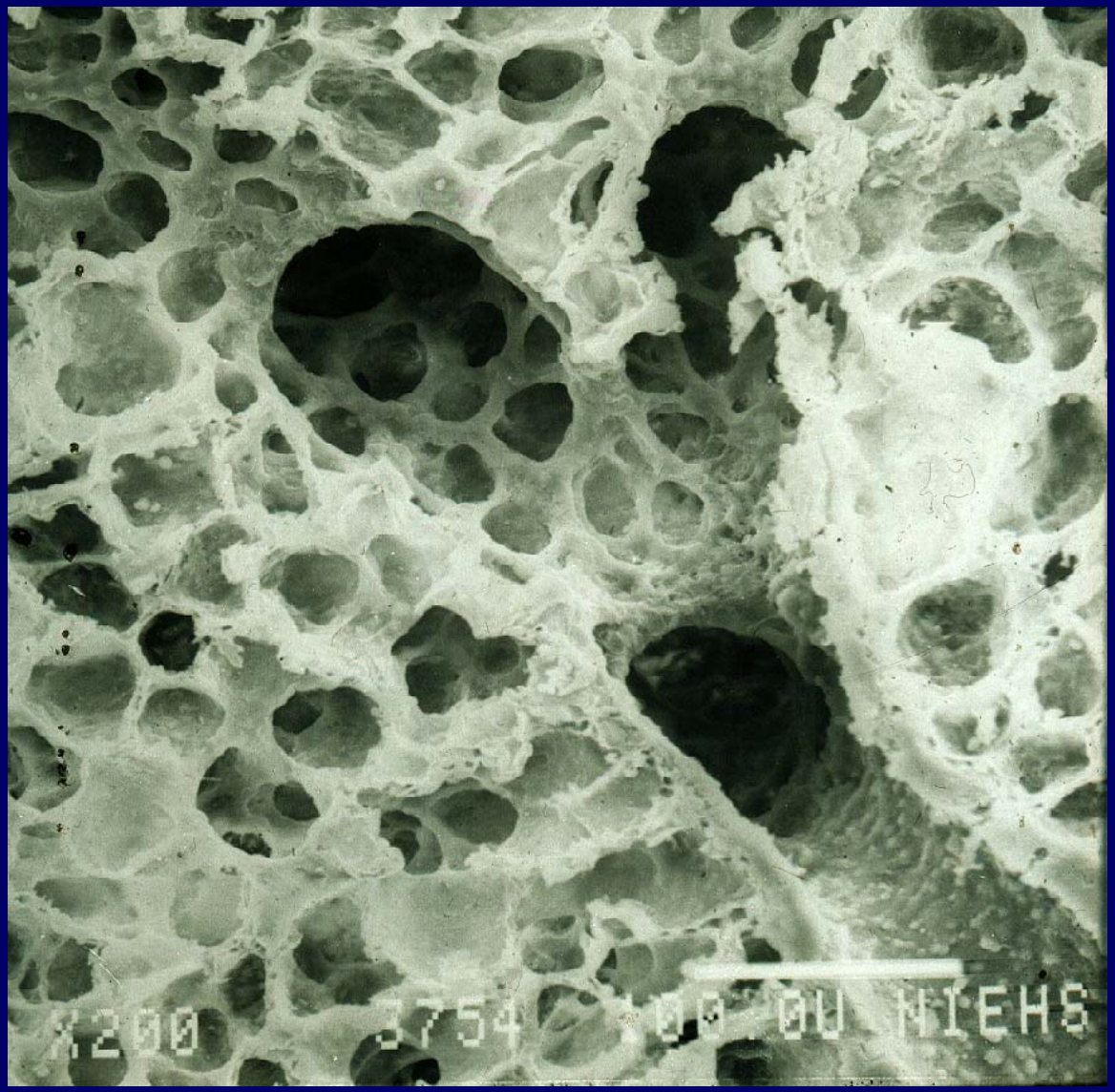
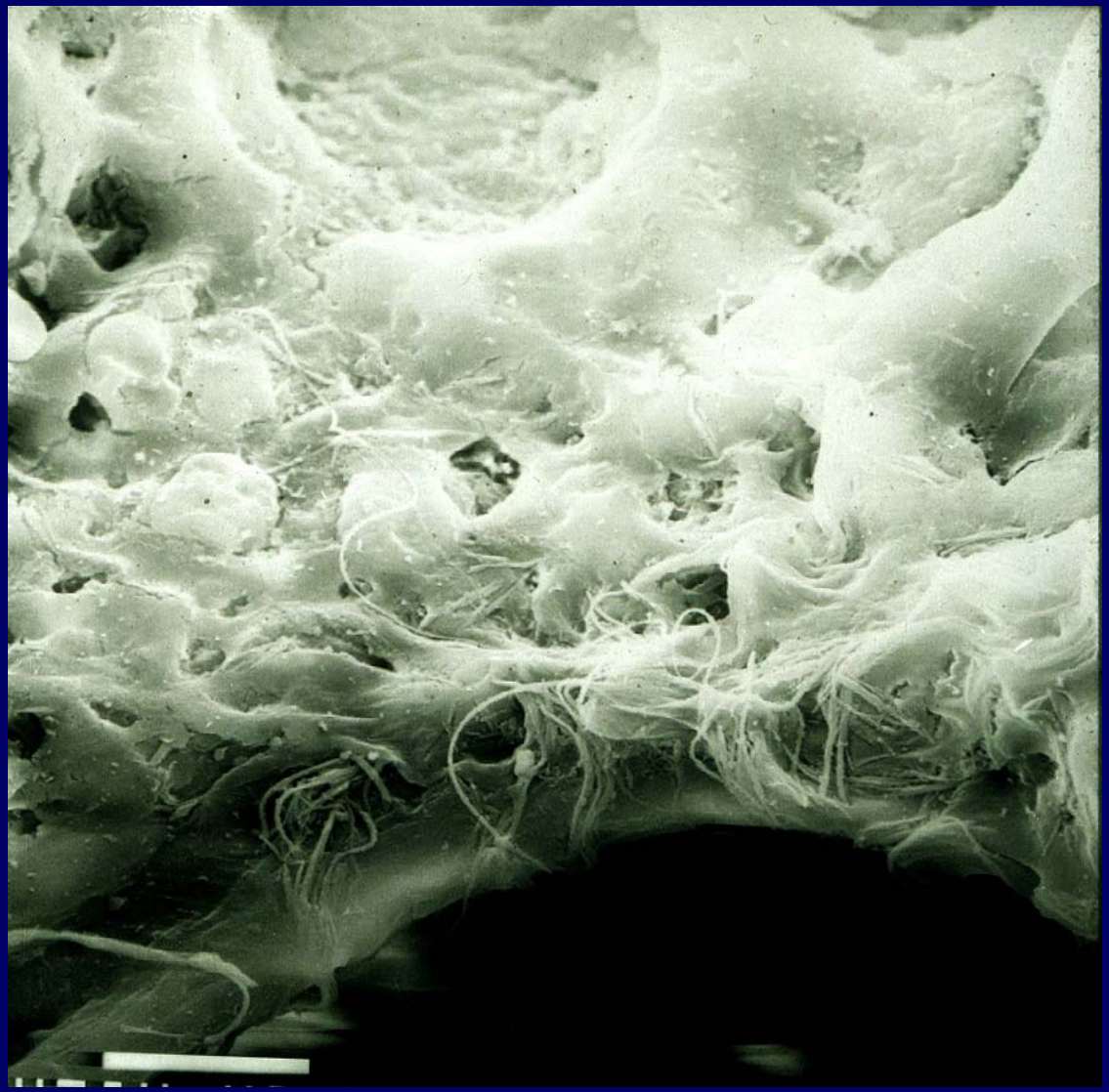
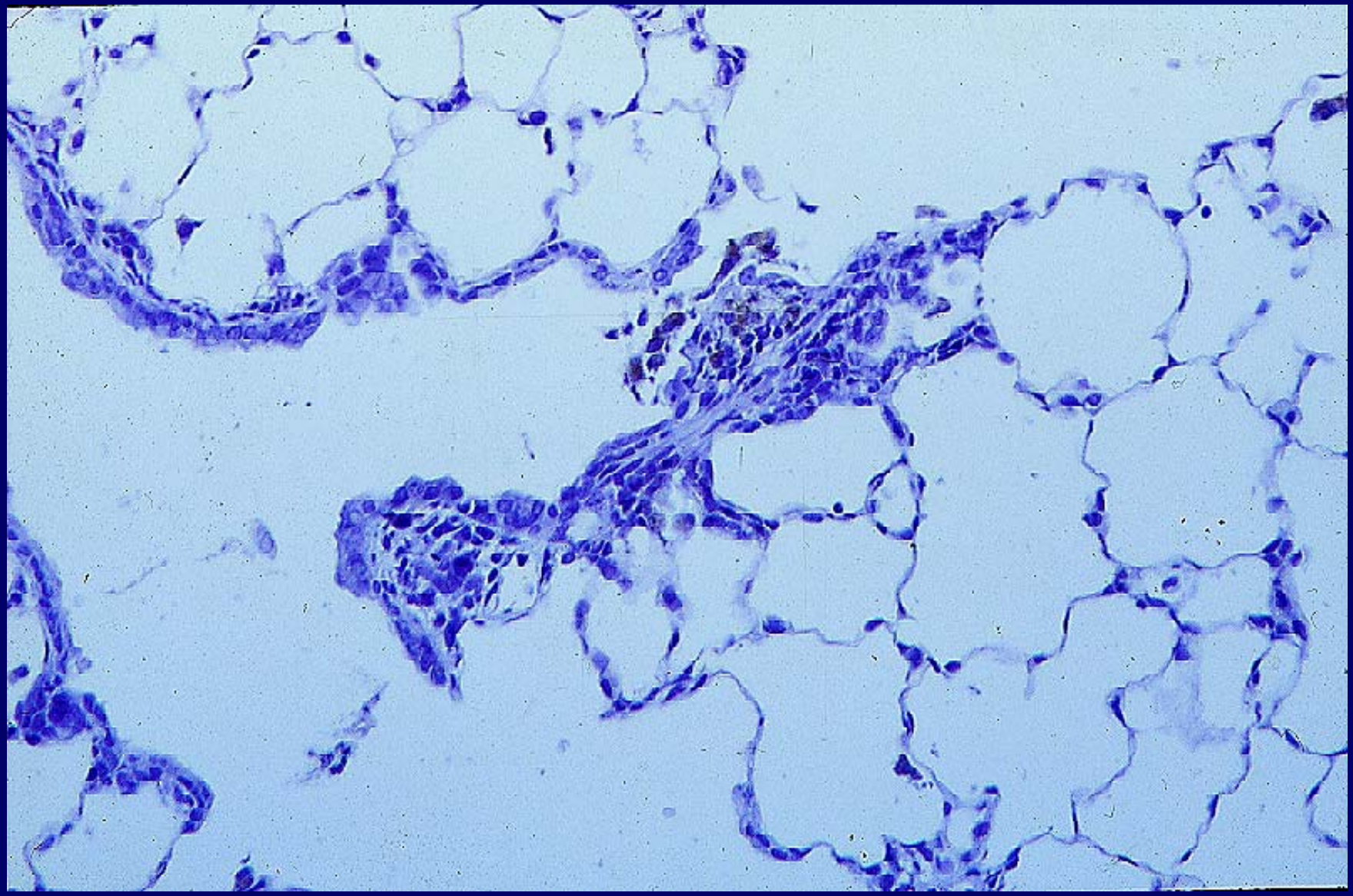
Interstitial Pulmonary Fibrosis (IPF)

