY GENE EXPRESSION DATA

Constantin Aliferis¹, Alexander Statnikov², Pierre Massion³

¹Biomedical Informatics, Vanderbilt University, 412 Eskind Library, 2209 Garland Ave, Nashville, TN, 37232, ²Biomedical Informatics, Vanderbilt University, Eskind Library, 2209 Garland Ave, Nashville, TN, 37232, ³Allergy, Pulmonary & Critical Care, Vanderbilt Ingram Cancer Center, Vanderbilt University, Vanderbilt University Medical Center, T1218 Mcn, Nashville, TN, 37232-2650.

Objectives: We propose a framework in which the (generally unknown) network of gene regulation is induced from data in order to produce high quality signatures and biologically interpretable markers of lung cancer from mass-throughput assays. **Methods:** We analyze human lung cancer gene expression data and elicit diagnostic signatures as well as biomarkers using several state of the art algorithms. We also conduct re-simulation experiments. We compare the state of the art methods with the novel algorithm in terms of signature accuracy, external reproducibility, marker compactness, stability, and pathway localization. **Results:** the novel network induction method returns significantly smaller marker sets. Markers are highly reproducible in cross-dataset validation. Surprisingly, most prior biomarker selection methods find markers that are barely better predictively than selecting markers at random. In re-simulated experiments the network induction method finds biomarkers closer to the target phenotype or gene in the gold standard generating networks, than purely associational methods. **Conclusions:** The network induction approach confers significant advantages over previous methods for the analysis of mass throughput data in lung cancer.

3.5

MULTISCALE STUDIES OF ENDOTHELIAL PHENOTYPES

Peter F Davies¹

¹Inst. Medicine & Engineering, University of Pennsylvania, Smith Walk, Philadelphia, Pennsylvania, 19104.

The plasticity of vascular endothelial phenotype is influenced by site-specific conditions. These relationships are likely to occur in the pulmonary as well as the systemic circulation. In the latter, the hemodynamic environment is a prominent site-specific determinant of phenotype that regulates vascular structural organization, signaling, molecular dynamics, and gene regulation. An important property is the spatial scale of biological relevance; blood flow forces vary significantly over short distances resulting in phenotypic heterogeneity. We conduct studies of normal arterial and aortic valve endothelial gene and protein expression in cells directly isolated from sub-regions that are susceptible to, or protected from, the development of atherosclerosis and/or calcification-sclerosis. Both protective and pro-pathological endothelial expression patterns co-exist in regions of hemodynamic instability suggesting greater sensitivity to (additional) pathological risk factors. Furthermore, differential endothelial protein expression and kinase activities were identified at similar sites. (NIH Grants HL62250, HL64388). Passerini, A., et al. Coexisting pro-inflammatory and anti-oxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. *Proc. Natl. Acad. Sci. USA* (2004) 101:2482-2487 First in vivo transcript profiles of endothelium. Magid, R., and P.F. Davies. Endothelial protein kinase C isoform identity and differential activity of PKC ζ in an atherosusceptible region of porcine aorta. *Circ. Research* (2005) 97:443-449 Extends differential endothelial phenotype profiling to candidate protein family PKC. Davies, P.F. Flow-mediated endothelial mechanotransduction. *Physiol. Rev.* (1995) 75:519-560. An in-depth review of mechanisms involved in flow regulation of endothelial biology.

4.0: PROTEOMIC AND GENOMIC APPROACHES TO DEVELOPING POTENTIAL THERAPEUTIC TARGETS

4.3

TRANSCRIPTIONAL REGULATION OF RESPIRATORY EPITHELIAL DIFFERENTIATION

Jeffrey A. Whitsett, M.D.¹

¹Chief Section of Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children's Hospital Medical Center.

Mammalian lung is lined by a diversity of epithelial cell types that vary during development, primarily along the cephalo-caudal and dorsal-ventral axes of the respiratory tract and among species. Formation and differentiation of the respiratory epithelium is strongly influenced by a number of transcription factors including TTF-1, GATA-6, NF-1, Foxa1, Foxa2, β -catenin, Foxj1, Ets family members, C/EBP α , Sox family members, p63 and others. The proteins are expressed in various pulmonary cell types to influence respiratory epithelial cell differentiation and gene expression. Gene deletion and addition studies in the mouse demonstrate the importance of these transcription factors in the formation and differentiation of the lung prior to birth and their roles in various aspects of lung function and homeostasis after birth. Many of the transcription factors interact at multiple levels, co-regulating each other, interacting directly via protein-protein interactions, and by both distinct and cooperative interactions at binding sites on specific transcriptional target genes. RNA microarray analyses of lung RNAs were utilized to identify shared and distinct transcriptional targets and participants in the networks. Many of these transcription factors are expressed postnatally and are regulated during repair of the lung following injury or during regrowth following unilateral pneumonectomy. Dynamic changes in the expression of TTF-1, β -catenin, Sox, Ets, Fox family members, and Stat-3 accompany the repair of the respiratory epithelium. Taken together, these studies support the concept that transcriptional programs that mediate epithelial cell differentiation during lung morphogenesis also play important roles during injury and repair. References Davé, V., Childs, T., Xu, Y., Ikegami, M., Besnard, V., Maeda, Y., Wert, S.E., Neilson, J.R., Crabtree, G.R. and Whitsett, J.A.: Calcineurin/NFAT signaling is required for perinatal lung maturation and function. *J. Clin. Invest.*, in press. Wan, H., Xu, Y., Ikegami, M., Stahlman, M.T., Kaestner, K.H., Ang, S-L. and Whitsett, J.A.: Foxa2 is required for transition to air breathing at birth. *Proc. Natl. Acad. Sci. USA* 101:14449-14454, 2004. Martis, P.C., Whitsett, J.A., Xu, Y., Perl, A-K.T., Wan, H. and Ikegami, M.: C/EBP α is required for lung maturation at birth. *Development* 133:1155-1164, 2006.

6.0: GENOMICS AND PROTEOMICS OF AIRWAY AND VASCULAR DISEASE

6.5

ASTHMA GENOMICS: MODELS AND MECHANISMS

David Erle¹

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DNA microarray analyses of samples from human subjects with lung disease are beginning to uncover gene expression changes associated with disease but this approach has important limitations. First, appropriate tissue samples may be difficult to obtain. Second, heterogeneity of human populations and diseases can limit the ability to detect important changes. Third, even when changes are identified it is difficult to determine how, if at all, these changes contribute to pathogenesis. As an alternative approach, we are using arrays to profile expression changes in mouse models of lung diseases, including asthma and emphysema, and in cell culture systems. Arrays have been particularly valuable for understanding relationships between different model systems and focusing our attention on important similarities and differences between models and human disease. In addition, array analysis of special transgenic models has helped to dissect how specific pathways and cell types contribute to changes in gene expression and pathogenesis of asthma and other inflammatory lung diseases. Novel potential therapeutic targets identified in array experiments can also be assessed further using these kinds of models.

7.0: ACUTE LUNG INJURY AND REJECTION

7.1

PROTEOMIC ANALYSIS OF TYROSINE PHOSPHORYLATED LUNG PROTEINS AFTER HIGH AIRWAY PRESSURE INJURY IN MICE

James Parker¹, Richard Frost², David Weber³, Lewis Pannell⁴

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Although the specific signaling pathways that increase vascular permeability after ventilator induced lung injury (VILI) are not fully understood, previous studies from our laboratory indicate that tyrosine phosphorylation of cellular proteins contributes to the permeability increase by activation of Src kinases but is simultaneously attenuated by Akt (protein kinase B) activation. To determine novel signaling proteins, we ventilated C57BL/6 mice for 60 minutes at either 10 or 55 cmH₂O peak inflation pressure (PIP), and harvested the lungs at 60 minutes. We homogenized the lungs, extracted the proteins and performed a western blot analysis for phosphotyrosine. Tyrosine phosphorylation increased primarily in 4 bands in the 50-65 kD range after high PIP ventilation. These bands were cut from identical gels, and the protein was extracted and trypsinized. The peptide mixtures were analyzed using a Micromass Ultima mass spectrometer and Mascot search software was used to identify specific proteins. Relevant mouse proteins previously found to be involved included integrin linked kinase (ILK) known to phosphorylate Akt, and pro-inflammatory cytochrome p450. Also present were mitochondrial, cytoskeletal and anti-oxidant proteins, aldehyde dehydrogenase, RAGE, and flavin monooxygenase. Thus, novel signaling constituents of pathways that both increase or suppress the permeability increase in VILI may be revealed by the proteomics approach. Supported by P01 HL66299.

7.2

DIAGNOSIS OF ACUTE LUNG REJECTION BASED ON GENE EXPRESSION IN BRONCHOALVEOLAR LAVAGE CELLS

Jeffrey Lande¹, Jagadish Patil¹, Na Li², Todd Berryman¹, Richard King¹, Marshall Hertl¹

¹Medicine, University of Minnesota, 420 Washington Avenue SE, Minneapolis, MN, 55455, ²School of Public Health, University of Minnesota, 420 Washington Avenue SE, Minneapolis, MN, 55455.

Acute lung rejection is a risk factor for chronic rejection, jeopardizing the long-term survival of lung transplant recipients. Our objective was to identify genes expressed in bronchoalveolar (BAL) cells that optimally classify acute rejection (AR) versus no-rejection (NR). BAL samples were collected from 32 unique subjects whose concurrent histology showed AR (n=14) or NR (n=18). Global BAL cell gene expression was measured using Affymetrix U133A microarrays. The nearest shrunken centroid method with 10-fold cross validation was used to define the model that best classified AR vs. NR BAL samples. Multiple runs of the algorithm were performed to determine the range of misclassification error and the most influential genes in determining classifiers. The estimated overall misclassification rate was below 20%. Genes that were optimal classifiers were overrepresented by ontology categories of immune and defense response. The proportion of neutrophils in BAL was more highly correlated with histologic rejection grade than was the proportion of lymphocytes. We conclude that BAL cell gene expression patterns are highly correlated with the histology of concurrent biopsies. T-cell activation genes are influential in determining optimal AR vs. NR classifiers, which perform better than using proportion of lymphocytes to diagnose histologic rejection.

7.3

GENE EXPRESSION AND CYTOKINE PROFILING OF LUNG TISSUE IN A MOUSE MODEL OF LPS TOLERANCE

Judie Howrylak¹, Wei Wu¹, Nafati Kaminski¹, Augustine MK Choi¹

¹Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh School of Medicine, MUH 628NW, Pittsburgh, PA, 15213.

Background: The phenomenon of tolerance to bacterial lipopolysaccharide (LPS) was described over fifty years ago, yet the molecular mechanisms of this process remain incompletely understood. **Methods:** In order to better characterize this process in the lungs, we performed gene expression and cytokine profiling of mouse whole lung tissue. The following four subgroups of mice were used for analysis: 1) naive mice exposed to a lethal dose of LPS, 2) mice pre-exposed to sub-lethal doses of LPS prior to a lethal exposure, 3) mice exposed to sub-lethal doses of LPS without a lethal exposure, 4) mice exposed to phosphate buffered saline. **Results:** Exposure of pre-conditioned mice to lethal doses of LPS resulted in significantly decreased expression of the following genes within the Jak-STAT signaling pathway: SOCS1, SOCS3, STAT1, STAT2, IL6, GCSF, IFN γ (p<0.05) compared to naive mice exposed to LPS. In addition, subsequent cytokine profiling revealed decreased levels of IL6, GCSF, and IFN γ in the pre-conditioned mice compared to the naive mice, suggesting transcriptional regulation of these cytokines. **Conclusions:** Decreased gene expression of key Jak-STAT pathway mediators appears to be involved in the development of tolerance to LPS. Modification of this pathway could potentially

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ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

alter the inflammatory response of the lungs to LPS. *Funding:* RO1-HL060234, RO1-HL55330, RO1-HL079904, and PO1 HL70807.

