Interstitial Pulmonary Fibrosis (IPF)

- Consists of-
  - Fibroblast stimulation
  - ECM deposition
  - Abnormalities in gas exchange
  - Progressive respiratory failure

- Our focus:
  - Cytokine growth factors involved in the development and progression of this injury
    - Tumor Necrosis Factor-α (TNF-α)
    - Transforming Growth Factor-β (TGF-β)
CHRYSOTILE ASBESTOS FIBER BUNDLE
Pseudocolor image of bifurcation after asbestos exposure; BrdU staining

labelled cell

unlabelled cell
3. Immunohistochemistry: PDGF-B

**A.** Unexposed control, 48 hrs.; **B.** PDGF-B, asbestos, 24 hrs. hrs.; the insert is high magnification of the section in the box; **C.** PDGF-B, asbestos, 48 hrs. **D.** The high magnification of the box in C.; positive for epithelial (arrowheads), interstitial cells (arrow) and macrophage (arrow). TB=Terminal Bronchiole, AD=Alveolar Duct; Bar=20 μm
Asbestos → PDGF-A → PDGF-B → PDGFRα → Mesenchymal Cell Proliferation
TNF-α Receptor Knockout Mice

- Have both the 55 and 75 KDa receptors for TNF-α knocked out
- Resistant to the fibrotic effects of bleomycin, silica and asbestos
TNF-α Receptor Knock-out Mice

BrdU-Labeling Index in Epithelial and Interstitial Cells of Bronchiolar-Alveolar Duct Bifurcations and Terminal Bronchioles Post exposure to Asbestos

Percentage of Positive BrdU cells

5 Hrs. | 48 Hrs. | 2 wks.

Time
SUSCEPTIBILITY TO ASBESTOS-INDUCED AND TGFβ-INDUCED FIBROPROLIFERATIVE LUNG DISEASE IN TWO STRAINS OF MICE

FROM THE LUNG BIOLOGY PROGRAM
TULANE UNIVERSITY HEALTH SCIENCES CENTER
NEW ORLEANS, LOUISIANA

Arnold R. Brody, Saku Warshamana, Patricia Sime, Derek Pociask
A single five hour exposure to chrysotile asbestos fibers induces fibroproliferative lesions at the bronchiolar-alveolar duct bifurcations. C57Bl/6 mice have more severe and extensive lesions than 129 strain mice despite identical deposition patterns and concentration of deposited fibers. These mouse strains were crossed to produce an F1 generation which was then backcrossed to the inbred founder strains. Results of these experiments are summarized in the following slides.
Histopathology scores of C57BL/6 and 129 Intercross and Backcross hybrids: 48 hrs post exposure to asbestos

- 18 unexposed
  - 0.36 ± 0.13
- 129
  - 1.8 ± 0.3
  - 2.7 ± 0.1
  - P = 0.003
- C57BL/6
  - 1.6 ± 0.6
  - 2.4 ± 0.1
  - P = 0.0009
- F1(C57x129)
- F1 x 129
- F1 x C57BL/6
CONTROL OF AIRWAY INFLAMMATION WITH AN ADENOVIRUS VECTOR TRANS DU CING EXPRESSION OF TGFβ

Arnold R. Brody, Saku Warshamana and Derek Pociask
Lung Biology Program
Tulane University College of Medicine, New Orleans LA
It had been determined in earlier studies that $10^6$ pfu of an adenovirus vector transducing active TGFβ1 was a “no-effect” level in the C57 and 129 strain mice. $5 \times 10^7$ causes minimal disease, and $10^8$ or $10^9$ induce a progressively worse diffuse fibroproliferative process. The two mouse strains were exposed to the three highest concentrations and studied at various times post-exposure to determine if there were differences in the severity or timing of disease development.
Construction of recombinant adenovirus AdTGFβ1^{223/225}

- hCMV
- TGFβ1^{223/225} cDNA
- poly A
- plasmid pACCMMV

3.7 μm

adeno derivative

AdTGFβ1^{223/225}

NH₂

COOH COOH

LAP

COOH COOH

Cys → Ser

active protein TGFβ1^{223/225}
B-galactosidase gene transfer to the lungs of C57BL/6 mice: day 4

Bar = 50µm
Histopathology scores of C57BL/6 and 129 mice post exposure to AVTGFβ1

Days post instillation of 10^8 (or 5 x 10^7) pfu of AVTGFβ1
INDUCTION OF FIBROSIS BY ADENOVIRUS-MEDIATED EXPRESSION OF ACTIVE TGF-β1 IN TNF-α RECEPTOR KNOCKOUT MICE (TNF-αRKO)

Lung Biology Program, Department of Pathology
Tulane University Medical Center
New Orleans, LA 70112, USA
J-Y Liu, G. S. Warshamana, D. A. Pociask,
T-J Wu, Shang-yi Tsai and Arnold R. Brody
C57bl/6 Histopathology Scores

Exposure Group

Histopathology Scores

- LTGF-β Vector
- Asbestos
- Asbestos + Vector Control
- Asbestos + LTGF-β
ACTIVATION OF LATENT TGFβ BY ASBESTOS-DERIVED OXYGEN RADICALS

DEREK A. POCIASK AND ARNOLD R. BRODY
**In-vitro Activation of Latent TGF-β₁ by Iron Mediated Reactive Oxygen Species**

**A**

![Graph A](image)

- **lat+Fe**
- **lat +asbFe+AA**
- **Lat+FeCl₃+AA**

**B**

![Graph B](image)

- Asbestos
- Asbestos +ascorbate
- Asbestos +citrate
- Asbestos +citrate +Ascorbate

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*ng/ml active TGF-β₁*
Activation of latent porcine TGF-β₁ by asbestos exposure in the human epithelial cell line A549.
Antioxidants (AO) prevent activation of TGF-β₁.
Cell death does not increase TGF-β₁ activation.
Deferoxamine treatment decreases activation of TGF-β₁.
Activation of TGF-β₁ through asbestos mediated ROS is biologically significant in mink lung cells as measured by 3H thymidine incorporation (A,B) and PAI-luciferase induction (C,D).
Conclusions

- TGF-β can be activated through iron catalyzed oxidative mechanisms
- TGF-β can be activated in cell culture in the presence of asbestos
  - increasing asbestos concentrations show increasing amounts of active TGF-β
- The use of SOD and catalase decrease the activation of TGF-β in cell culture
  - Increasing the units of AOS decrease the activation of TGF-β
  - This protection does not appear to be linked to cell survivability
- Iron from asbestos appears to be a key element in the activation of TGF-β1 by ROS.
TNF-α induces expression of TGF-β₁ mRNA and protein in Swiss 3T3 fibroblasts in a dose and time dependent manner
TNF-α and Chrysotile Asbestos Induce the Expression of TGF-β₁ mRNA in A549 Lung Epithelial Cells
## Lung Biology Personnel

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