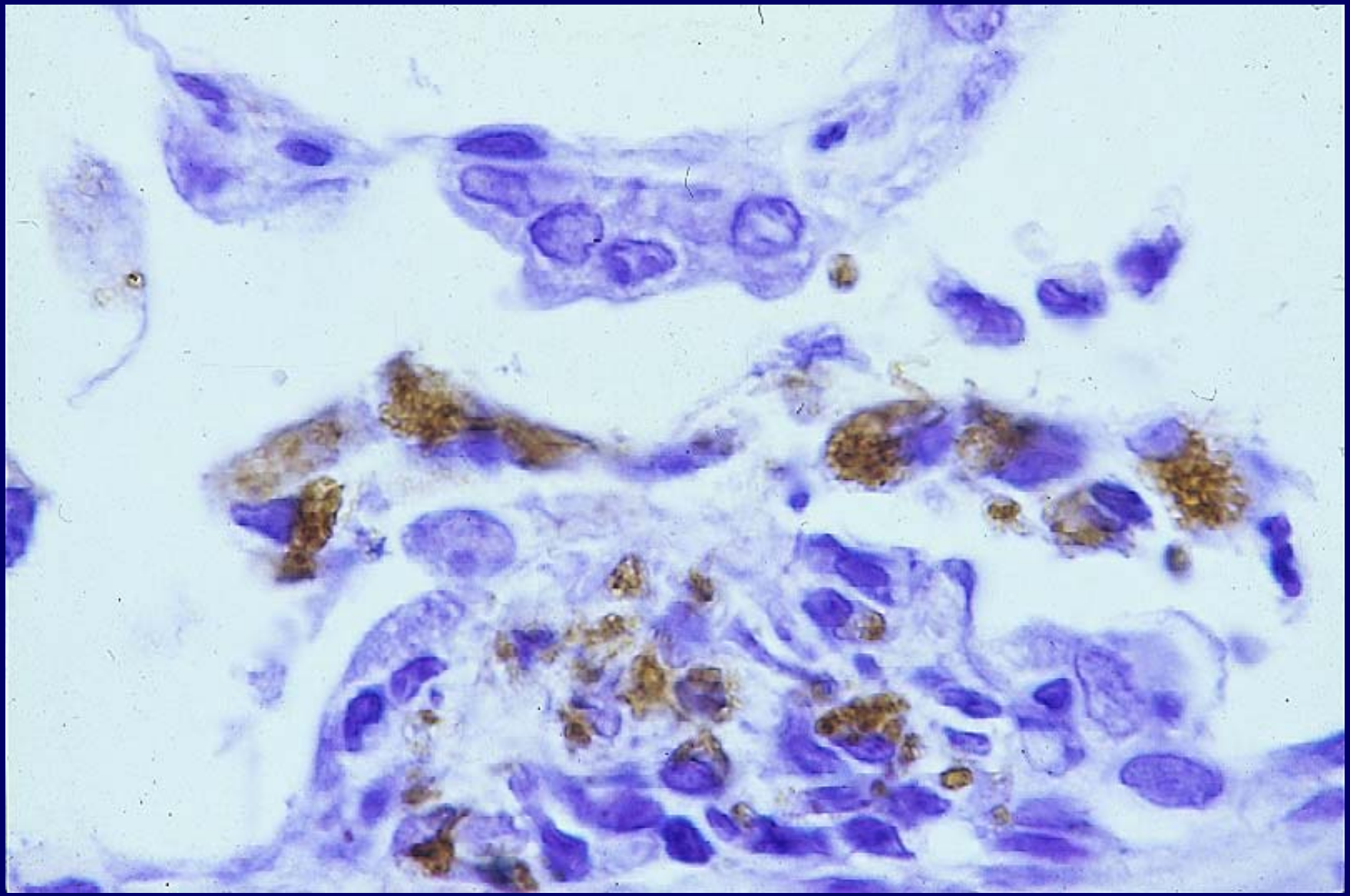
- Consists of-
  - Fibroblast stimulation
  - ECM deposition
  - Abnormalities in gas exchange
  - Progressive respiratory failure
- Our focus:
  - Cytokine growth factors involved in the development and progression of this injury
    - Tumor Necrosis Factor-α (TNF-α)
    - Transforming Growth Factor-β (TGF-β)