7.4 CARBOXYL METHYLATION AND ENDOTHELIAL UNFOLDED PROTEIN RESPONSE

Sharon Rounds¹, Qing Lu¹, Elizabeth Harrington¹, Matthew Jankowich¹
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Isoprenylcysteine carboxyl methyltransferase (ICMT) causes post-translational carboxyl methylation of C-terminal CAAX motifs, such as those encoded in Rho GTPases. We have shown that chemical inhibition of ICMT decreases Ras and RhoA GTPase carboxyl methylation and activation and causes endothelial cell apoptosis through a mechanism involving increased intracellular S-adenosylhomocysteine (SAH) and disruption of focal adhesion complexes. We examined the effects of chemical inhibition of ICMT on the proteome of human pulmonary artery endothelial cells (PAEC). Proteomic analysis of PAEC incubated for 24 hours with an ICMT chemical inhibitor, N-acetyl-geranylgeranyl cysteine (AGGC), demonstrated a shift in the isoelectric point of the glucose regulated protein 94 (GRP94), a component of the Unfolded Protein Response (UPR). Inhibition of ICMT by AGGC also caused relocalization of GRP94 from the perinuclear area to a more diffuse cytoplasmic localization. While GRP94 encodes an internal CAAX motif, no carboxyl methylation was detected. Inhibition of ICMT by AGGC or siRNA decreased GRP94 protein level, an effect that was blunted by caspase inhibition. Furthermore, knockdown of GRP94 by siRNA exacerbated endothelial cell apoptosis induced by ICMT inhibition. Over-expression of RhoA, but not Ras, GTPase blunted the effects of ICMT inhibition on GRP94 relocalization and protein expression. Taken together, these results suggested that decreased expression and altered post-translational modification and subcellular localization of GRP94 upon ICMT inhibition may be important in induction of endothelial cell apoptosis. (HL69436, HL67795, American Lung Association RG1140N, and Parker B. Francis Fellowship).

7.5 MEASUREMENT OF AN EXTENSIVE PANEL OF CHEMOKINES IN BRONCHOALVEOLAR LAVAGE FLUID FOLLOWING MECHANICAL VENTILATION

Christelle Douillet¹, Tomas Navratil², Stephanie Beidler¹, Aaron Stitzel¹, Paul Riesenman¹, Preston Rich¹

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Introduction: Lung pathophysiology are often associated with inflammation regardless of the etiology. The measurement of chemokines in the airway space provides useful information about the extent of inflammation but until recently, these determinations were limited to few cytokines, especially in small-size samples. Here, we determined the concentration of 23 chemokines in bronchoalveolar lavage (BAL) fluid from rats submitted to either protective or injurious mechanical ventilation. **Methods:** Anesthetized rats were divided into 3 groups (n=5 each): unventilated control (C) and ventilated groups with small (S, 6 ml/kg) or large (L, 40 ml/kg) tidal volumes (40 bpm, room air). After 1 hour, BAL was performed on the left lung, and sample aliquots (25 µl) were analyzed by xMAP-Luminex. Procedures were approved by IACUC. **Results:** IL-2, IL-9, IL-10, IL-18, MCP-1, leptin and MIP-1α significantly increased in L group as compared to C or S, while GRO/KC significantly decreased in L as compared to C or S. IL-6, TNFα, eotaxin, GM-CSF, IL-13 and IP10 tended to increase, whereas IL-1α, IL-4, IFNγ tended to decrease without reaching statistical significance. IL-1β, IL-17 and VEGF were unchanged. IL-5, RANTES, and G-CSF were below detection levels. **Conclusion:** Injurious mechanical ventilation is associated with an alteration of most measured chemokines. The xMAP technology allows an accurate measurement of a large number of chemokines in small samples from BAL after mechanical ventilation.

7.6 PROTEOMIC BIOMARKERS OF CHRONIC LUNG ALLOGRAFT REJECTION

Chris Wendt¹, Tereza Cervenká¹, Madelaine Haddican¹, Yan Zhang², Gary Nelsestuen²
¹Medicine, Univ. of Minnesota, 420 Delaware St. SE MMC 276, Minneapolis, MN, 55455, ²Biochemistry, Univ. of Minnesota, 420 Delaware St. SE, Minneapolis, MN, 55455.

Lung transplantation has become effective therapy for many end stage lung diseases, however, chronic allograft rejection remains the leading cause of mortality. We hypothesize that there are distinct and reproducible sets of protein products within the lung that characterize chronic rejection and can be characterized into a biosignature of rejection. **Methods:** We used mass spectrometry (MALDI-TOF and iTRAQ labeling with MS/MS) to identify biomarkers of chronic allograft rejection (BOS), in bronchoalveolar lavage fluid from lung transplant recipients. **Results:** Our initial analysis of a cohort of well-characterized lung transplant recipients using MALDI-TOF identified several potential biomarkers for BOS including: elevated levels of human neutrophil peptide (HNP), declining Clara cell secretory protein (CCSP), and certain peak ratios by profile analysis of the MALDI-TOF spectrum, including unidentified proteins that predicted those that would develop BOS. These changes occurred up to 20 months prior to the clinical onset of BOS. Thus far we have prospectively tested 137 patients (411 samples) and found elevated levels of HNP associated with a 2.7 fold increase in developing BOS. We have applied an expanded proteomic method, iTRAQ, to our cohort samples and have identified over 265 proteins, several of these proteins had greater than 10-fold increases in BOS patients compared to controls. Two proteins, matrix metalloprotein-9 (MMP-9) and proteinase 3 had the largest fold increases up to 66 and 9-fold respectively at the time of BOS diagnosis and had elevated levels prior to developing BOS. Preliminary evidence suggests these biomarkers will be an early warning of lung transplant rejection.

7.7 PERIPHERAL BLOOD GENE EXPRESSION PROFILES AS A SURROGATE BIOMARKER IN CHRONIC LUNG GRAFT REJECTION

Sean Studer¹, Thomas Richards¹, Yingze Zhang¹, Wei Wu¹, Naftali Kaminski¹
¹Division of PACCM, University of Pittsburgh, MUH 628 NW; 3459 Fifth Avenue, Pittsburgh, PA, 15213.

Rationale: Chronic lung allograft dysfunction (OB) is the leading cause of late death following lung transplantation. Early noninvasive detection of OB would allow earlier therapeutic intervention. We hypothesized that the changes in gene expression in peripheral blood mononuclear cells (PBMCs) could distinguish between OB and no evidence of rejection (NER)

following lung transplantation. **Methods:** We evaluated gene expression profiles in a cross sectional study of 30 lung transplant recipients (LTRs) divided between OB (n=14) and no evidence of rejection (NER, n=16). Blood from consented subjects, obtained by venipuncture at time of routine outpatient visits in CPT tubes, was stored as PBMCs at -80 degrees in Trizol until batched RNA isolation. We utilized an Illumina platform representing 23454 genes. **Results:** Preliminary analysis of our data reveals a gene expression profile in circulating PBMCs that distinguishes OB from NER. CCR2 expression was significantly upregulated in OB compared to NER while CCR5 and CXCR4 were not differentially expressed. **Conclusions:** Early results of PBMC gene expression profiles suggest a noninvasive biomarker signal in LTRs' PBMCs that may distinguish OB from NER. Specifically, CCR2 expression was increased consistent with published data (J Clin Invest, 2001;108:547-6) demonstrating increased CCR2 expression in a murine model of OB. Furthermore, the lack of differential expression of CCR5 and CXCR4 suggests that the PBMC profile in OB may be distinct from the profile we have previously observed in acute lung graft rejection. We expect that these results will be amenable to future validation in a longitudinal study of LTRs.

7.8 ALTERATIONS IN THE PROTEOME OF PULMONARY ALVEOLAR TYPE II CELLS IN THE RAT AFTER LIVER ISCHEMIA

Jan Hirsch¹, Claus Niemann¹, Kirk Hansen², SooJinNa Choi¹, Anil Sapru³, Alma Burlingame², Michael Matthay³

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Hepatic ischemia is well known to be a potential cause of lung injury. Alveolar epithelial type II cells (ATII) play a key role in lung injury and repair. We investigated the impact of liver ischemia on the proteome of these cells. Male lean Zucker rats were anesthetized with isoflurane under spontaneous ventilation. Vascular supply to the left and medial lobe (70 % of the liver) was clamped for 75 min with subsequent reperfusion for 8h. Sham operated rats were used as controls. Bronchoalveolar lavage (BAL) was performed and ATII were isolated (purity >90%) and lysed by sonication. Proteins were tryptically digested into peptides that were labeled using the novel isobaric tagging reagent iTRAQ. Then, controls and samples were pooled and purified by cation exchange chromatography, separated by HPLC and identified using ESI-MS-MS mass spectrometry. Spectra were interrogated and quantified using ProteinProspector. After ischemia, we observed a significantly increased neutrophil percentage (48±26%) compared to controls (5±3%) in BAL (p<0.01). Quantitative proteomics showed significant differences especially in proteins of the mitochondrial energy metabolism, the oxidant antioxidant system and cellular enzymes. Proteins with altered content included ATP synthase, catalase, and superoxide dismutase. Liver ischemia in rats lead to acute lung injury that can be detected by quantitative mass spectrometry. The identified proteins have important roles in cell metabolism, host defense and the clearance of pulmonary edema fluid. The alterations in ATII after liver ischemia may be clinically relevant and merit further investigation.

7.9 Abstract withdrawn by author.

7.10 COMPUTATIONAL APPROACHES AND LUNG TRANSCRIPTIONAL NETWORKS

Kevin Harrod¹, M. Juanita Martinez¹

¹Infectious Diseases, Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr., SE, Albuquerque, NM, 87108.

Recent advances in computational approaches provide unique opportunities to understand transcriptional networks in well-defined systems. In light of these advances, little has been done in the area of bioinformatics to understand transcriptional networks in lung epithelia. One important application is the utilization of coregulated gene subsets to discover transcriptional regulatory motifs in gene regulatory loci. Previous work from this lab has utilized such an approach to identify DNA-binding proteins *a priori*. Using this approach, both transcription factors previously proposed to have function in lung epithelia were identified as well as novel transcription factors whose elucidation requires experimental validation. The use of phylogenetic comparisons across various promoter regions of lung specific genes likewise can be utilized to identify novel transcriptional regulators. Lastly, an investigation of cooperative transcription factors in clusters of motifs common across certain lung specific gene subsets such as surfactant proteins and secretoglobins suggest that currently available algorithms may be useful in defining novel gene expression patterns specific to distinct transcriptional networks. The use of these computational tools provide a genomic approach to understanding lung transcriptional networks. Coupled with the recent elucidation of gene array tissue compendiums, bioinformatic approaches can now be used for elucidating genome-wide molecular mechanisms of lung epithelial in normal and diseased states.