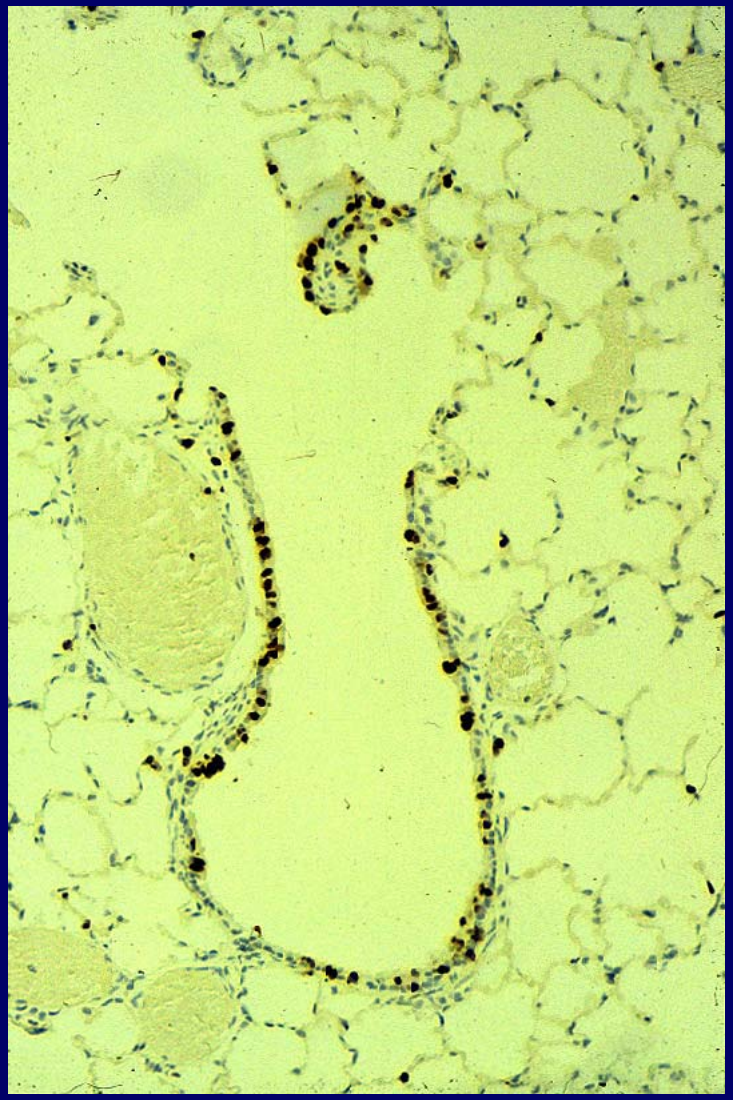
CHRYSOTILE ASBESTOS FIBER BUNDLE

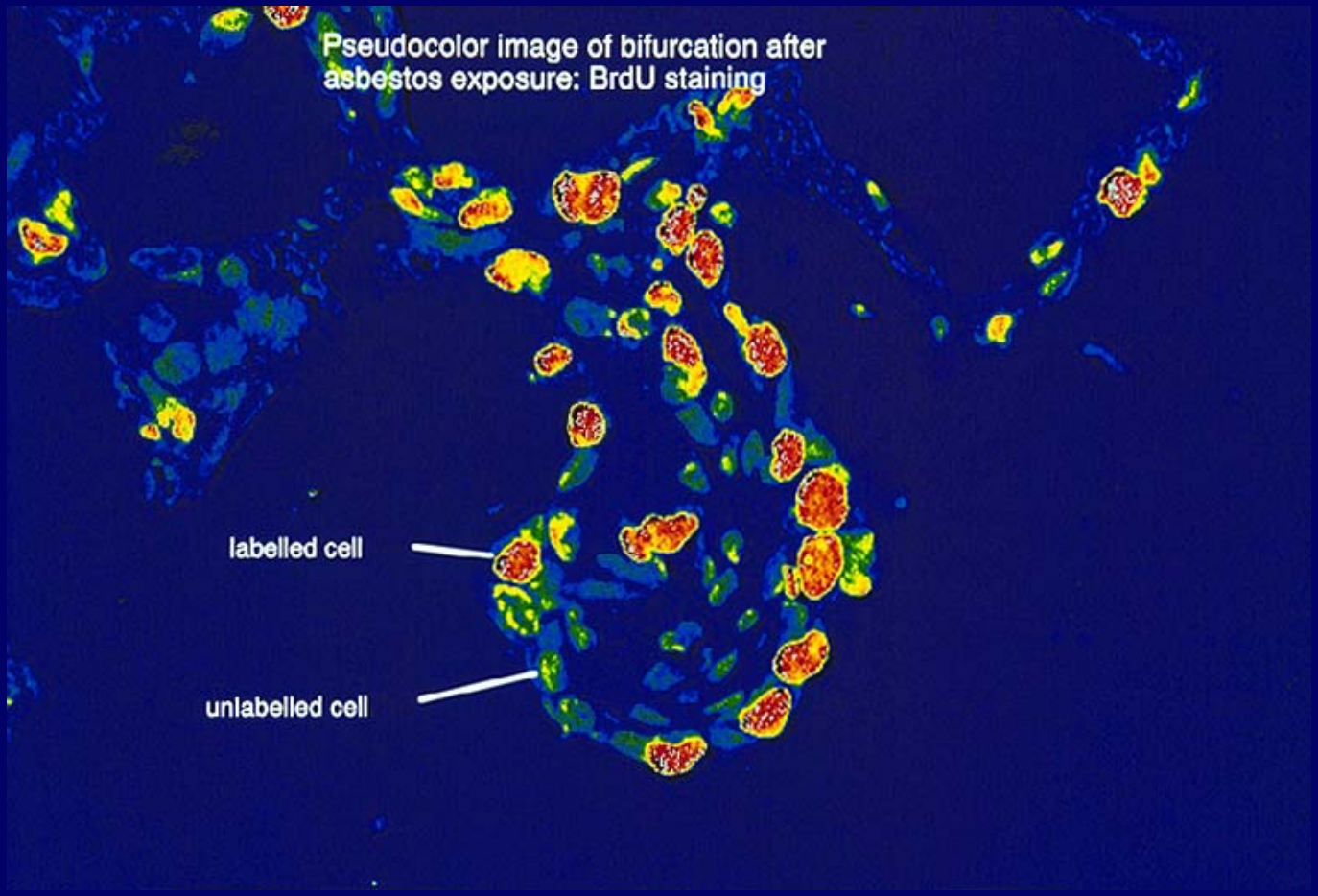

X200 3754 00 0U NIEHS







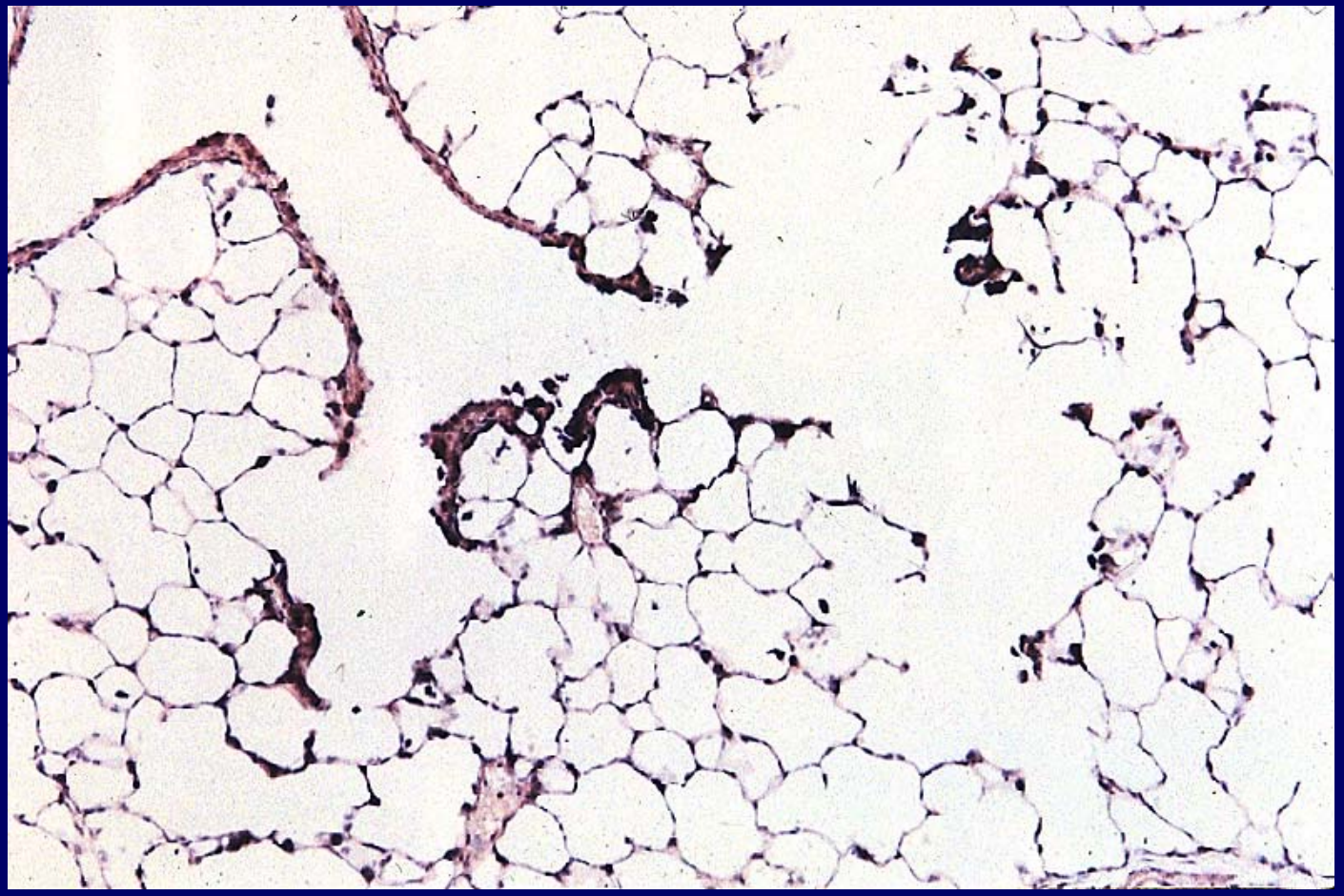


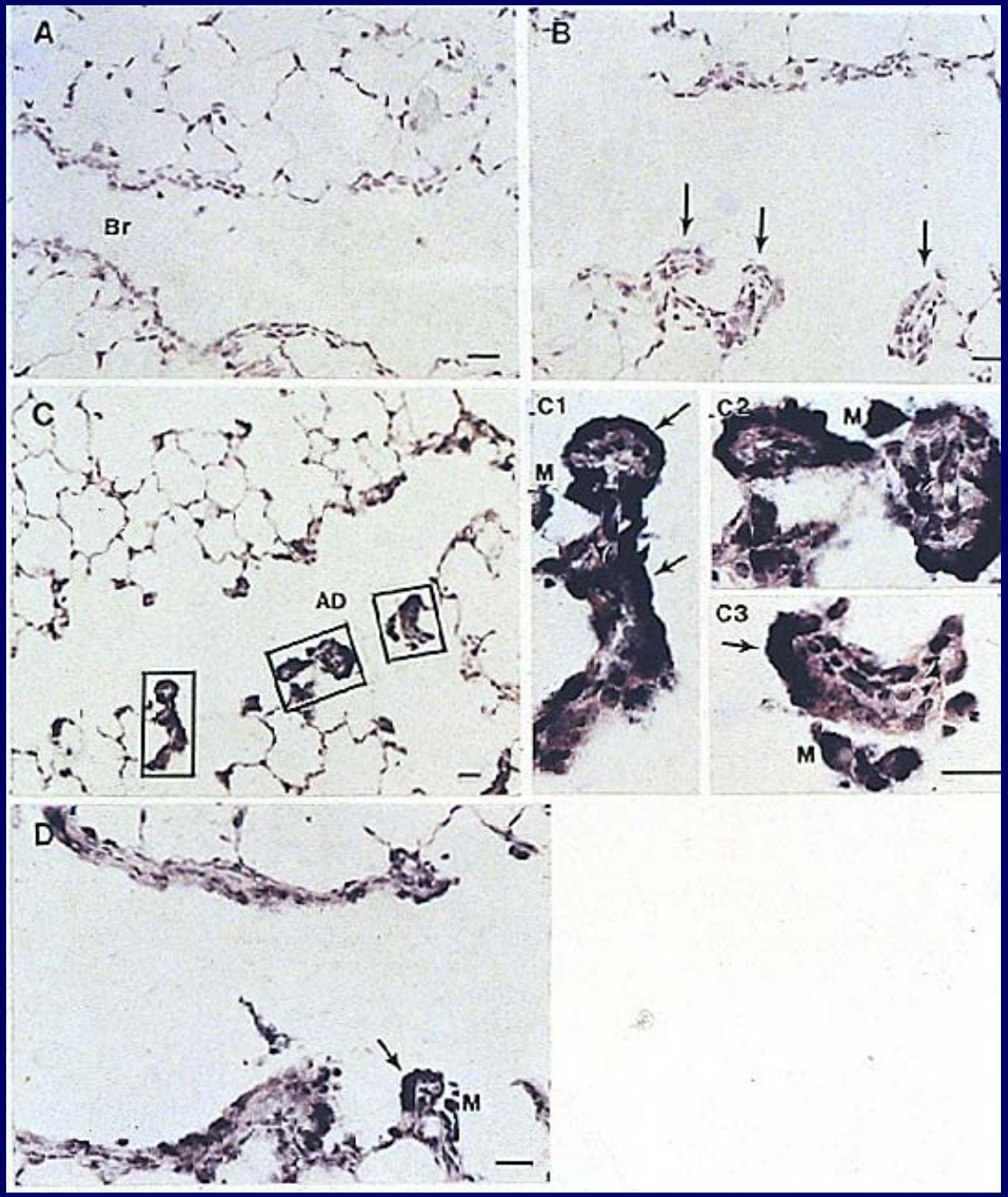

Pseudocolor image of bifurcation after asbestos exposure: BrdU staining
labelled cell
unlabelled cell

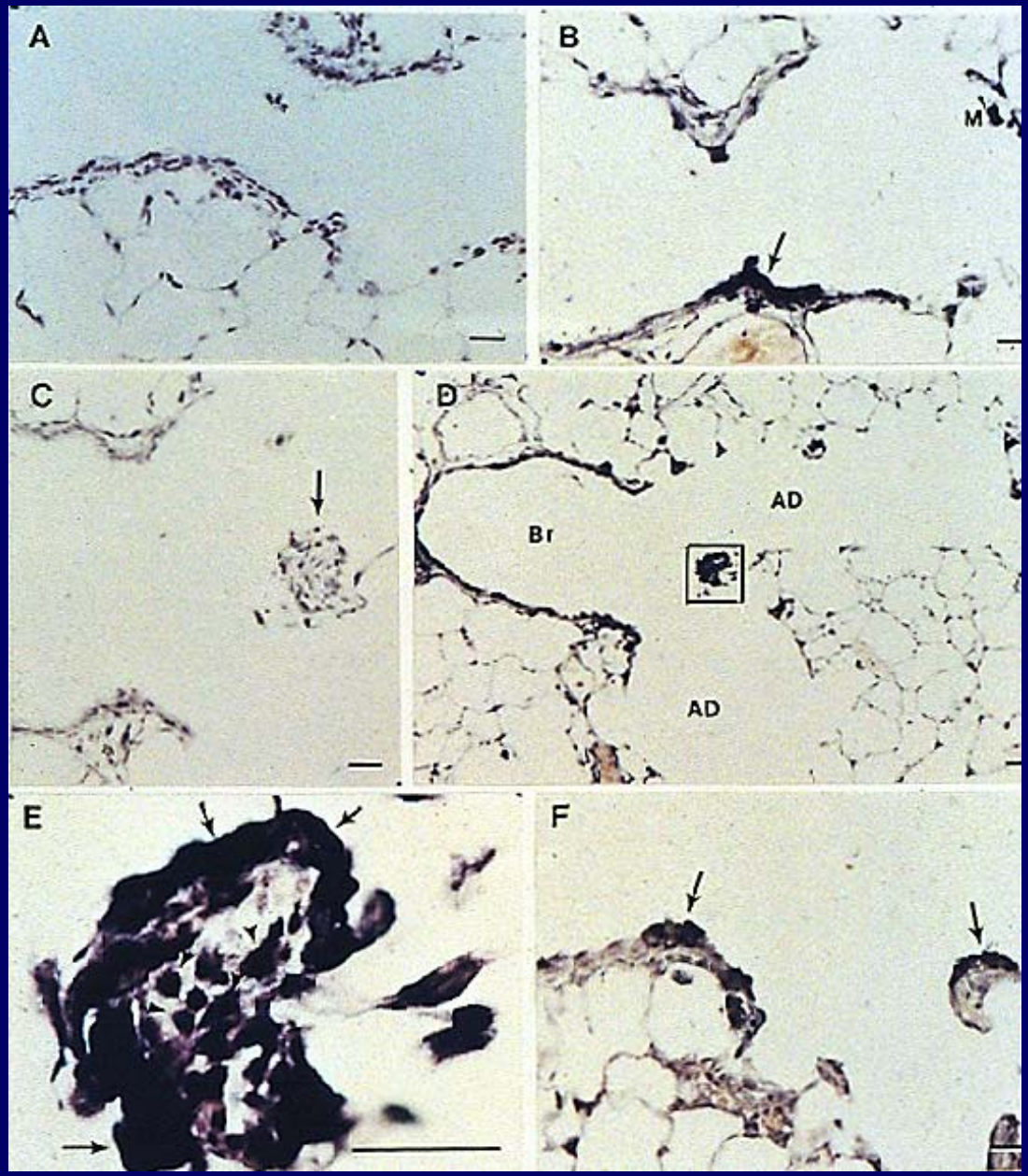
Cytokine Biology...

Human: TGF-β1, TGF-β1.2, TGF-β2, TGF-β3, TGF-β5, LAP, TGF-α, TGF-β sRII, sTNF RI, sTNF RII, TNF-α, TNF-β, PDGF, PDGF-AA, PDGF-BB, PDGF-AB, HGF, VEGF, β-NGF, EGF, IGF-I, IGF-II, HB-EGF, FGFa, FGFb, FGF-4, FGF-5, FGF-6, KGF, SLPI, PTN, IFN-γ, β-ECGF, ANG, OSM, PD-ECGF, ENA-78, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-6 sR, IL-1ra, gp130, IL-4 sR, IL-1 sRII, IL-5 sR, M-CSF, GM-CSF, G-CSF, EPO, LIF, SCF, MIP-1α, MIP-1β, GROα, GROβ, GROγ, MCP-1, RANTES

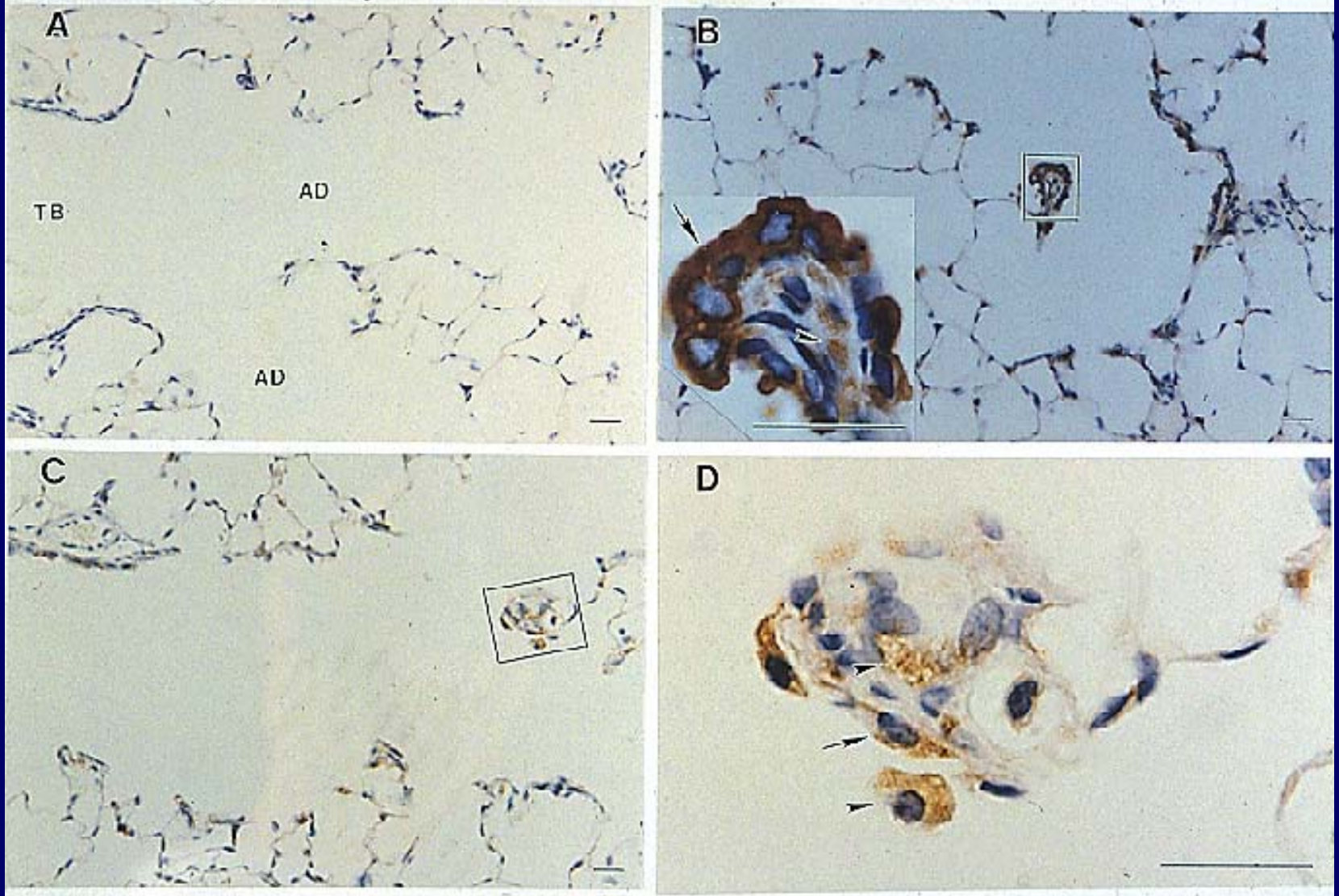
Murine: IL-1α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-13, MIP-1α, MIP-1β, GM-CSF, LIF, TNF-α, SCF