7.11 CHRONIC ALCOHOL INGESTION RENDERS THE LUNG EPITHELIUM SUSCEPTIBLE TO ACUTE INJURY BY ALTERATIONS IN GRANULOCYTE/MACROPHAGE COLONY-STIMULATING FACTOR SIGNALING AND ALVEOLAR EPITHELIAL PERMEABILITY

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Alcohol abuse predisposes individuals to acute lung injury (ALI), and the "alcoholic lung" phenotype is characterized by abnormal alveolar permeability. Using a rat model, we found that chronic alcohol ingestion (6 wks) alters tight junction protein expression by type I alveolar epithelial cells, with claudin-1 and claudin-7 decreased and claudin-5 increased. Claudin localization to tight junctions also was impaired by alcohol ingestion, however, claudin expression by type II cells was not affected by alcohol. Previously, we found that granulocyte/macrophage colony-stimulating factor (GM-CSF) rapidly enhances barrier function in the alcoholic lung. Consistent with this, GM-CSF treatment of alveolar epithelial cells isolated from alcohol-fed rats increased claudin localization to tight junctions. Further, alcohol ingestion decreases GM-CSF receptor expression by alveolar epithelial cells and macrophages, however recombinant GM-CSF treatment *in vivo* restores GM-CSF receptor expression and signaling via the PU.1 transcription factor. This implicates a feed-forward cycle of GM-CSF signaling in the alveolar space that is dampened, but not blocked, by alcohol. Taken together, these findings suggest that alcohol abuse dampens GM-CSF signaling which, in turn, contributes

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to the alcoholic lung phenotype and renders the lung susceptible to edematous injury. GM-CSF treatment, in part by restoring tight junction protein assembly, may decrease the risk of ALI in susceptible patients.

7.12

INFLAMMATORY RESPONSES IN INBRED MICE WITH DIFFERENT SUSCEPTIBILITY PHENOTYPES TO INFLUENZA A VIRUS INFECTION

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Introduction: Disease severity during influenza infection may be related to the magnitude of the inflammatory response in the lung. Inoculation with a given dose of influenza virus causes greater mortality in BALB/cByJ (B) mice vs. C57BL/6J (C) mice. We assessed whether facets of the early immune response to influenza infection differ in B and C mice. Methods: B and C mice (10 per strain) were infected intranasally with influenza virus and killed 30 hours later. Lung was assayed for viral titers (TCID50), cytokine levels (Luminex; pg/lung), and myeloperoxidase (MPO) (ELISA; ng/mg protein). Mean \pm SEM values were compared between strains using Student's t-test. Studies were approved by the SIUSM IACUC. Results: Viral titers did not vary significantly between strains ($10^{5.1}$ vs $10^{4.9}$). However, infected B mice had higher levels of cytokines in lung than did C mice, as follows: IL-1 α , 1252 \pm 84 vs. 478 \pm 75; IL-1 β , 2510 \pm 236 vs. 1606 \pm 126; G-CSF, 9994 \pm 1390 vs. 1849 \pm 465; KC, 1858 \pm 51 vs. 625 \pm 97; IP-10, 23375 \pm 755 vs. 11394 \pm 838; MCP-1, 4465 \pm 309 vs. 2927 \pm 657; MIP-1 α , 2750 \pm 280 vs. 1485 \pm 276; TNF α , 1573 \pm 114 vs. 612 \pm 160; and MPO, 3312 \pm 1132 vs. 1785 \pm 467 ($p < 0.01$). IL-6, IL-10, IL-12, IFN γ and GM-CSF did not differ significantly between strains. Conclusion: Although viral titers are equivalent, B mice develop a greater proinflammatory response during influenza infection than C mice, which may contribute to the differential mortality in these strains. Supported by NIH Grants HL-70522 and RR-17543.

7.13

MICROARRAY ANALYSIS OF GENE EXPRESSION IN LUNG OF INFLUENZA-INFECTED C57BL/6J AND BALB/CBYJ MICE

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Introduction: BALB/cByJ (B) and C57BL/6J (C) mice differ in their responses to influenza infection. To assess the basis for these differences, we used cDNA microarray technology to evaluate gene expression related to influenza infection in both strains of mice. Methods: B and C mice (N=4/group) were inoculated intranasally with influenza virus or saline and were euthanized 30 hours later. Biotin-labeled cRNA was prepared from lung and hybridized to Mouse U420A chips for 16 h. Probe arrays were stained and scanned using Affymetrix technology. Data were analyzed using GeneSpring software and Mas 5 methods. The SIUSM IACUC approved all animal use. Results: 238 genes varied in expression by 3 fold or more across the 4 groups (ANOVA, $p < 0.01$). Of these, 47 were immune-related genes (IRG). In uninfected mice, 6 IRG were expressed to a greater degree in B (mean ratio of B:C=28 fold) compared to 21 in C (mean C:B=11 fold). 42 IRG increased expression to a greater degree in infected B (mean B:C=24 fold) than in C (mean C:C<3 fold). Lung viral titers did not differ between strains. Conclusions: After administration of saline, a greater number of IRG are activated in lung of C vs. B mice. However, B mice show more robust activation of IRG in response to influenza virus. This variation may underlie differences in the pathophysiology and severity of disease in the two strains. Supported by HL070522 and RR017543.

8.0: OXIDATIVE STRESS

8.1

OXIDATIVE STRESS, PROTEIN UBIQUITINATION, AND METABOLIC ALTERATIONS IN DIAPHRAGM MUSCLE INDUCED BY DIABETES: EVIDENCE FROM GENE EXPRESSION PROFILING

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Diabetes impairs respiratory muscle contractile performance, and hyperglycemia has adverse effects on outcomes in critically ill patients. The cellular mechanisms which underlie the deleterious effects of diabetes on the respiratory system are not well-delineated. The present study used array technology to examine global changes in gene expression in diaphragm muscle of rats rendered diabetic with streptozotocin (glucose level 279 vs control of 70 mg/dl; n=3 each; $P < 0.001$). Diabetic diaphragm had 105 genes with statistically significant changed expression of at least ± 2 -fold in magnitude. The 55 genes with upregulated expression were assigned to statistically over-represented gene ontology groups dealing with palmitoyl-CoA hydrolase activity (a component of lipid metabolism), ubiquitination (a mechanism for protein degradation), oxidoreductase activity (which includes genes involved in oxidative stress), and morphogenesis/organogenesis. In contrast the 50 genes with reduced expression were related to carbohydrate metabolism, extracellular matrix/collagen, and organogenesis. Several of these themes are consistent with previous data on diabetic limb muscles, whereas others had only been identified previously in other organ systems such as kidney and pancreas. These data indicate that diabetes has wide-ranging effects on diaphragm muscle; further studies are needed to evaluate diabetic effects on other components of the respiratory system.

8.2

CADMIUM AND MERCURY CAUSE AN OXIDATIVE STRESS-INDUCED ENDOTHELIAL DYSFUNCTION IN BOVINE PULMONARY MONOLAYERS

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We investigated the ability of cadmium and mercury ions to cause endothelial dysfunction in bovine pulmonary artery endothelial cell monolayers. Exposure of monolayers for 48 hours to metal concentrations greater than 3-5 μ M produced profound cytotoxicity (increased lactate dehydrogenase, LDH, leakage), a permeability barrier failure, depletion of glutathione and ATP and almost complete inhibition of the activity of key thiol enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glucose-6-phosphate dehydrogenase (G6PDH). In contrast, metal concentrations less than 1-2 μ M induced increases in glutathione and thiol-enzyme activities with minimal changes in LDH leakage, barrier function and ATP content. At shorter incubation times (24 hours or less), high concentrations of cadmium caused glutathione induction rather

than depletion. Thus, oxidative stress and cytotoxicity induced by lower concentrations of the metal ions stimulate compensatory responses, including increased synthesis of glutathione, which presumably preserved the activity of key thiol enzymes, however these responses were not sustainable at higher metal ion concentrations. We conclude, while high concentrations of heavy metals are cytotoxic, lower concentration induce a compensatory protective response, which may explain threshold effects in metal-ion toxicity.

8.3

LUNG-GENOME RESPONSES TO OZONE (O₃) IN ALPHA-TOCOPHEROL TRANSFER PROTEIN (ATP^{-/-}) DEFICIENT MICE

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O₃ is a major component of urban air pollution and is a regulated environmental toxicant. Epidemiologic and toxicologic studies suggest that AT plays a protective role in O₃-induced lung pathobiology. The present study screens genomic responses of lungs to O₃ (0.5 ppm, 6 hrs/day for 3 days) in C57BL6 ATP^{+/+} and ATP^{-/-} mice using Affymetrix 430A 2.0 high density oligonucleotide arrays. Hepatic ATP preferentially incorporates AT into plasma, thus maintaining systemic AT levels. ATP^{-/-} mice lung AT levels are <10% of those in ATP^{+/+} mice. Global gene expression analysis demonstrated that O₃ exposed ATP^{-/-} mice were more changed from baseline air exposure than ATP^{+/+} mice (99 genes were differentially expressed in ATP^{-/-} mice compared to 52 genes being differentially expressed in ATP^{+/+} mice). The data showed an O₃-induced upregulation of genes related to cell proliferation/DNA repair and inflammatory-immune responses in both ATP^{+/+} and ATP^{-/-} mice, the expression of 22 genes being common to both, whereas 30 and 77 genes were unique to ATP^{+/+} and ATP^{-/-} mice, respectively. In O₃ exposed ATP^{-/-} mice the expression of genes such as cyclin A2, cyclin B1, cyclin B2, serum amyloid A3, macrophage receptor with collagenous structure (MARCO), lipocalin 2, glutamate cysteine ligase were upregulated and were unique to this group. The present study provides further documentation for the role of AT in O₃-induced lung pathobiology.

8.4

UP-REGULATION OF THE EXPRESSION OF FC γ RIIIB (CD16B) ON THE SURFACE OF HUMAN BLOOD NEUTROPHILS BY ANTI-IL-8:IL-8 COMPLEXES

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Previous studies from our laboratory revealed that the presence of anti-IL-8 autoantibody:IL-8 complexes in lung fluids from patients with ALI/ARDS is an important prognostic indicator for the development and outcome of ALI/ARDS, and that purified anti-IL-8 autoantibody:IL-8 complexes trigger chemotaxis of neutrophils, induce activation of these cells, and prolong the lifespan of neutrophils via IgG receptors, Fc γ RIIa. Sepsis is considered the major risk factor for development of ALI/ARDS, and LPS has been recognized as a causative factor for progression to ALI/ARDS. Importantly, we showed that both expression of Fc γ RIIa in neutrophils as well as cellular activation mediated by Fc γ RIIa is increased upon LPS treatment. Therefore, microarray analysis was performed to identify changes in gene expression of neutrophils treated with LPS alone or LPS and anti-IL-8:IL-8 complexes. We detected up-regulation of several genes, including Fc γ RIIIB (CD16b). The increase in level of CD16b was confirmed by confocal microscopy. It is known that CD16b mediates recruitment and activation of neutrophils, and enhances activity of Fc γ RIIa. Further, a soluble form of this receptor is elevated in BAL fluids from patients with ARDS. Soluble CD16b, which is generated by proteolysis of a membrane bound receptor, has also capability to trigger activation of monocytes. This study provides an additional evidence in support of our hypothesis that anti-IL-8:IL-8 complexes may contribute to the pathogenesis of lung inflammation in ALI/ARDS. Supported by the NIH grant R01 HL073245.

8.5

PEROXYNITRITE-INDUCED NITRATION AND CROSS-LINKING OF HUMAN SURFACTANT PROTEIN D (SP-D)

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We hypothesized that peroxynitrite (ONOO-) might modify SP-D structure and function by oxidizing and/or nitrating residues in the lectin domain. We exposed purified trimeric human SP-D neck+CRD domain (30 mg) to various concentrations of ONOO-. Samples were subjected to MS/MS for analysis of protein modifications. Parallel aliquots were analyzed for nitrotyrosine by immunoblotting using specific antibodies. Immunoblotting assays and MS/MS analysis demonstrated specific, dose-dependent crosslinking to form larger immunoreactive aggregates when examined by SDS-PAGE in the presence of reducing agents. We found nitration of tyrosines at positions 228 (in neck domain) and at 306 and 314 (in the CRD domain). Oxidative modifications were also detected in some of the regions between cysteines in positions 331 and 345 within the C-terminal end of the lectin domain. Our results show evidence of ONOO-mediated tyrosine nitration within functionally important regions of the C-terminal domains. We speculate that these structural modifications and the associated cross-linking can lead or contribute to functional inactivation of the protein at sites of active inflammation. Funded By: NIH HL075540,NIH-HL31197,NIH HL72871, NIH HL51173.

8.6

QUANTITATIVE PROTEOMIC PROFILES OF BALF IN WILD TYPE AND SP-A KO MICE AFTER EXPOSURE TO OZONE

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Millions are exposed to ozone levels above recommended limits, impairing lung function, causing epithelial damage and inflammation, and predisposing some to pneumonia, asthma, and other conditions. Surfactant protein A (SP-A) plays a role in host defense, the regulation of inflammation, and repair of tissue damage. We hypothesized that SP-A(-/-) (KO) mice would be

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more susceptible to ozone-induced damage and have different bronchoalveolar lavage (BAL) protein patterns than wild type (WT) mice +/- ozone exposure. WT and KO mice were exposed to ozone (2 ppm) for 3 hours or to filtered air (FA). BAL was performed 4hr after exposure and 2-dimensional difference gel electrophoresis (2-DIGE) was used to compare the BAL proteome. Proteins were identified by matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry (MALDI-ToF/ToF). We detected 443 protein spots and picked 96 for MALDI-ToF/ToF. Comparisons showed 130 and 96 significant differences in WT FA vs ozone, KO FA vs ozone. Comparisons between FA-exposed WT vs KO and ozone-exposed WT vs KO, revealed significant differences in 33 and 28 spots, respectively. The differences included a variety of classes and functional categories of proteins. These findings show that the BAL proteome of KO mice differs from that of WT after exposure to ozone or FA and indicate that the susceptibility to ozone-induced toxicity may be altered in mice lacking SP-A compared to WT. Supported by NIEHS 09882.

9.0: ENDOTHELIAL CELLULAR PERMEABILITY AND PULMONARY EDEMA

9.1 ENDOTHELIAL-DERIVED NITRIC OXIDE INHIBITS ALVEOLAR FLUID REABSORPTION

Stephanie Kaestle¹, Christian Reich¹, Jörg Weimann¹, Wolfgang Kuebler¹
¹Institute of Physiology, Charité-University Medicine Berlin, Arnimallee 22, Berlin, Germany. While hydrostatic lung edema has previously been attributed solely to increased fluid filtration, impaired alveolar fluid reabsorption (AFR) may contribute importantly to its pathogenesis. Recently, we identified pressure-induced endothelial NO formation as a potential mechanism inhibiting AFR. Here, we tested whether inhibition or deficiency of endothelial NO synthase (eNOS) may preserve AFR and thus, attenuate hydrostatic lung edema. In isolated perfused lungs of eNOS^{-/-} and wild-type mice, left atrial pressure (P_{LA}) was elevated from 2 to 6 cmH₂O for 25 min. Resulting hydrostatic lung edema was determined as wet/dry weight ratio. In isolated perfused rat lungs, alveolar fluid influx and reabsorption were quantified over 60 min by a double-indicator dilution technique at P_{LA} of 5 or 15 cmH₂O. In wild-type mice, P_{LA} elevation from 2 to 6 cmH₂O increased wet/dry weight ratio from 4.1±0.2 to 10.1±0.4 (p<0.05). In both, lungs of eNOS^{-/-} mice or lungs of wild-type mice perfused with the NOS blocker L-NAME, hydrostatic lung edema was significantly attenuated (wet/dry weight ratios of 6.3±0.2 and 6.5±0.4). In rat lungs, P_{LA} elevation increased alveolar fluid influx from 0.93±0.37 ml/h to 2.1±0.6 ml/h (p<0.05) while reducing AFR from 1.28±0.38 ml/h to 1.7±1.1 ml/h (p<0.05). At elevated P_{LA} of 15 cmH₂O, L-NAME completely reconstituted AFR to 1.7±0.8 ml/h while alveolar fluid influx remained unchanged (2.7±0.8 ml/h). These data demonstrate that eNOS-derived NO promotes the formation of hydrostatic lung edema by inhibiting AFR. Sponsored by DFG GRK 865 and Ku 1218/4-1.

9.2 NO-REGULATED FEEDBACK LOOP PROTECTS LUNG MICROVASCULAR BARRIER IN HYDROSTATIC STRESS

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¹Institute of Physiology, Charité-University Medicine Berlin, Arnimallee 22, Berlin, Germany, ²Department of Anesthesiology, German Heart Center Berlin, Augustenburger Platz 1, Berlin, Germany. In lung capillary endothelial cells, hydrostatic stress increases cytosolic Ca²⁺ concentration ([Ca²⁺]_i), resulting in an elevated capillary filtration coefficient (K_f) and activation of endothelial NO synthase (eNOS). Here, we studied the functional significance of pressure-induced NO formation. In isolated perfused rat lungs, endothelial [Ca²⁺]_i and NO production were quantified by *in situ* fluorescence imaging of fura-2 and DAF-FM, and K_f was measured by gravimetry. For pressure stress, left atrial pressure (P_{LA}) was elevated from 5 cmH₂O to 15 cmH₂O for 30 min. In control lungs, P_{LA} elevation increased endothelial [Ca²⁺]_i from 100±4 nM to 162±5 nM, NO production to 212±16% (of baseline), and K_f from 0.4±0.1 to 2.6±0.7 ml·min⁻¹·cmH₂O⁻¹·100 g⁻¹. Inhibition of eNOS by L-NAME amplified the pressure-induced [Ca²⁺]_i and K_f responses to 194±7 nM and 3.7±0.3 ml·min⁻¹·cmH₂O⁻¹·100 g⁻¹ while the NO donor GSN0 attenuated both responses (125±5 nM and 1.2±0.4 ml·min⁻¹·cmH₂O⁻¹·100 g⁻¹). Endothelial [Ca²⁺]_i response and K_f were also augmented by the soluble guanylate cyclase (sGC) inhibitor ODQ. In contrast, both the sGC activator BAY 41-2272 and the cGMP analogue 8-Br-cGMP attenuated the pressure-induced endothelial [Ca²⁺]_i response, NO production, and K_f increase. Pressure-induced NO formation may establish a negative feedback loop limiting the endothelial [Ca²⁺]_i response and thus, deterioration of the lung microvascular barrier by a cGMP-dependent inhibition of Ca²⁺ influx channels. Sponsored by Kaiserin-Friedrich Foundation, Berlin.

9.3 ACTIN REARRANGEMENT CAUSES LUNG ENDOTHELIAL DYSFUNCTION IN CONGESTIVE HEART FAILURE

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¹Institute of Physiology, Charité-University Medicine Berlin, Arnimallee 22, Berlin, Germany. Congestive heart failure (CHF) impairs endothelial NO production in lung microvessels. Here, we analyzed cellular mechanisms underlying this endothelial dysfunction. In rats, CHF was induced by supracoronary aortic banding 8 weeks prior to investigations. Western blot analyses were performed with protein of freshly isolated endothelial cells or homogenized lungs. Endothelial NO production, Ca²⁺ concentration ([Ca²⁺]_i) and F-actin expression were determined by *in situ* fluorescence imaging of DAF-FM, fura-2, and alexa-phalloidin. NO and [Ca²⁺]_i images were obtained at baseline left atrial pressure (P_{LA}) of 5 cmH₂O and during 30 min of P_{LA} elevation to 15 cmH₂O. Western blot analyses revealed preserved eNOS expression in CHF lungs. Yet, P_{LA} elevation increased NO production only in control (239±18% of baseline) but not in CHF lungs (98±2%). Analogously, P_{LA} elevation increased [Ca²⁺]_i from 102±3 nM to 172±8 nM in control but not CHF lungs (86±2 nM vs. 91±4 nM). β-actin protein expression in lung endothelial cells was increased 13.2-fold in CHF as compared to control rats, while fluorescence imaging revealed a 9.3-fold increase in actin density as compared to control rats. Disruption of the actin cytoskeleton by cytochalasin D reconstituted endothelial [Ca²⁺]_i (176±10 nM) and NO (212±16%) responses to P_{LA} elevation in CHF lungs. Congestive heart failure causes overexpression of β-actin in pulmonary capillary endothelial cells. Cytoskeletal remodeling impairs endothelial responses and thus, contributes to lung microvascular pathology in CHF. Sponsored by EU-IP "Pulmotension"

9.4 THE IMPACT OF CYTOKINE TREATMENT ON GENE EXPRESSION IN LUNG ENDOTHELIAL CELLS

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¹Department of Pathology, University of Sao Paulo - School of Medicine, Av. Dr. Arnaldo, 455, Cerqueira Cesar, Sao Paulo, 01246903, Brazil. Endothelial cells (EC) of lung microcirculation present specific biological properties that differentiate them from other circulatory territories' ECs. Although several lung endothelial cell-specific genes have been identified, little is known about the molecular framework underlying the unique features of lung microcirculation EC. In order to study lung EC's response to cytokine stimulation, we compared microarray dataset for unstimulated and cytokine-treated (IFN[gamma], IL4 and TNF[alpha]) human ECs representing both lung and systemic (aortic, iliac, dermal, and colonic) territories from a public collection of genomic data (GDS498, 499, 500, 501, 502). Data were log intensity-filtered and lowess-normalized or median array-normalized (for unchallenged ECs). Comparison of unstimulated lung to systemic ECs detected 2484 differentially expressed genes while the differences among non-pulmonary ECs were less clear (<600 genes). Multivariate analysis has grouped all arrays for cytokine-treated lung EC into a single cluster. Therefore, they were compared as one class with cytokine-treated non-pulmonary ECs and yielded 1037 genes. Interestingly, there was no substantial overlap between the differentially-expressed geneset for unchallenged and cytokine-treated lung EC. Our results suggest that molecular phenotype of untreated lung EC is distinct from other ECs and that these differentially expressed genes are not the same genes that underly lung EC's response to cytokine