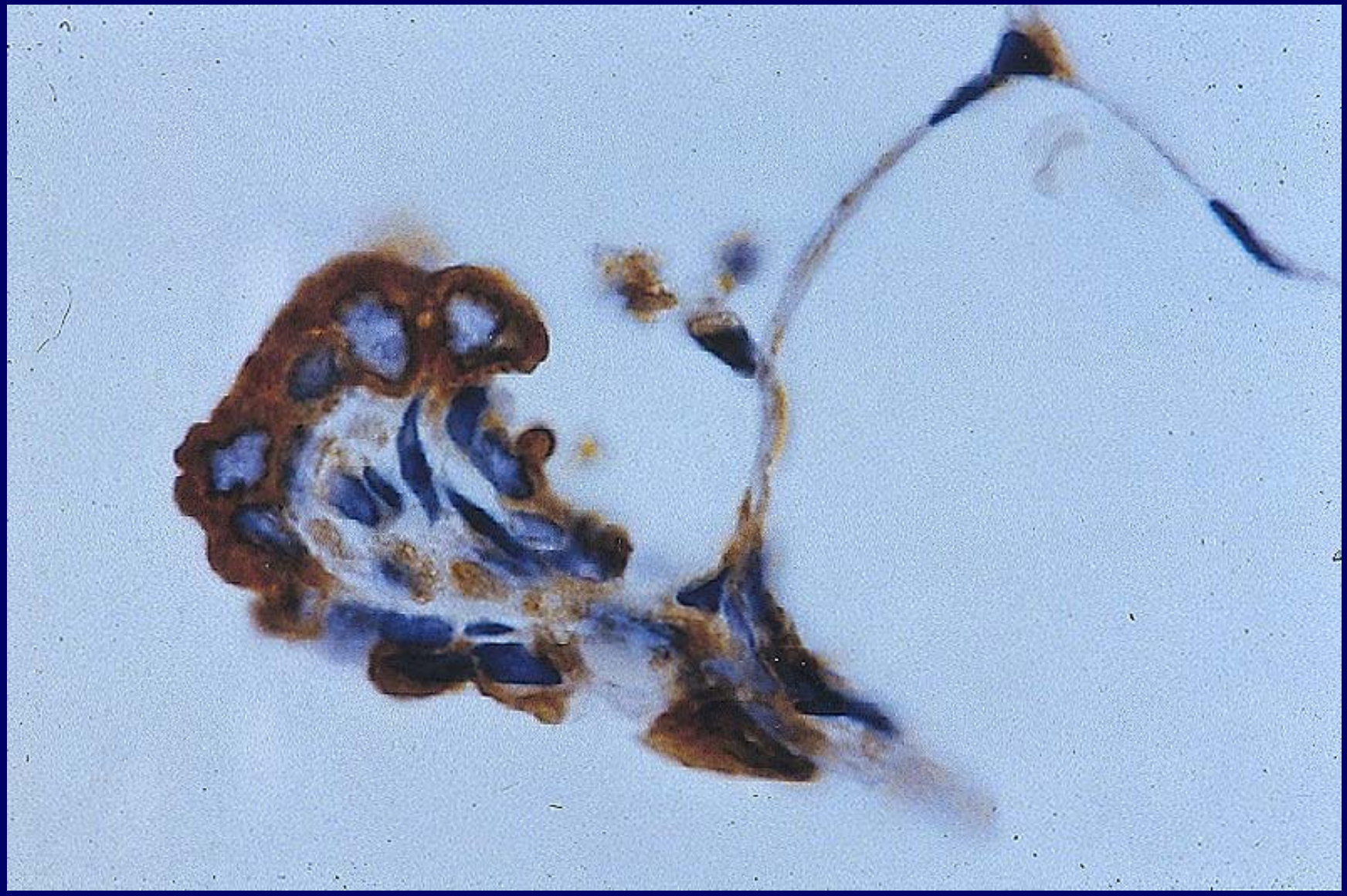

A
Br
B
C
AD
C1
M
C2
M
C3
M
D
M

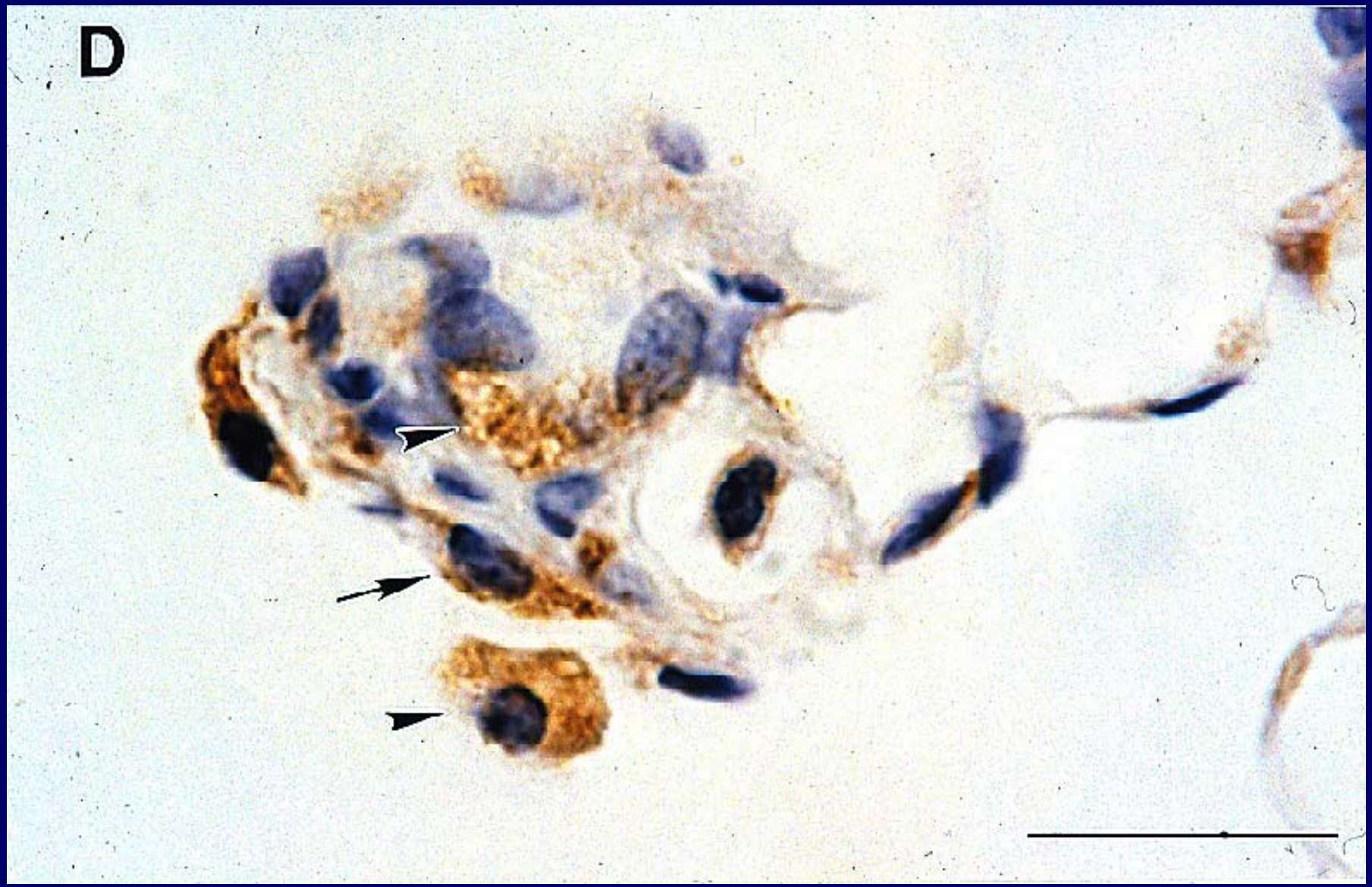

A
B
M
C
D
AD
Br
AD
E
F

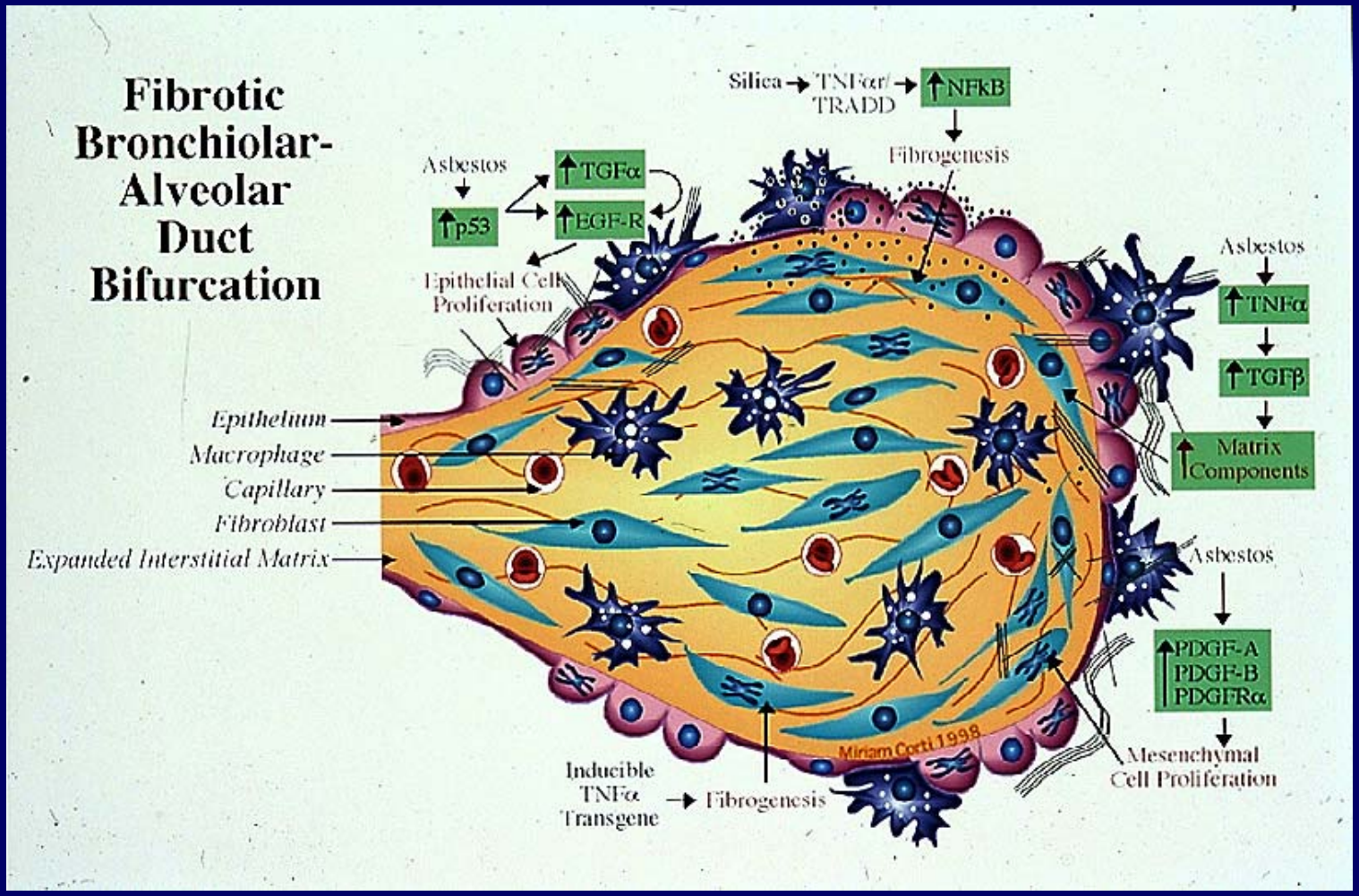
3. *Immunohistochemistry:* PDGF-B

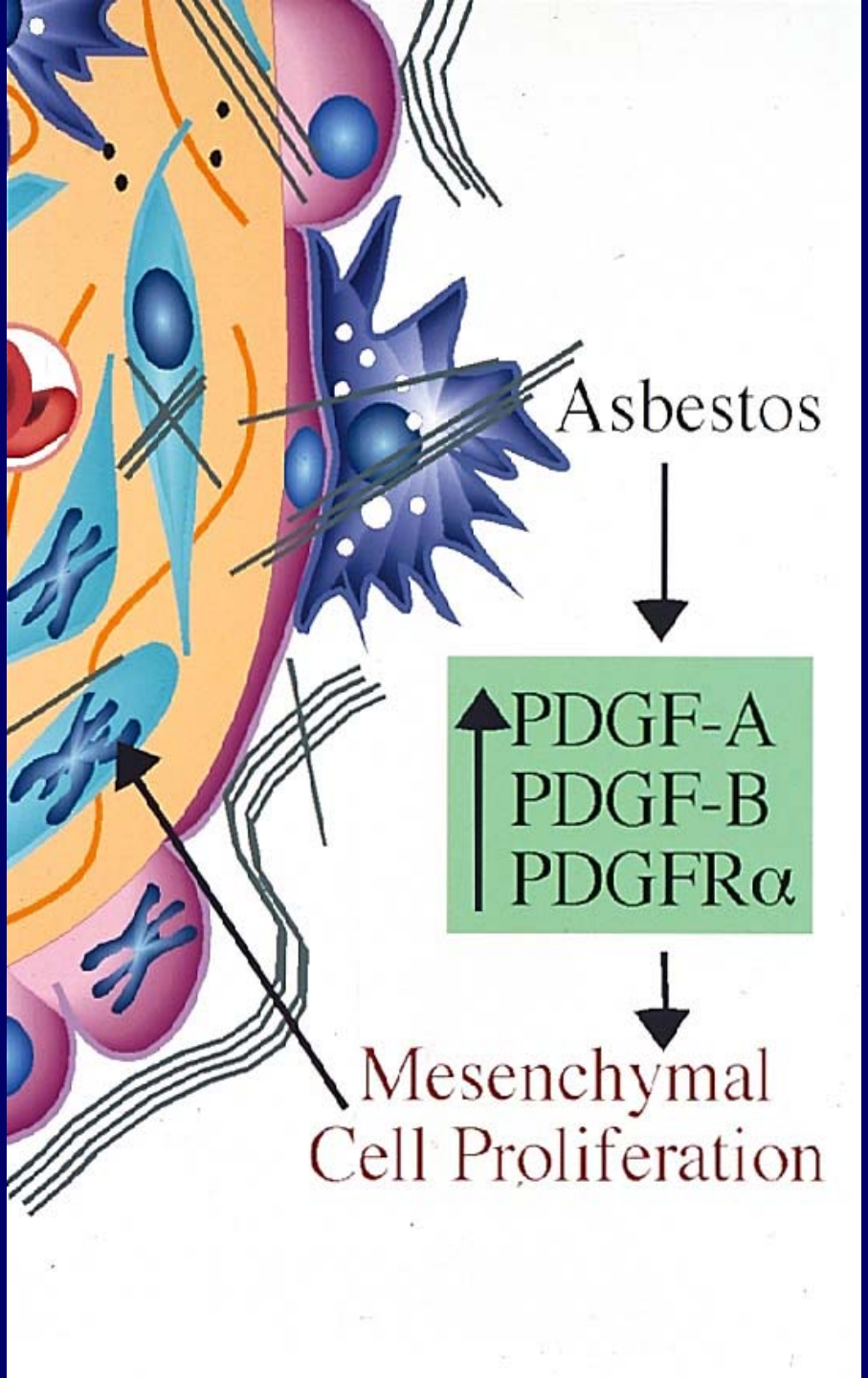


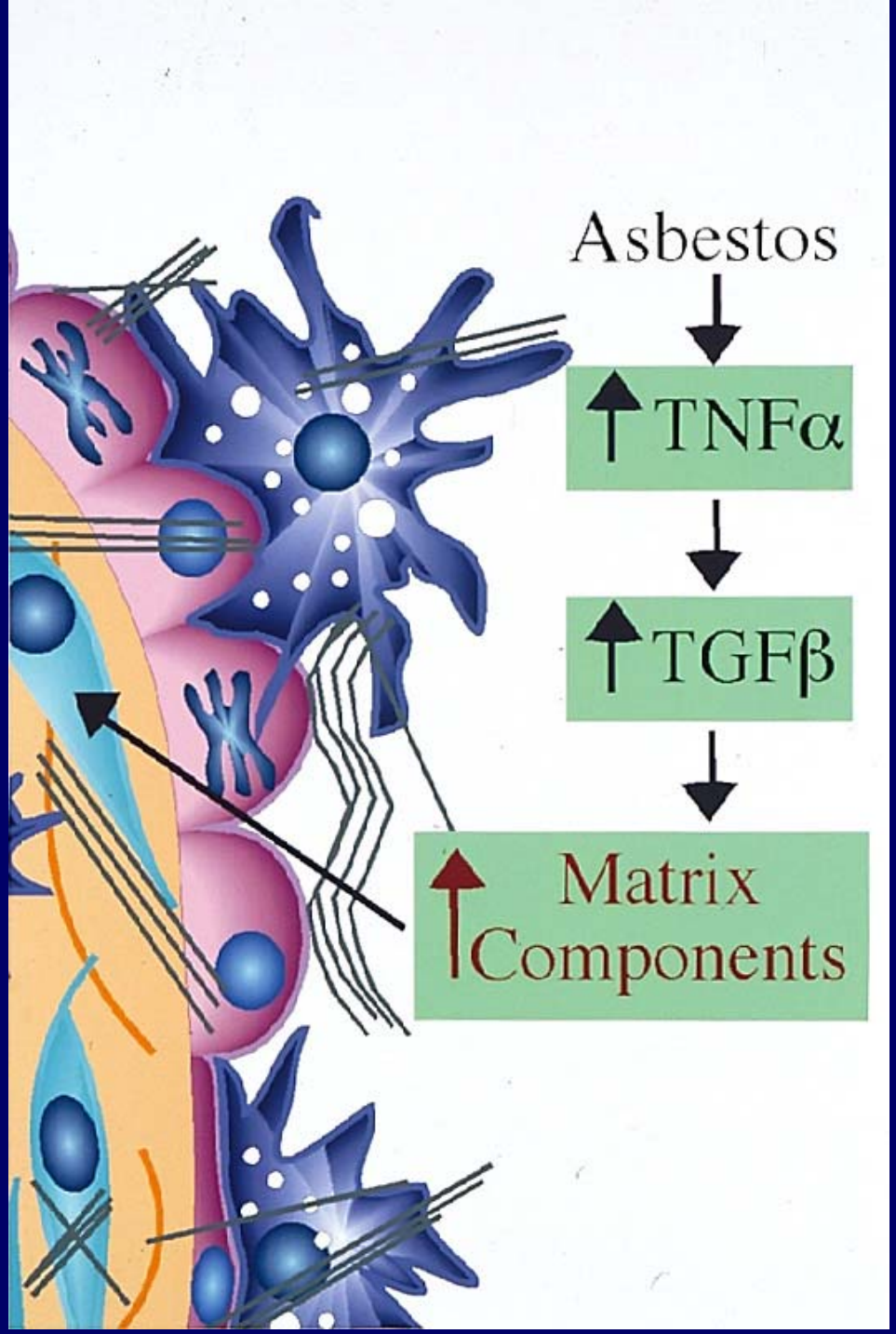
A. Unexposed control, 48 hrs.; B. PDGF-B, asbestos, 24 hrs. hrs.; the insert is high magnification of the section in the box; C. PDGF-B, asbestos, 48 hrs., D. The high magnification of the box in C.; positive for epithelial (arrowheads), interstitial cells (arrow) and macrophage (arrow). TB=Terminal Bronchiole, AD=Alveolar Duct; Bar=20 μm