11.0: GENOMICS AND PROTEOMICS OF ENVIRONMENTAL LUNG DISEASE

11.5 GENE PROFILING OF EPITHELIAL CELL REMODELING AFTER INHALATION OF ASBESTOS.

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*Department of Environmental Health Sciences, University of South Carolina, and Department of Pathology, University of Vermont College of Medicine. Chronic exposure to asbestos, a family of naturally occurring fibrous minerals, is associated with the development of lung cancers, malignant mesotheliomas, and pleural and pulmonary fibrosis (asbestosis). Bronchiolar epithelial cells are initial targets of asbestos fibers after inhalation and pivotal cells in the development of a number of airway diseases and fibrosis. Lung remodeling may involve epithelial signaling pathways such as extracellular signal-regulated kinases (ERKs) (1) and interactions with underlying mesenchymal cells and/or cells of the immune system. To elucidate genes important in development and/or repair of asbestos-induced lung diseases, global gene expression (Affymetrix) in whole lung homogenates was examined from C57BL/6 mice after inhalation of chrysotile asbestos for 3, 9, and 40 days (2). These studies revealed increased expression of genes linked to proliferation, inflammation, and epithelial/ matrix remodeling including *mclca3* (*gob5*), a gene implicated in mucin production, that was confirmed in epithelial cells at the protein level using immunohistochemistry. We next created mice expressing a dominant-negative-mitogen-activated protein kinase kinase-1 (dnMEK1) transgene targeted to bronchiolar epithelium using the CC10 promoter and evaluated epithelial cell proliferation, differentiation and lung gene expression that were altered after inhalation of crocidolite asbestos in comparison to transgene-negative C57BL/6 mice. Using laser capture microdissection (LCM) and subsequent analysis by the quantitative real-time polymerase chain reaction (QRT-PCR) and gene array approaches (3), we are exploring altered gene expression in distal bronchiolar epithelial cells isolated from the lungs of sham and asbestos-exposed dnMEK1 and C57BL/6 mice (transgene-negative littermates) to determine possible candidates altered in lung injury and remodeling. These approaches may aid in defining mechanisms of epithelial cell injury by airborne particulates and in the development of therapeutic strategies for asbestos-associated lung diseases. Supported by P01 HL 67004 from the National Heart, Lung and Blood Institute (BM,NH,DT), K01 CA104159-03 from the National Cancer Institute (MRN), and a TIPS award (KES014742A) from the National Institute of Environmental Health Sciences (TZA). References: 1. Cummins AB, Palmer C, Mossman BT, and Taatjes DJ: Persistent localization of activated extracellular signal-regulated kinases (ERK1/2) is epithelial cell specific in an inhalation model of asbestosis. *Am J Pathol* 162: 713-720, 2003. 2. Sabo-Attwood T, Ramos-Nino M, Bond J, Butnor KJ, Heintz N, Gruber AD, Steele C, Taatjes DJ, Vacek P, and Mossman BT: Gene expression profiles reveal increased mClca3 (Gob5) expression and mucin production in a murine model of asbestos-induced fibrogenesis. *Am J Pathol* 167: 1243-1256, 2005. 3. DJ Taatjes and BT Mossman, eds.: *Cell Imaging Techniques: Methods and Protocols* (Methods in Molecular Biology, vol. 319), Humana Press, Totowa, NJ, pp. 1-490, 2006.

11.6 GENE-ENVIRONMENT INTERACTIONS IN LUNG DISEASE

Steven Kleebberger¹
¹Laboratory of Respiratory Biology, NIEHS, 111 T.W. Alexander Drive, Building 101, Rm. D240, Research Triangle Park, North Carolina, 27709. It is generally agreed that genetic background is an important determinant of susceptibility to many lung diseases, including asthma and chronic obstructive pulmonary disease, and that these diseases are complex (i.e., multigenic). The complexity stems from the number of subphenotypes that characterize the disease. A gene or set of genes may confer susceptibility or predisposition to each phenotype, and genome-wide searches for linkages of diseases and disease subphenotypes have revealed a number of candidate genes for the phenotype in question. However, genetic background alone cannot account for most diseases. Expression of the disease or its subphenotypes is likely dependent on environmental factors (i.e., gene-environment interaction). That is, relative risk of disease for a specific combination of genes may depend on frequency of exposure to the environmental agent, strength of interaction between the genotype and the agent, and specificity of the environmental effect with respect to the genotype. Important environmental exposures include indoor (NO₂, tobacco smoke) and outdoor (ozone, PM_{2.5} and ultrafine, NO₂, and SO₂) air pollutants, and allergens (house dust mite, cockroach, pollen, and cat dander). Understanding the interaction of environmental and genetic factors important in lung disease will lead to improved evaluation of risk of a specific genotype(s) for a given environmental exposure, and a potential means for intervention to prevent disease.

13.0: PULMONARY FIBROSIS

13.1 COMPARATIVE EXPRESSION OF SPLUNC1, SPLUNC2 AND LPLUNC1 IN NORMAL AND DISEASED LUNG.

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ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

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The human PLUNC gene locus on chromosome 20 encodes a family of at least 10 proteins that are expressed in the upper airways, nose and mouth. The family subdivides into short (S) and long (L) proteins, which contain domains structurally similar to one or both of the domains of bactericidal/permeability-increasing protein. PLUNCs are secreted into airway and nasal lining fluids and saliva where they may play a role in innate immune defence. A number of studies have suggested that these proteins are differentially expressed in lung diseases. We have performed a comparative analysis of the localisation of SPLUNC1, SPLUNC2 and LPLUNC1 in normal and diseased (Cystic Fibrosis [CF]) lung tissue and associated salivary and mucosal glands. The results show that PLUNC proteins are expressed in distinct cell types within the epithelial and glandular tissues of the respiratory tract. In CF expression of SPLUNC1 and LPLUNC1 is significantly elevated in diseased airways and positive staining was noted in some of the inflammatory infiltrates. Submucosal gland staining of LPLUNC1 is also significantly increased in CF. SPLUNC2 predominantly stains salivary and minor mucosal glands of the mouth. It is not found in the normal airways nor was expression found to be altered in CF tissues. Our results show unique expression domains for PLUNCs within the airways and suggest that alterations in expression of these putative innate immune molecules may be associated with lung disease.

13.2

LASER CAPTURE MICRODISSECTION AND MICRORRAY ANALYSIS REVEAL MMP19 AS A POTENTIAL NEW REGULATOR OF FIBROSIS

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Introduction: Lung phenotype in IPF is determined by the epithelial/fibroblast crosstalk and signals detected in alveolar epithelium may provide significant insights about lung microenvironments. **Methods:** We used LCM and microarrays to profile gene expression in IPF microenvironments to identify and verify the IPF regulators in lung epithelium adjacent to fibrotic regions. Protein expression was validated by Western Blotting and immunohistochemistry (IHC). To explore the role of MMP19 in lung fibrosis we used Bleomycin model in MMP19 knockout mice. **Results:** One of the top expressed genes in epithelium adjacent to fibrotic areas was MMP19, a protease involved in normal degradation and maintenance of basement membrane. Protein analysis confirmed overexpression of activated MMP19 in IPF and IHC localized MMP19 in epithelial regions adjacent to fibrosis. MMP19 deficient mice developed significantly severe fibrosis in response to bleomycin compared to controls. **Conclusion:** Microarray analysis of LCM obtained cells allows identification of differentially expressed genes not detected by microarray analysis of whole human lungs. MMP19 is increased in epithelium adjacent to fibrotic regions in humans. In the bleomycin model lack of MMP19 is associated with severe fibrotic response suggesting a protective role for this MMP in fibrosis. NIH HL07374501.

13.3

MOLECULAR PROFILING OF LUNG HOMEOSTASIS AND FIBROSIS

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Objective: To molecularly profile lung fibroblasts characteristic of homeostasis and fibrosis. **Methods:** Genome-wide microarray (Affymetrix), and metabolomics based on stable isotope labeling and mass spectrometry. **Background:** Lung fibroblasts determine alveolar structure, function and homeostasis, as determined by up-regulation of the Parathyroid Hormone-related Protein (PTHrP) signaling pathway, which down-regulates the Sonic Hedgehog/Wnt/beta catenin (SWC) signaling pathway, mediated by Peroxisome Proliferator Activated Receptor (PPAR) gamma. Inhibition of the lung fibroblast PTHrP pathway by a variety of pathophysiologic agents known to cause lung fibrosis causes down-regulation of the PTHrP pathway, and up-regulation of the SWC pathway, leading to up-regulation of alpha-SMA and myofibroblast proliferation, a process referred to as transdifferentiation. **Results:** Cluster analysis of the microarray data exhibited down-regulation of the fatty acid and cholesterol synthetic genes, and up-regulation of the fatty acid degradation and SWC-related genes, a pattern highly characteristic for the transdifferentiation of lipofibroblasts to myofibroblasts. Consistent with these microarray data, metabolomic analysis revealed decreased expression of lipogenic pathways and increased expression of lipid degradative pathways. Treatment of transdifferentiating fibroblasts with PPAR gamma agonists prevented the molecular and metabolic changes detected by microarray and metabolomics. **Conclusions:** molecular profiling of the PTHrP and SWC pathways provides key targets for the early diagnosis and treatment and prevention of lung fibrosis. Supported by grants from the NIH (HL55268 and HL075405) and TRDRP (14RT-0073).

13.4

WISP-1, A NOVEL MEDIATOR AND THERAPEUTIC TARGET IN PULMONARY FIBROSIS

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Lung fibrosis is characterized by a loss of respiratory function due to alveolar epithelial type II (ATII) cell hyperplasia, enhanced extracellular matrix deposition, and fibroblast proliferation. Repetitive epithelial injuries with altered ATII cell gene expression represent a trigger in lung fibrosis, but the molecular mechanisms linking ATII cell repair with fibrosis are poorly understood. Here, we report increased proliferation (186 ± 225%, 95% C.I.) and altered gene expression profiles of ATII cells derived from mice with lung fibrosis, using whole genome microarray analysis. WISP-1, a member of the CCN family, is highly upregulated on RNA and protein level in lung fibrosis (induced by bleomycin), and in IPF lungs. Enhanced WISP-1 protein expression localized to hyperplastic ATII cells. ATII cells stimulated with WISP-1 showed increased proliferation (154 ± 220%, 95% C.I.), mediated by enhanced phosphorylation of protein kinase B (PKB/Akt). WISP-1 treatment leads to epithelial-to-mesenchymal transition, as analyzed by increased expression of the mesenchymal markers smooth muscle actin and vimentin, as well as decreased expression of the epithelial markers tight junction protein-1 and E-Cadherin. Depletion of WISP-1 in vivo (using neutralizing antibodies) resulted in marked

attenuation of bleomycin-induced lung fibrosis, as assessed by histological analysis, flat-panel volumetric computed tomography, and measurement of lung function. Our study thus identified WISP-1 as a key regulator of the hyperplastic ATII cell phenotype in lung fibrosis in vitro and in vivo, and unravels WISP-1 as a novel mediator and potential therapeutic target in pulmonary fibrosis. Funding: German Research Foundation.

14.0: CHRONIC OBSTRUCTION PULMONARY DISEASE

14.1

Abstract withdrawn by author.

14.2

EARLY TRANSCRIPTOMIC PREDICTORS OF CIGARETTE SMOKE (CS) CARCINOGENESIS

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Simultaneous modulations in the expression of multiple genes are implicated in carcinogenic processes. We have screened the genome-wide lung responses to CS in an attempt to identify gene-clusters that may be associated with early events in CS-induced tumors. This study compared genome-wide lung responses of tumor-prone AJ mice with those of the tumor resistant C57BL6 lungs to whole smoke (WS) or gas phase of CS. Recent studies suggest that the GP of CS is equipotent to WS in increasing lung tumors in AJ mice. Mice (n=5/group) were exposed to air or to incremental WS (90-125 mg/m³) or to GP for 10 days (6h/day). Total RNA was isolated from each lung tissue and processed for GeneChip (Affymetrix, 430A 2.0) analysis as described by the manufacturer. Data from a total of 30 GeneChips were analyzed with D-chip Microarray analysis software to obtain the effects of the three different exposures on each-, and between each of the two mouse strains. ~2000 genes were differentially expressed between the two strains of mice breathing air. Further analysis focused on 314 GP sensitive genes of AJ lungs because they are most likely to be early predictors of CS-induced tumors. Comparative analysis of these genes in C57BL6 mice identified 3 WS and GP sensitive gene clusters unique to AJ lungs and included immune responsive genes (suppressed), membrane proteins and receptors (induced) and select members of Nr2f driven, phase II genes (induced). These preliminary data suggest that clusters of genes responsive to oxidant stress, inflammatory processes and cell-cell interactions cooperate in CS-induced carcinogenic process in the lungs of AJ mice.

14.3

CIGARETTE SMOKE (CS) SENSITIVE LUNG TRANSCRIPTOME IS MODULATED BY TOCOPHEROL TRANSFER PROTEIN (TTP)

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Oxidant and inflammatory processes are implicated in CS induced lung diseases. We hypothesized that these are multigenic processes affected by the lung's intrinsic antioxidants such as alpha-tocopherol (AT), a potent diet derived lipophilic antioxidant. Lung's AT levels can also be affected by the hepatic TTP-gene which regulates systemic AT levels. Our hypothesis was tested in C57BL6 wild-type (WT) mice fed diets varying in AT content (<10IU-1000IU AT/kg diet) or in TTP-deficient (TTP-KO) mice. One month old mice (n=5/group) were fed the diets for 4 months and then exposed to either air or CS (60mg/m³, 6h/day, for 3 or 10 days). Post-exposure, total RNA was extracted and 10ug RNA from each lung was pooled group-wise and processed for GeneChip analysis (Affymetrix 430A 2.0). Differential analysis of the transcriptomes (~15,000 mRNAs) identified CS sensitive genes that were modulated by lung AT-levels. CS activated two AhR driven genes, cyp1a1 and cyp1b1 whose induction was increased by AT. In contrast, lung AT did not affect the expression of Nr2f driven genes. Largest clusters of CS-AT sensitive genes included lymphocyte and leukocyte specific genes. These gene-clusters included those encoding immunoglobulins and cytokines, which were repressed by CS and were modulated by the lung AT levels. These preliminary data from genome-wide analysis of lung's response to CS support our hypothesis and suggest reciprocal actions of CS on the expression of xenobiotic and immune-inflammatory genes that are further modulated by lung AT concentrations.

14.4

EVIDENCE FOR ATTEMPTED REGIONAL ELASTIC FIBER REPAIR IN SEVERE EMPHYSEMA

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The rate of emphysematous lung tissue destruction and regional heterogeneity of disease within individuals can be highly variable, and yet the mechanisms for progressive alveolar destruction are poorly understood. We generated maps of alveolar destruction via ³He diffusion MRI in explanted human lungs with severe COPD and in control lungs. The images directed selection of 6 tissue cores (3 mild and 3 severe) from each of two emphysematous lungs and 6 cores from a human control lung, for gene expression profiling using Affymetrix HU 133 arrays. Among genes regulated differentially between normal and diseased specimens were the elastin gene (ELN) and 2 other genes important in elastic fiber assembly, MFAP2 and Emilin-1. These data were validated by realtime RT-PCR and expanded in further studies of explanted lungs with end-stage COPD (n=10, 6 cores/lung) and control lungs (n=10, 3-6 cores/lung). Expression of all genes studied showed marked variation between specimens of differing disease severity from the same lung, highlighting spatial heterogeneity of disease within lungs; much less variation was observed between samples from control lungs. In-situ hybridization showed that the upregulated ELN expression in diseased specimens localized to alveolar walls--sites of ELN expression during normal lung development. This evidences attempted alveolar elastic fiber repair in end-stage emphysema.

14.5

Abstract withdrawn by author.

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14.6

DEFECTIVE FGF SIGNALING INDUCES ABERRANT ELASTOGENESIS IN MICE WITH POSTNATAL AIRSPACE ENLARGEMENT

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 Previous studies have shown congenital airspace enlargement in mice bearing compound mutations of FGFR3 and 4. Gene expression profiling of newborn lung tissue revealed few changes in compound FGFR3/FGFR4 deficient mice, suggesting the sequence of events leading to the phenotype initiates after birth in this model. Profiling of 1 and 4 week-old lung tissues revealed an induction of genes related to elastic fiber assembly in compound mutants. Real time PCR (qPCR) validated significant increases (p<0.05) in the expression of tropoelastin, microfibrillar associated protein-5, lysyl oxidase, lysyl oxidase-like 1, fibrillin 1, fibulin 1, fibulin 5, biglycan and asporin in compound mutant lungs. Biochemical analysis confirmed increased elastin deposition beginning in 1-week old compound mutant lungs. To define the spatial pattern of airspace elastogenesis, we used histochemical analysis. These data revealed an excess of fibers deposited throughout the alveolar wall beginning at 1 week, not restricted to the tip of secondary septae as is observed in control animals. To determine the mechanisms mediating the aberrant elastin production, we isolated lung mesenchymal cells and evaluated gene expression in vitro. No difference in baseline expression or FGF-stimulated repression was observed between FGFR3/FGFR4 mutant and control cells. These data suggest that paracrine signaling is necessary for abnormal elastogenesis in FGFR3/FGFR4 mutant mice. Funded By: NIH HL71885.

14.7

MOLECULAR MARKERS FOR QUANTITATIVE AND DISCRETE COPD PHENOTYPES

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 To identify gene expression markers for chronic obstructive pulmonary disease (COPD), we performed genome-wide expression profiling of lung tissue from 56 patients with a solitary pulmonary nodule undergoing surgical resection. Correlation of gene expression with a quantitative measure of airflow obstruction (FEV1), using both Pearson and Spearman coefficients (p<0.01), identified a set of 65 marker transcripts. Analysis of differential expression between cases (FEV1<70% predicted, FEV1/FVC<0.7) and controls (FEV1>80% predicted, FEV1/FVC>0.7) identified a set of 65 transcripts representing discrete markers associated with COPD. A total of 43 transcripts were identified that showed evidence of significant correlation (p<0.05) with quantitative traits and differential expression between cases and controls. Finally, we intersected our results with those generated by applying similar methods to a data set generated from patients with severe emphysema undergoing lung volume reduction surgery (Spira et al., AJRCMB, 2004). Although these data sets represent distinct populations of COPD patients, we identified 8 shared marker transcripts (CTSK, HPGD, COL10A1, KIAA0265, ARHGAP12, OR2J3, RWDD1, UPP3A), all derived from quantitative phenotypic analysis. Our data contribute to the understanding of gene expression changes occurring in the lung tissue of patients with obstructive lung disease and provide additional insight into potential mechanisms involved in the disease process. Funded by: NIH HL72303.

15.0: ASTHMA

15.1

GENETIC EFFECT OF CYSLTR2 POLYMORPHISMS ON ITS MRNA SYNTHESIS AND STABILIZATION

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 We previously demonstrated that single nucleotide polymorphism (SNP) and haplotypes were associated with aspirin hypersensitivity in asthmatics. In this study, we investigated the genetic effects of the SNPs and haplotypes on the expression of the CYSLTR2 gene. We found that the expression of CysLTR2 protein was higher in B cell lines of asthmatics having ht2+/+ than in those having ht1+/+. PMA/ionomycin induced higher mRNA expression of CysLTR2 in B cell lines from ht2+/+ asthmatics than those from ht1+/+ asthmatics. A nuclear protein from the B cell lines showed stronger DNA binding affinity with a probe containing c-819T than one containing c-819G. The luciferase activity of the c-819T type of CYSLTR2 promoter was higher than that of the c-819G type. EGFP expression was higher in the EGFP-c.2078T 3'-UTR fusion construct than in the c.2078C construct. In conclusion, the sequence variants of CYSLTR2 may affect its transcription and the stability of its mRNA, resulting in altered expression of CysLTR2 protein, which in turn causes some asthmatics to be susceptible to aspirin hypersensitivity. This study was supported by a grant of the Korean Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG6-01GN14-0003).

15.2

PROTEOMIC ANALYSIS OF HUMAN BALF: COMPARISON OF BRONCHIAL ASTHMA WITH NORMAL CONTROL

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 Bronchoalveolar lavage fluid (BALF) is presently the most common way of sampling the components of the epithelial lining fluid and the most faithful reflect of the protein composition of the pulmonary airways. In this study, we had compared the BALF protein profiles of bronchial asthma patient (n=8) and healthy normal subjects (n=8). The airway protein pattern changes in bronchial asthma BALF were analyzed by 2-DE. Twenty proteins were found to be differentially expressed in BALF between asthma patients and normal healthy control. These proteins, which were identified by matrix assisted laser desorption ionization time of flight

(MALDI-TOF) MS. Including Vitamin D binding protein, 10 proteins were up-regulated whereas 10 proteins including apolipoprotein AI were down-regulated. Among them, Vitamin D binding proteins levels in BALF were validated by using ELISA. The concentrations of vitamin D binding protein were higher in asthma patients (n = 52) than in normal control (n = 14). Identification of proteins in the BALF and their expression changes at different stages of asthma could provide further insights into the complex molecular mechanisms involved in this disease. A large portion of these proteins and their expression changes were identified for the first time from BALF, thus providing new insights for finding novel pathological mediators and biomarkers of asthma. These results suggest the involvement of the vitamin D binding protein in the pathogenesis of bronchial asthma.

15.3

B₂-ADRENERGIC RECEPTOR GENOTYPIC VARIANTS IN AFRICAN AMERICANS WITH ASTHMA

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 Two single nucleotide polymorphisms (SNPs) identified in the β₂-adrenergic receptor (β₂-AR) gene that result in amino acid substitutions at positions 16 (Gly or Arg) and 27 (Gln or Glu) may influence response to β₂-agonists. Asthma disproportionately affects African Americans (AAs); yet little is known about SNP variations in asthmatic AAs at these loci. As part of a multicenter trial that explored β₂-agonists use in AA asthmatics (n=155; mean FEV₁ 60% predicted, mean age 38 yrs), combined loci 16-27 haplotypes were determined. Consistent with previous reports in healthy AAs, in these asthmatics the Arg16Gly and Gly16Gly variants and the Gln27Gln alleles were the most frequent (Table). The most common haplotype combination was Arg16Gly-Gln27Gln. β₂-AR genes at these 2 loci may meaningfully impact the bronchodilator response in AA subjects, as median FEV₁ response varied from 17.8% to 28.9% for different haplotype combinations.

Genotype (n=155)	Frequency, %	Haplotype combination Frequency ^a , %	Median % change FEV ₁
Arg16Arg	25	Arg16Arg-Gln27Gln 24	23.7
Arg16Gly	43	Arg16Gly-Gln27Gln 26	21.7
Gly16Gly	32	Arg16Gly-Gln27Glu 14	28.9
Gln27Gln	63	Gly16Gly-Gln27Gln 10	17.8
Gln27Glu	32	Gly16Gly-Gln27Glu 17	27.7
Glu27Glu	5	Gly16Gly-Glu27Glu 4	26.1

^a5% with other combinations not shown
 Supported by Sepracor, Inc

15.4

DIFFERENTIAL GENE EXPRESSION IN PRIMARY HUMAN AIRWAY EPITHELIUM EXPOSED TO ISOMERS OF ALBUTEROL

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 Inhaled β₂-agonists are the mainstay of treatment for bronchoconstriction. A growing body of evidence suggests that exposure to the (S)-isomer of albuterol may have negative effects on airway function. The objective of this study is to understand the effects of the isomers of albuterol on airway epithelial cells. To do this total mRNA was isolated from well-differentiated primary cultures of human airway epithelial cells after acute (1 day) and chronic (8 days) of exposure to either (R)-, (S)-, and racemic albuterol at concentrations of 1 nM, 10 nM, 100 nM, and subjected to gene expression profiling using the Affymetrix Genechip™. Additionally, real-time polymerase chain reaction was used to confirm differentially expressed genes. At 1 nM level, (R)-albuterol caused greater changes in gene expression than observed with (S)-albuterol. However, chronic treatment diminished this difference, yet an isomer-specific gene expression pattern was still detectable. Treatment for 24 hours with 10 nM concentration of either isomer resulted in the expression of similar genes being up- and down-regulated. The genes that increased included ARG2, TM4SF3, FABP5, CLCA2, DAF, EDN2, SSA2 and PFDN4; and some that decreased were TRIM31, TNFAIP2, KDR, APOBEC3B, CX3CL1, KRT23, INDO, TXNIP, CCND1 and VGLL1. Interestingly, CYP3A5 mRNA levels markedly increased both in acute and chronic exposure to (S)- but not (R)-albuterol. Further studies with prolonged exposure will help to elucidate the global change in gene expression patterns associated with the isomers. Supported by Sepracor Inc.