D

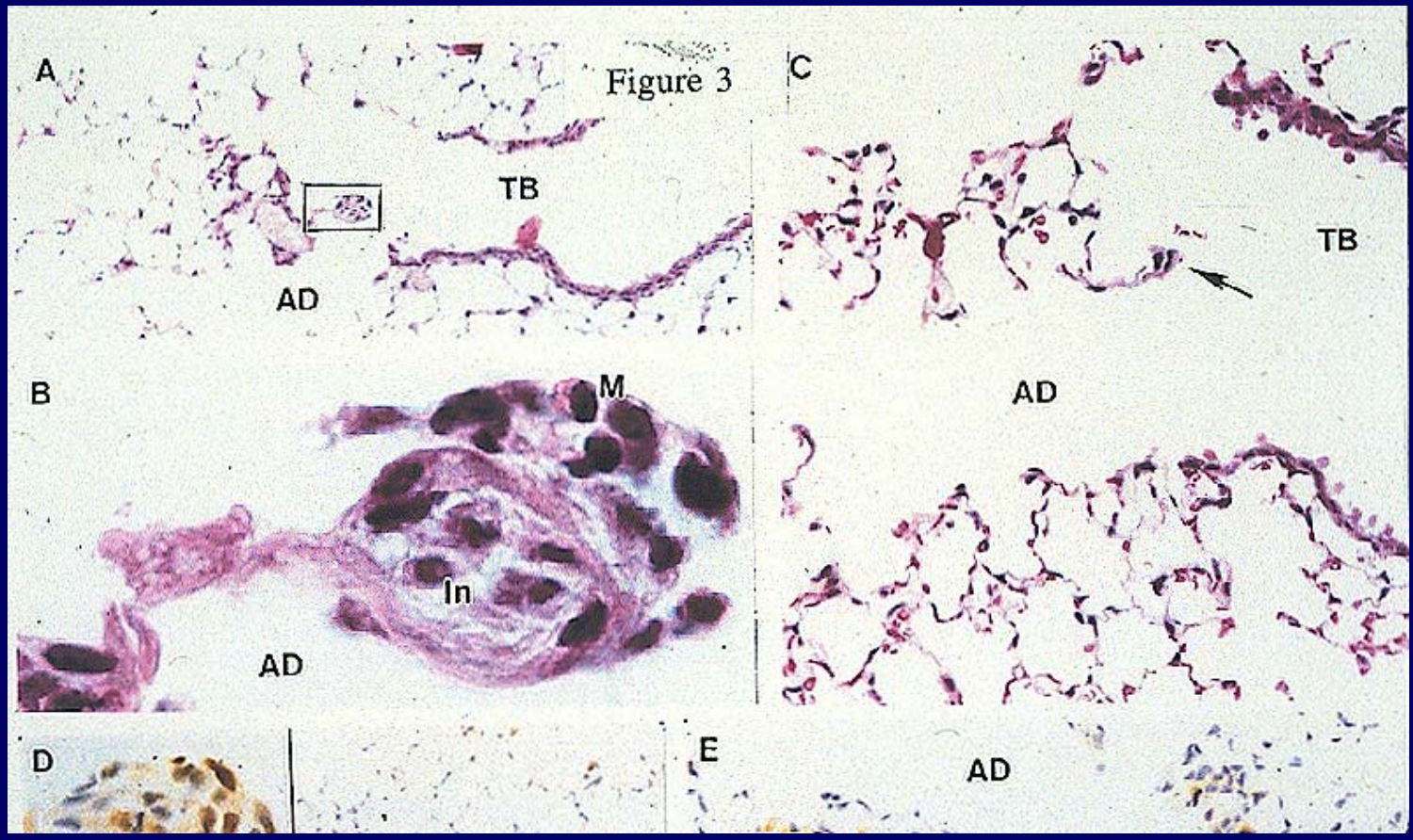

Fibrotic Bronchiolar-Alveolar Duct Bifurcation
Silica
TNFαr/ TRADD
↑NFkB
Fibrogenesis
Asbestos
↑TGFα
↑p53
↑EGF-R
Epithelial Cell Proliferation
Asbestos
↑TNFα
↑TGFβ
↑Matrix Components
Epithelium
Macrophage
Capillary
Fibroblast
Expanded Interstitial Matrix
Asbestos
↑PDGF-A
PDGF-B
PDGFRα
Mesenchymal Cell Proliferation
Inducible TNFα Transgene
Fibrogenesis
Miriam Corti 1998


Asbestos
PDGF-A
PDGF-B
PDGFRα
Mesenchymal
Cell Proliferation

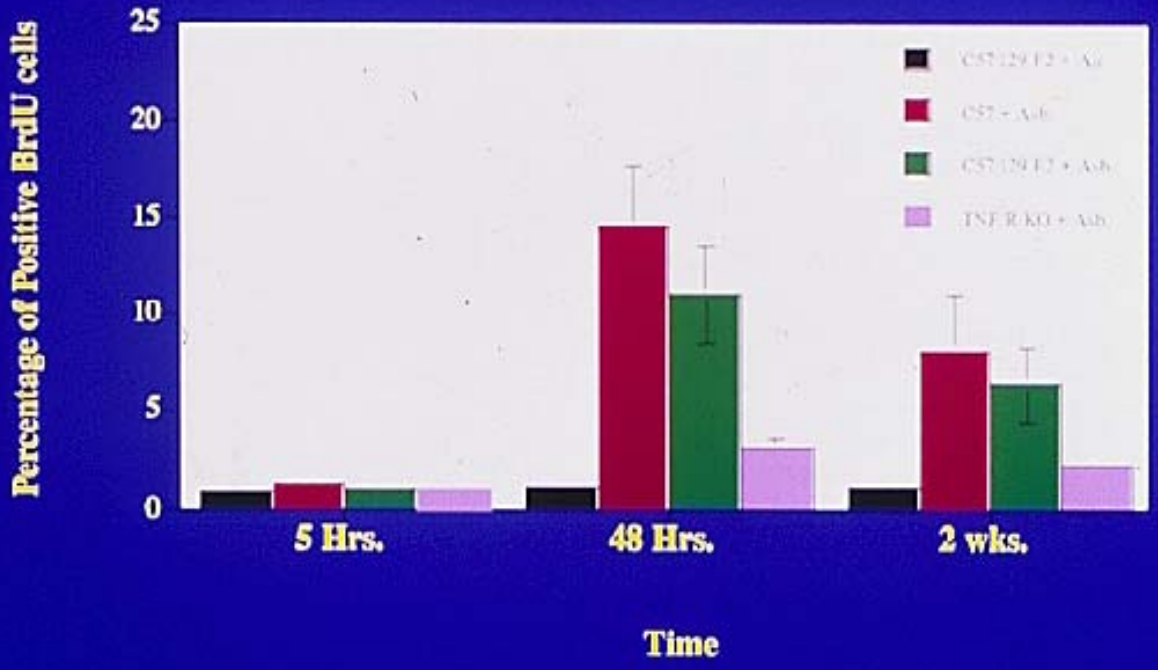

Asbestos
↑TNFα
↑TGFβ
↑Matrix Components

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


A
Figure 3
TB
AD
C
TB
B
M
In
AD
AD
D
E
AD

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



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

(In Press, Amer. J. Resp. Cell and Mol. Biol. 2002)

FROM THE LUNG BIOLOGY PROGRAM

TULANE UNIVERSITY HEALTH SCIENCES CENTER

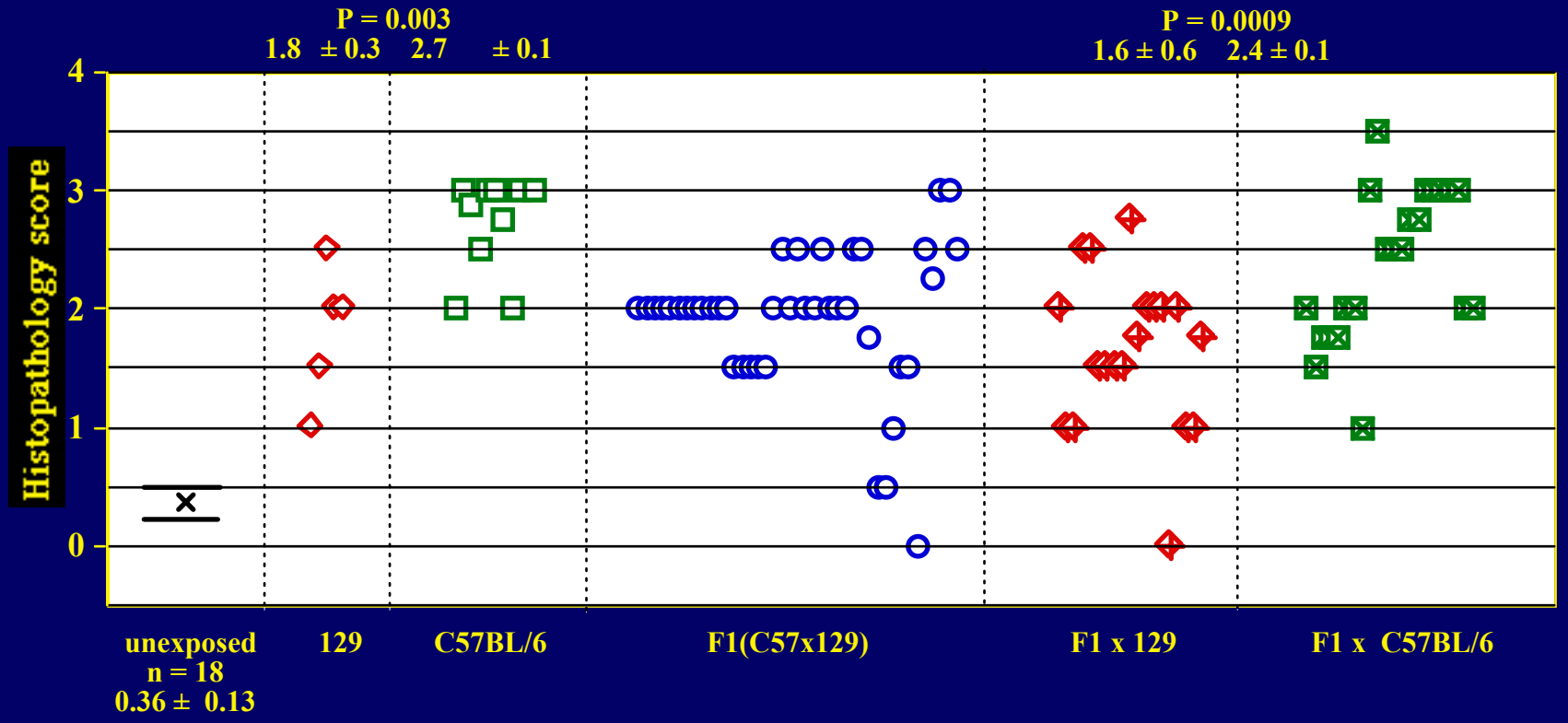
NEW ORLEANS, LOUISIANA

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



CONTROL OF AIRWAY INFLAMMATION WITH AN ADENOVIRUS VECTOR TRANSDUCING EXPRESSION OF TGFb

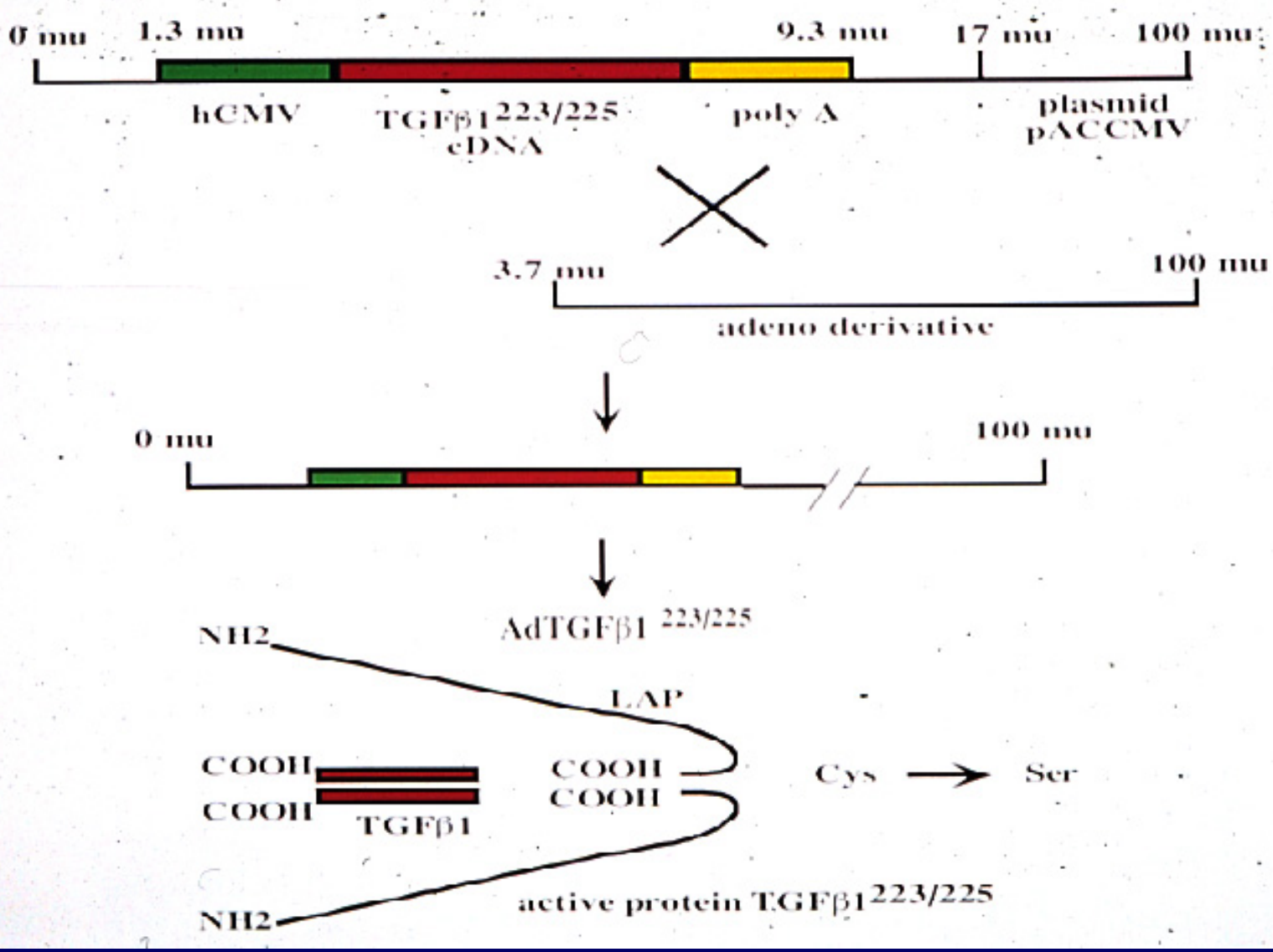
Arnold R. Brody, Saku Warshamana and Derek Pociask