15.5

MYCOPLASMA PNEUMONIAE (MP) DECREASES AIRWAY EPITHELIAL VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) GENE EXPRESSION IN MICE: MICROARRAY AND LASER CAPTURE MICRODISSECTION (LCM) APPROACHES.

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 Mp is involved in chronic asthma. Angiogenesis may be a pathologic feature of asthma. VEGF is critical in angiogenesis. However, the role of an infection (e.g., Mp) in regulating VEGF expression remains unclear. Here, we determined the effects of Mp on VEGF in mouse lung tissues especially airway epithelial cells. Additionally, the direct effects of Mp on VEGF protein from human primary bronchial epithelial cells were examined. BALB/c mice (n=6/group) were intranasally inoculated with Mp (10⁸ CFU/mouse) or saline (control). On day 3 post Mp or

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saline, lung tissues were collected for microarray (Affymetrix mouse genechip Moe 430.2.0) analysis or for LCM. Airway epithelial cells were isolated using LCM, and analyzed for VEGF mRNA by real-time RT-PCR. In addition, normal human primary bronchial epithelial cell air-liquid interface culture was performed to determine the direct effects of Mp (1-50 CFU/cell) on VEGF protein. As compared to control mice, Mp-infected mice showed a 3.7-fold reduction of VEGF mRNA in the whole lungs as analyzed by microarray. Using the LCM and RT-PCR approach, VEGF mRNA was shown to be reduced (3 to 9-fold, $p < 0.05$) in large, medium and especially small airway epithelial cells. Mp infection in human epithelial cells also reduced (about 40%) the production of VEGF protein. Our results suggest that infection of human pathogen Mp reduces lung (e.g., airway epithelial cells) VEGF at both gene and protein levels. Funded by: NIH PO1 HL073907 and FAMRI.

16.0: LUNG DEVELOPMENT

16.1 CFTR-DEPENDENT LUNG ORGANOGENESIS: MOLECULAR MECHANISM

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There is growing evidence for the role of CFTR in lung development and differentiation. The mechanism by which the chloride channel could affect lung organogenesis, however, is unknown. In utero gene transfer that over expressed CFTR in the fetal rat lung results in the increased expression of a number of genes primarily associated with muscle structure and function. Histological and biochemical characterization of myosin heavy chain, heat shock protein 27, and isoforms of myosin light chain show that CFTR over expression has a profound effect on smooth muscle contraction-related proteins. These effects were shown to be related to chloride and extracellular ATP modulation by CFTR and dependent upon the PI3 Kinase and Phospholipase C pathways. Mechanical forces influence lung development through pulmonary distension. CFTR over expression in the fetal lung increases muscle contraction and accelerated functional maturation in the normal perinatal rat lung. In addition, in utero CFTR gene therapy reverses the functional lung phenotype of the CFTR knockout mouse. These data demonstrate that CFTR influences lung development by regulating the muscle contractions associated with cytoskeletal tension and stretch induced differentiation. The implications for the CF disease phenotype are significant. First, reduction of stretch induced differentiation would result in an immature lung phenotype similar to but more severe than that seen in extremely immature neonates. Recovery of normal lung phenotype post-natally would not be possible, because of changes in the lung environment from fluid to air driven stretch. Additionally, any gene that either positively or negatively modulates muscle contraction would act to affect the CF phenotype.

16.2

IN VITRO INCREASE ON TRACHEAL SMOOTH MUSCLE (TSM) CONTRACTILITY AFTER RECOVERY FROM MODERATE HYPEROXIA

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The aim of this work was to evaluate the long-term effects of neonatal moderate hyperoxia exposure on TSM contractility and airway architecture. 46 newborn Wistar rats were exposed during two weeks to 50%FiO₂. After that period 30 animals (age=15days) were euthanised with CO₂, the 16 remaining returned to ambient air during 21 days and were euthanised at their 36th day. The experimental groups (EG) were compared with age-matched controls (CG). *In vitro* isometric force of tracheal rings in response to methacholine (10^{-7M}-10^{-1M}) were measured and both sensitivity (EC_{50%}) and reactivity (F_{max}) were compared between EG and CG. Tracheal wall collagen and muscle of EGs (n=5, each) were qualitatively compared with CG (n=5, each) by optic microscopy. TSM area (TSMa) was also assessed (courtesy of Prof. J. Martin, McGill Univ, Canada). Only the EG returned to ambient air after hyperoxia had higher EC_{50%} and F_{max} to cholinergic stimulation than CG: EC_{50%} 5.7*10^{-3M} vs 1.8*10^{-3M}; F_{max} 4.36g±1.03g vs 3.04±1.05g, $p < 0.001$. There was an increase in collagen in the lamina propria and adventitia after 50%FiO₂ which didn't persist after recovery. There was also a trend to TSMa increase in both EGs: after 50%FiO₂ -7.34mm² vs 5.46mm² in CG and 6.65 mm² in the recovery group vs 4.81 mm² in CG. In conclusion, although changes in airway wall collagen were reversible, 50%FiO₂ induced an increase in TSMa and changes in contractility that were not present in the immediate post-exposure period. This chronic model can be used to study the molecular biology of TSM in response to hyperoxia as seen in bronchopulmonary dysplasia.

16.3

DEXAMETHASONE-MEDIATED REGULATION OF BETA-CAROTENE 15,15'-MONOOXYGENASE GENE EXPRESSION IN HUMAN PULMONARY EPITHELIAL CELLS

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Retinoids (vitamin A) play a critical role in pulmonary alveolar development and post-injury regeneration. Glucocorticoids (dexamethasone, DEX) also promote fetal lung maturation and injury recovery. However, vitamin A promotes but DEX inhibits alveolar growth and regeneration. β , β -carotene 15, 15'-monooxygenase 1 (CMO1), expressed in lung tissue, catalyzes the first step in vitamin A biosynthesis from provitamin A carotenoids. We hypothesized that glucocorticoid interacts with retinoid pathways at the level of pulmonary CMO1 gene regulation. We determined that CMO1 expression is developmentally regulated in mouse and human alveolar epithelium and is expressed in several pulmonary epithelial cell lines including A549. DEX treatment of A549 cells reduced CMO1 mRNA, protein and promoter activity. The decrease in transcriptional activity was mediated via PPAR γ and MEF2. DEX treatment significantly decreased PPAR γ binding to the CMO1 promoter. In contrast, DEX increased expression of PPAR α which, in turn, inhibited CMO1 transcription via suppression of PPAR γ /RXR α heterodimer formation. Conversely, PPAR α siRNA abolished the DEX-mediated inhibition of CMO1. DEX also suppressed activation of MAPK p38 and its downstream target, MEF2. DEX did not affect MEF2 isoform expression. These findings provide the first evidence that DEX inhibits CMO1 gene expression via multiple signaling pathways in pulmonary epithelial cells. These results suggest a mechanism by which DEX antagonize effects of retinoids

on alveolarization during development and in lung diseases and may lead to therapeutic targets for BPD and emphysema. (NIH HD42174, RR18728).

17.0: PULMONARY HYPERTENSION

17.1

PERINATAL HYPOXIA INDUCES LONG-TERM EFFECTS ON PULMONARY ARTERY SMOOTH MUSCLE CELLS POTASSIUM CHANNELS

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Adverse perinatal events, like perinatal hypoxia (PH), have been associated with adult diseases. Smooth muscle cells potassium channels (K⁺) play an important role in the regulation of pulmonary vasoreactivity in the fetus as well in the newborn and in the adult. In the fetus, calcium-activated potassium channels (KCa) are predominantly implicated and, from birth, voltage-dependent K⁺ (Kv) prevail in the adult. We postulated that PH could alter this maturation shift and modify pulmonary vasoreactivity in relation to K⁺ channels in adulthood. We developed an experimental model of PH in mice and evaluated the activity of Kv and KCa at the level of the main pulmonary artery by techniques of electrophysiology and pharmacology. In patch-clamp studies, charybdotoxin (CTX), a selective blocker of KCa, induced a greater inhibition of the outward current in adult pulmonary artery smooth muscle cells (PASMC) following PH as compared to controls (19.4±4.9% vs 0.5±3.9%, at 40mV, $p < 0.05$). Inhibition with 4-aminopyridine (4-AP), a selective blocker of Kv, was also more important following PH as compared to controls (30.9±2.1 vs 21.8±2.8%, at 40 mV, $p < 0.05$). In isolated pulmonary artery tension studies, relaxation induced by NO donors (SNAP, DEA/NO) in animals born under hypoxia was more inhibited by both CTX and 4-AP than in controls. These results suggest that PH induces long-term effects on PASMC K⁺ channels in the main pulmonary artery. We postulate that these perturbations could contribute to modifications of pulmonary vasoreactivity and predispose to pulmonary vascular pathologies in adulthood. (Financed by the Leenards foundation).

17.2

THE INTERLEUKIN 13 RECEPTOR SYSTEM: A NOVEL PATHOGENETIC MECHANISM IN PULMONARY ARTERIAL HYPERTENSION

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Interleukin (IL)-13 controls airway hyperresponsiveness and airway smooth muscle cell (SMC) hypertrophy, but its effect on pulmonary artery SMC has not been investigated thus far. Here, we examined the expression and activity of the IL-13 system in pulmonary arterial hypertension (PAH), including the receptor isoforms IL-4R, IL-13RA1, and IL-13RA2. Using quantitative RT-PCR, we detected increased expression of IL-13RA2 in lungs of PAH patients compared with controls (transplant donors). In contrast, no differences were observed in IL-13, IL-4R, or IL-13RA1 expression. Similar results were obtained in lungs of mice and rats subjected to hypoxia- or monocrotaline-induced PAH, respectively. Immunohistochemical and microdissection analysis demonstrated selective localization of IL-13RA2 to pASMC. Freshly isolated pASMC displayed a dose-dependent growth inhibition upon IL-13 treatment, without inducing apoptotic effects. The antiproliferative effect of IL-13 was due to G0/G1 cell cycle arrest and phosphorylation of STAT3 and STAT6, which was diminished by overexpression of IL-13RA2. In addition, pASMC subjected to hypoxia (24h) displayed a significant up-regulation of IL-13RA2 expression on mRNA and protein level. Our studies thus demonstrate that IL-13 is a potent antiproliferative factor in pASMC. Upregulation of the decoy receptor IL-13RA2 on pASMC in PAH or hypoxia may therefore lead to a loss of this antiproliferative effect and therefore enhanced pASMC proliferation during the pathogenesis of this disease. Funding: German Research Foundation.

18.0: GENERAL CATEGORY

18.1

PHARMACO-INTERACTOMICS OF CHAPERONE-MEDIATED RESCUE OF Δ F508 CFTR FROM ERAD

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Cystic Fibrosis (CF) is an autosomal recessive disorder caused by mutations in CFTR. The severe CFTR mutation, Δ F508, is retained in the ER and degraded by the ubiquitin-proteasome pathway. Mutant CFTR can be rescued by 4-phenylbutyrate (4PBA). We hypothesize that a subset of proteins binding to CFTR and HSC70 during restoration of Δ F508-CFTR trafficking, will correlate with wtCFTR expression. Total cellular lysates from IB3-1 CF bronchial epithelial cells (Δ F508/W1282X), non-CF (IB3-1 plus wtCFTR) and rescued CF (IB3-1 treated with 4PBA) were immunoprecipitated with anti-CFTR and anti-HSC70 antisera and resolved on 2-DE. Proteins were identified by PMF using MALDI-TOF, and tracked in ER, cytosol and plasma membrane (PM) fractions. Informative proteins belonged to the HSP70 family, and are known to assist mainly in ERQC and ERAD. Together with HSP70 and HSC70, ERAD associated chaperones (GRP94, 78, 75, 58 and HSP84) were modulated by 4PBA in ER, cytosol and PM. Several 4PBA regulated ER stress chaperones interacted with B form of CFTR (160 kDa) in the ER fraction, but not with folded C form of CFTR (180 kDa). GRP94, 58 and HSP84 were the only ER-resident proteins to be equally regulated by 4PBA and associated with HSC70. 4PBA mediated rescue of CFTR is associated with changes in the network of ER resident chaperones, and mimics patterns of interaction observed with wtCFTR expression. This work was supported by RO1 HL 59410 and NO1 BAA HL 02-04.

18.2

MICROARRAY ANALYSIS OF MDM4 FUNCTION IN A MESOTHELIAL CELL LINE USING SHRNA TECHNOLOGY

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The p53 tumor suppressor gene is the most frequently inactivated gene in human cancer. However, p53 mutations are rare in human mesothelioma patients, suggesting that aberrant p53 function may be due to alterations in its regulatory pathways. MDM4 has been shown to be a key

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regulator of p53 activity, both independently as well as in concert with its structural homolog, MDM2. MDM4 also maps to a chromosomal region (1q32.1) frequently altered in mesotheliomas. In this study, we used genomic technology to investigate the impact of the suppression of MDM4 mRNA and protein levels. Short hairpin RNA (shRNA) molecules were used to silence the MDM4 gene in a "normal" mesothelial (MeT5a) cell line. Pre-tested MDM4 shRNA vectors (Mission, Sigma-Aldrich) were packaged in a lentiviral vector and used to transduce human MeT5a cells. Cells were then selected for puromycin resistance and clones isolated. To account for off-target gene activity, two distinct MDM4 shRNA isoforms were used. Suppression of the MDM4 gene was confirmed by showing loss of the MDM4 protein using a western immunoblot. Global changes in gene expression of MDM4 suppressed cells compared to shRNA controls were analyzed by microarray before and after Ultraviolet (UV) exposure to address the role of MDM4 in exponentially growing and stressed cells. These results demonstrate the importance of MDM4 in cell cycle regulation as well as a possible role in the pathogenesis of human malignant mesotheliomas. This abstract was supported by Grant Number RR017670 (NCRR, NIH).

18.3

GM-CSF REGULATES MACROPHAGES AND INNATE IMMUNE RESPONSES IN TUBERCULOSIS

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Granulocyte macrophage colony stimulating factor is a critical homeostatic factor in the lung that is involved in the pathogenesis of tuberculosis but the mechanisms are incompletely understood. We demonstrate that attenuated and virulent mycobacteria establish similar infection rates in the lungs of WT, GM-CSF knockout (GMKO), and GMKO mice with high topical expression of GM-CSF in lung (GMOE), despite marked differences in macrophage phenotypes as evidenced by expression of ER-MP20 and the MARCO scavenger receptor. Both GM-CSF deficiency and unregulated GM-CSF expression in the lung altered granuloma formation. SP-A and SP-D decreased transiently following pulmonary mycobacterial infection. Peripheral macrophages from spleen and bone marrow of GMOE mice but not dendritic cells were hyporesponsive producing low levels of TNF and IL-12. The expression of MARCO in spleen was inversely correlated to the level of GM-CSF in the lung. We conclude that lung GM-CSF regulates granuloma formation by influencing alveolar epithelial cells and inter-organ communication of macrophages.

18.4

A COMPARATIVE PROTEOMIC ANALYSIS OF BRONCHOALVEOLAR LAVAGE FLUID IN RATS WITH AGING USING 2-DIGE AND MALDI-TOF/TOF

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There are many changes associated with normal aging of the lung that can play a role in the way the lung responds to environmental insults and contribute to the increased incidence of lung disease in the elderly. This study describes the quantitative comparison of the proteomic profiles of bronchoalveolar lavage (BAL) from F344 rats of different ages, including juvenile (1mo), adult (2mo), and aged rats (18mo), using 2-dimensional difference gel electrophoresis (2-DIGE) for protein quantitation and with matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry (MALDI-ToF/ToF) for protein identification. We detected and compared 427 proteins, of which 130 were subsequently analyzed by MALDI-ToF/ToF. Age group comparisons of BAL proteins showed significant changes in protein levels between juvenile and adult rats, juvenile and aged rats, and adult and aged rats for 80, 66, and 49 of the proteins, respectively. Of these changes, about twice as many increases were detected vs decreases. In each set of comparisons, about half of the significant changes were greater than 1-fold. Differences in protein expression covered many classes and functional categories of proteins. The results reported here could provide new insight into the basis for age-related differences in the incidence of various respiratory ailments and provide potential therapeutic targets for the treatment of lung diseases, especially those that affect the elderly.

18.5

GENE ALTERATION ANALYSIS OF ASBESTOS EXPOSED MURINE LUNGS

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Gene expression analysis in a mouse model was used to gain new insights into asbestos-related diseases (ARD) by identifying novel candidate genes involved in the asbestos response and grouping them into specific functional pathways for future analysis. Though the use and production of asbestos has decreased in recent years, ARD remain a significant problem due to both a worldwide distribution of the fibers and the long latency period before disease manifestation. While the effects of asbestos are not completely understood, recent studies in mice have shown that asbestos exposure induces a complex network of cytokines, growth factors, and receptors involved in inflammation, asbestosis and carcinogenesis. It has yet to be determined how genetic differences in these genes influence resistance or susceptibility to ARD and cancer in humans. The results from microarray analysis following a six-month asbestos exposure in C57Bl/6 mice identified previously unexplored expression alterations in several genes. The significance of gene expression changes in these critical pathways, including apoptotic, carcinogenic, fibrotic, and immune, was determined using the GoMiner program. Expression alterations of specific genes involved in these pathways were confirmed via semiquantitative RT-PCR of mRNA and immunohistochemistry detection of proteins on lung tissue sections. Overall, these results indicate that specific, novel genes are involved in the asbestos response and alterations in these genes may play a role in ARD susceptibility and resistance. This work was supported by RR017670 (NCRR/NIH).

18.6

THE ROLE OF NEUROFIBROMATOSIS TYPE 2 (NF2) IN THE DEVELOPMENT OF MALIGNANT MESOTHELIOMA.

Amy Erbe, Melisa B. Schelvan, Corbin Schwanke, Shane T. Heivly, and Mark Pershouse

Malignant mesotheliomas are uniformly fatal human tumors of the lung heavily linked to asbestos exposure. While much is known of the initial host response to asbestos inhalation, the molecular mechanisms that lead to the development of these malignant tumors is not well understood. The single most common genetic alteration in human mesothelioma is the inactivation NF2, which occurs in 80% of tumors. Thus, NF2 may play a central role in the

development of mesothelioma. The objective of this research is to analyze the downstream effects following the inactivation of NF2 in a normal mesothelial cell line (MeT5a) using small hairpin RNA (shRNA). Five different shRNA directed against NF2 (Mission, Sigma-Aldrich) were delivered to MeT5a cells using a lentiviral packaging system to reduce NF2 expression. After transduction, five single colonies surviving puromycin selection were chosen for each individual shRNA vector. Immunoblot analysis of NF2 expression was used to choose the best representative clones per shRNA treatment group for subsequent gene expression studies using a custom 10K human oligo array. By analyzing at least two different shRNA clones in gene expression studies against cells transduced with an shRNA control vector, off-target suppression was addressed. Genes significantly and reproducibly altered in expression relative to control cells will be presented. GOMiner and Pathways Architect analysis of these genes will also be described. A better understanding of the genetic mechanisms of tumor progression specifically resulting from inactivation of NF2 will be useful for future design of novel therapeutics, early detection of neoplasms, and better staging of disease.

19.0: ACUTE LUNG INJURY AND INFLAMMATION

19.4

PROTEOMIC ANALYSIS OF PLASMA IN ARDS

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Studies of individual biological markers in small single center studies of patients with ARDS have revealed major alterations in the cytokine, inflammatory and coagulation cascades both in the lung and systemically. Despite the wealth of information gained from these biomarker studies, no single biomarker can unequivocally diagnose ARDS or differentiate survivors from non-survivors. Because of the complexity of ARDS, new approaches and methods are needed that can address the clinical pathophysiology of ARDS at a more comprehensive level. The development of plasma protein profiles that can diagnose ARDS and predict outcome has the potential to be a powerful tool both for clinical use, facilitating application of appropriate therapies and stratification in clinical trials and to improve our understanding of the pathogenesis of clinical ARDS. Findings from a multi-disciplinary clinical proteomics approach to develop biomarker panels for diagnosis and prognosis in ARDS will be presented. Funded by NIH HL U01081332 REFERENCES: Tzouveleki A et al. Serum biomarkers in acute respiratory distress syndrome an ailing prognosticator. *Respir Res.* 2005;6:62. A current review of serum and plasma biomarkers in ARDS Uchida T et al. Receptor for advanced glycation end-products is a marker of type I cell injury in acute lung injury. *Am J Respir Crit Care Med* 2006;173:1008. Study of a new marker of alveolar epithelial injury in ARDS. Ware LB et al. Significance of von Willebrand factor in septic and non-septic patients with acute lung injury. *Am J Respir Crit Care Med* 2004;170:766. Large multicenter study of a marker of endothelial injury in ARDS.

19.6

USING GENETIC VARIATION IN THE HUMAN TOLL-LIKE RECEPTOR PATHWAY TO UNDERSTAND THE ROLE OF INNATE IMMUNE INFLAMMATION IN CRITICAL ILLNESS.

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The toll-like receptor (TLR) pathway is critical for effective innate immune responses. Genetic variation in genes of the TLR pathway can influence inter-individual variability in innate immune responses and could alter susceptibility to poor outcomes in sepsis or septic shock and related organ failure. We have comprehensively tested for associations between single nucleotide polymorphisms (SNPs) in genes of the TLR pathway and responses to TLR agonists in peripheral leukocytes from healthy volunteers *ex vivo*. We genotyped tag SNPs from 45 genes in the TLR pathway and measured responses to a panel of TLR agonists in an *ex vivo* whole blood assay. We found an association between 20 SNPs from 14 genes and TLR-mediated responses, including a novel association with SNPs in the TLR1 gene. Using a cohort of critically ill patients with sepsis and septic shock, we found these same TLR1 SNPs to be associated with increased mortality, greater organ failure, and higher prevalence of infection with gram-positive organisms. These data illustrate that an understanding of the genetic control of inter-individual variation in TLR-mediated innate immune responses in the normal population can lead to new insights on sepsis, septic shock and related organ failure. The potential applications of this approach will be discussed.

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