Lung Biology Program

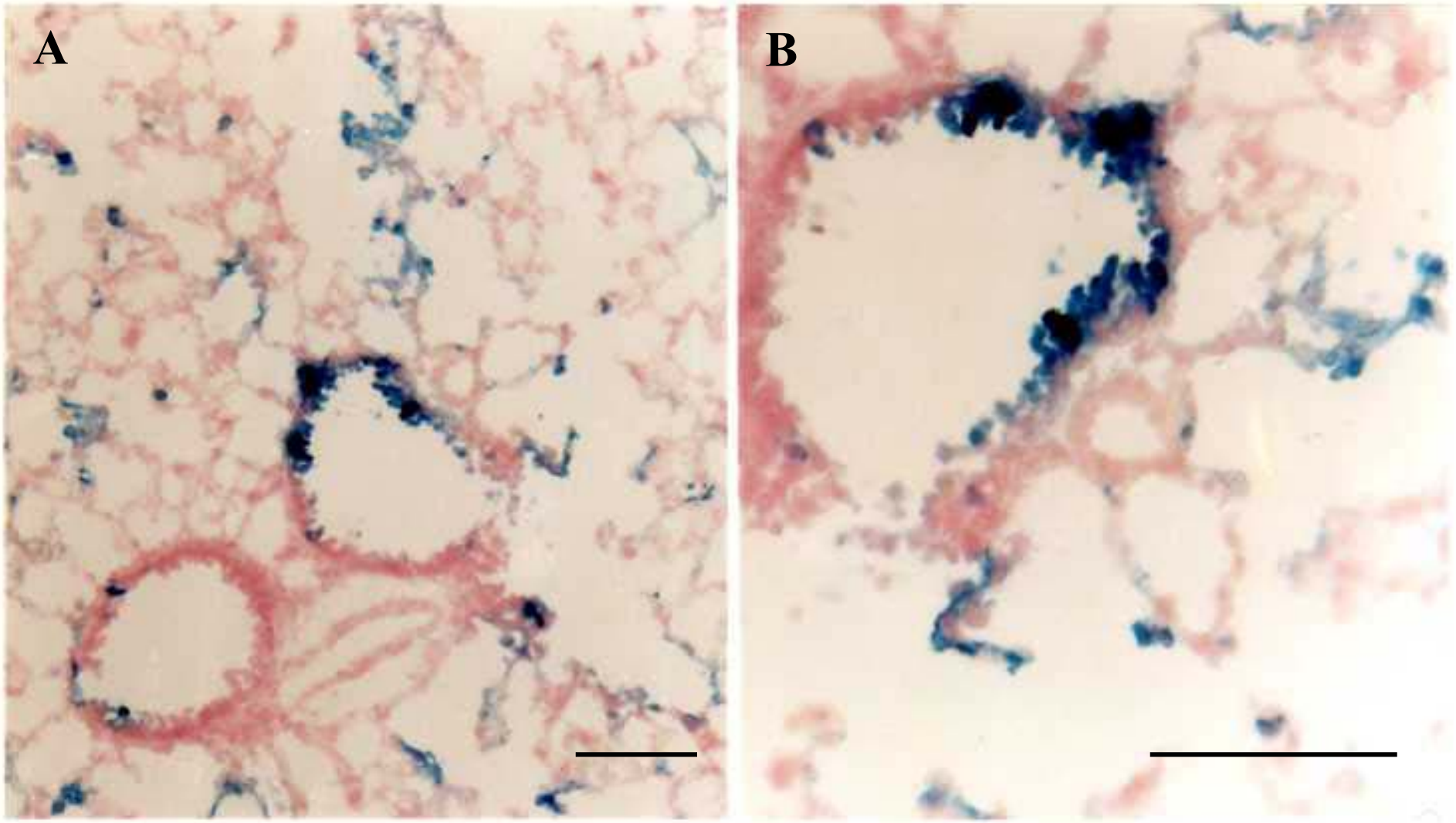
Tulane University College of Medicine, New Orleans LA

It had been determined in earlier studies that 10^6 pfu of an adenovirus vector transducing active TGFb1 was a "no-effect" level in the C57 and 129 strain mice. 5X10^7 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

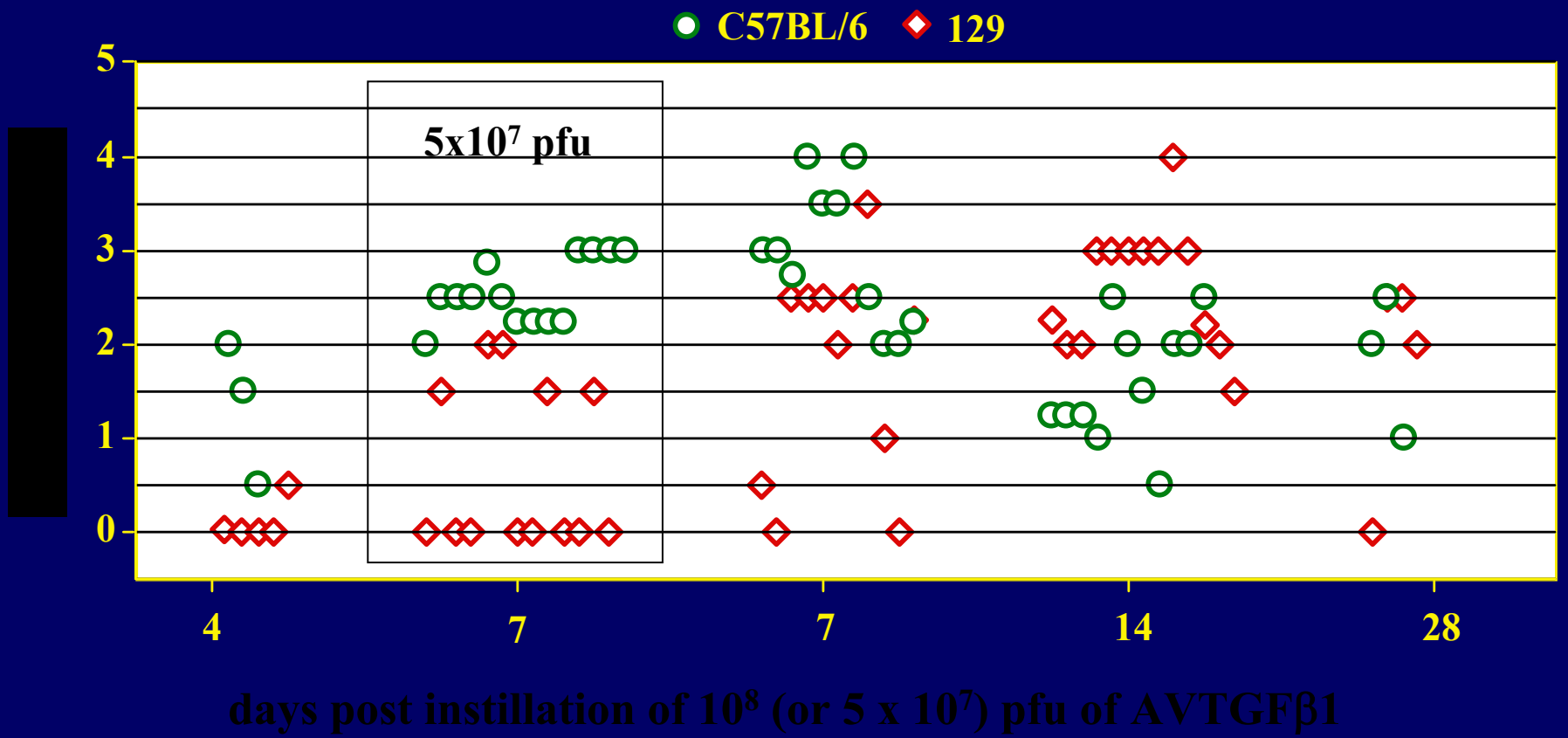


B-galactosidase gene transfer to the lungs of C57BL/6 mice: day 4



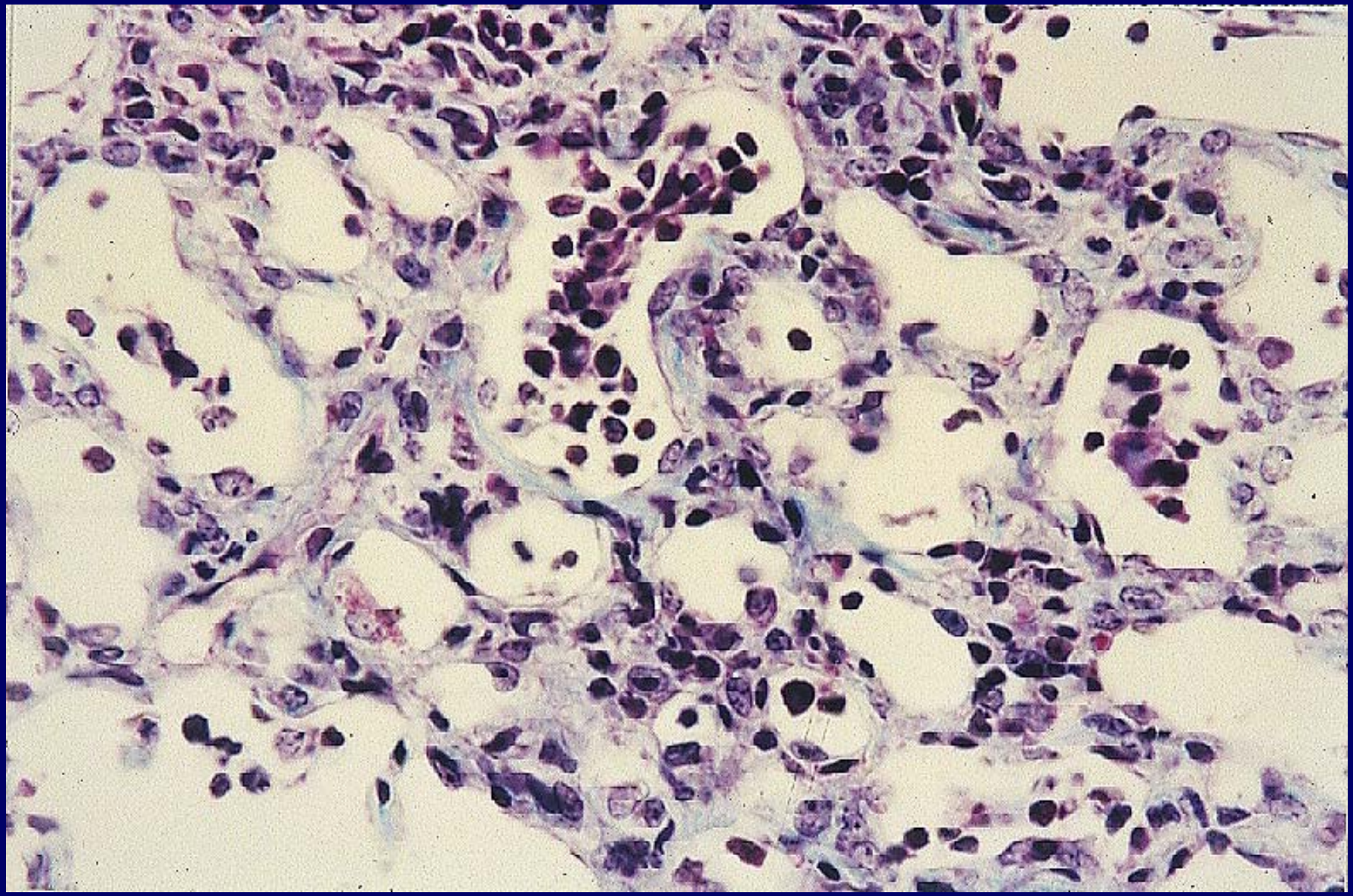
Bar = 50μm

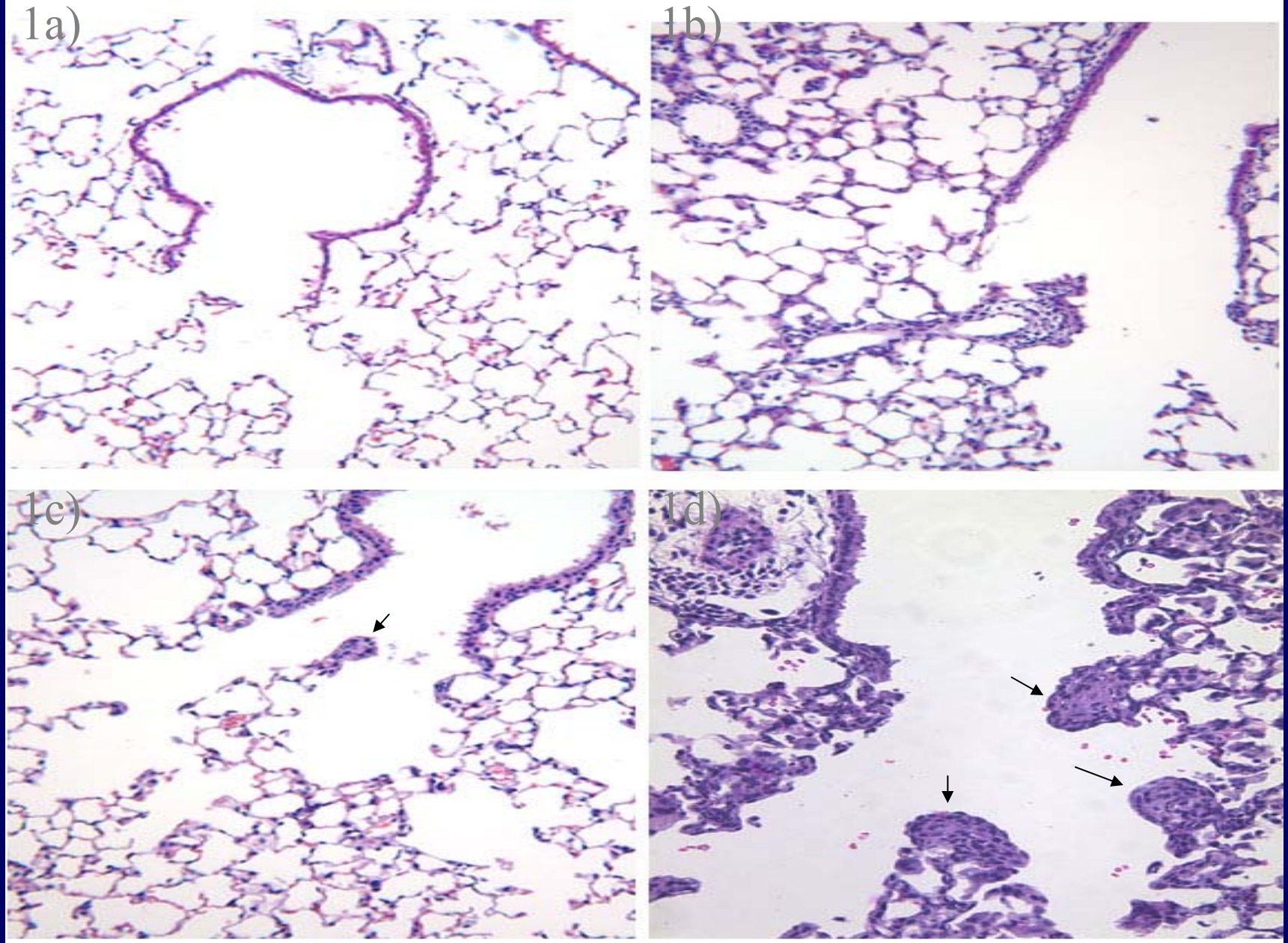
Histopathology scores of C57BL/6 and 129 mice post exposure to AVTGFβ1



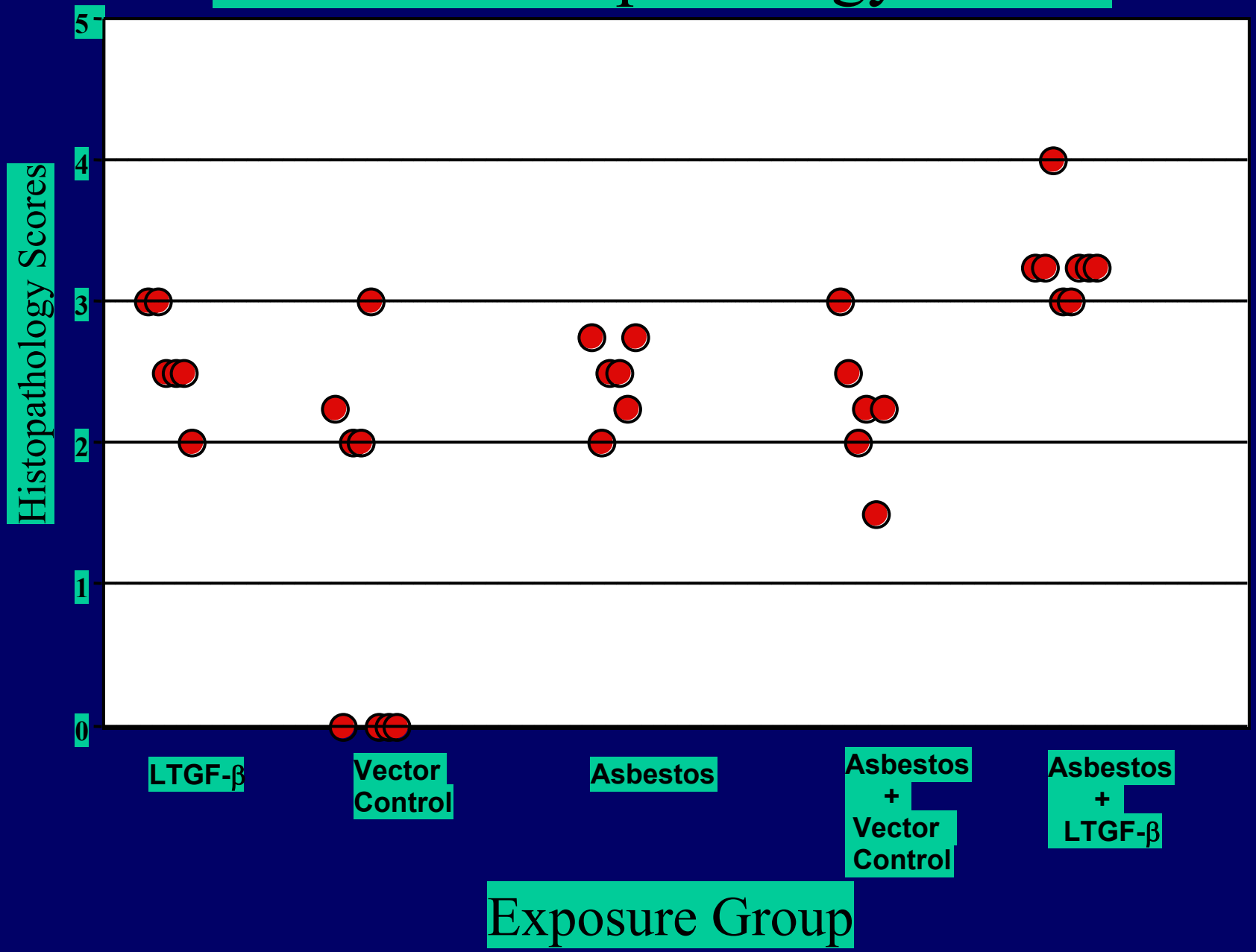
INDUCTION OF FIBROSIS BY ADENOVIRUS-MEDIATED EXPRESSION OF ACTIVE TGF-β1 IN TNF-α RECEPTOR KNOCKOUT MICE (TNF-αRKO)

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



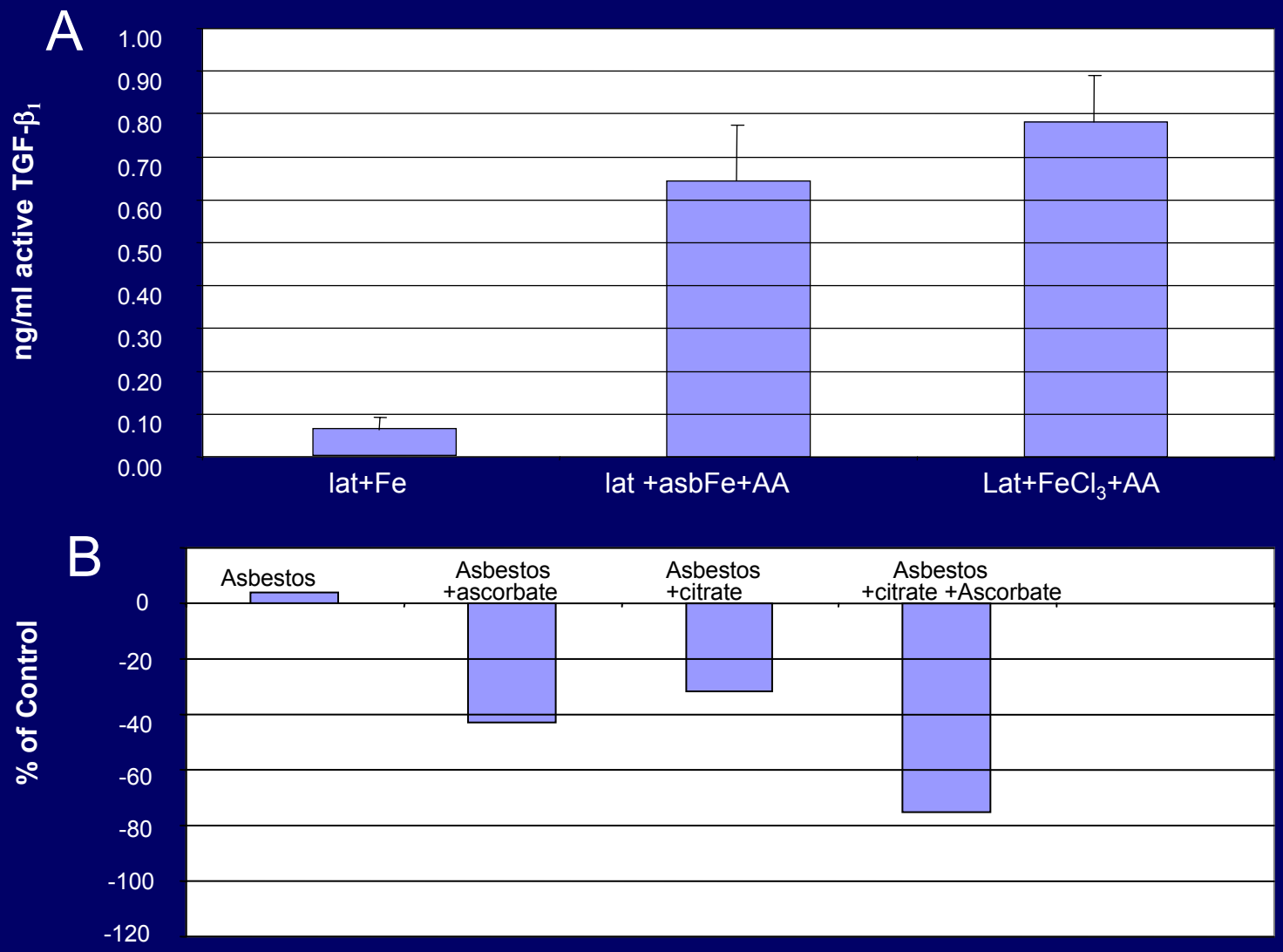

1a)
1b)
1c)
1d)

C57bl/6 Histopathology Scores

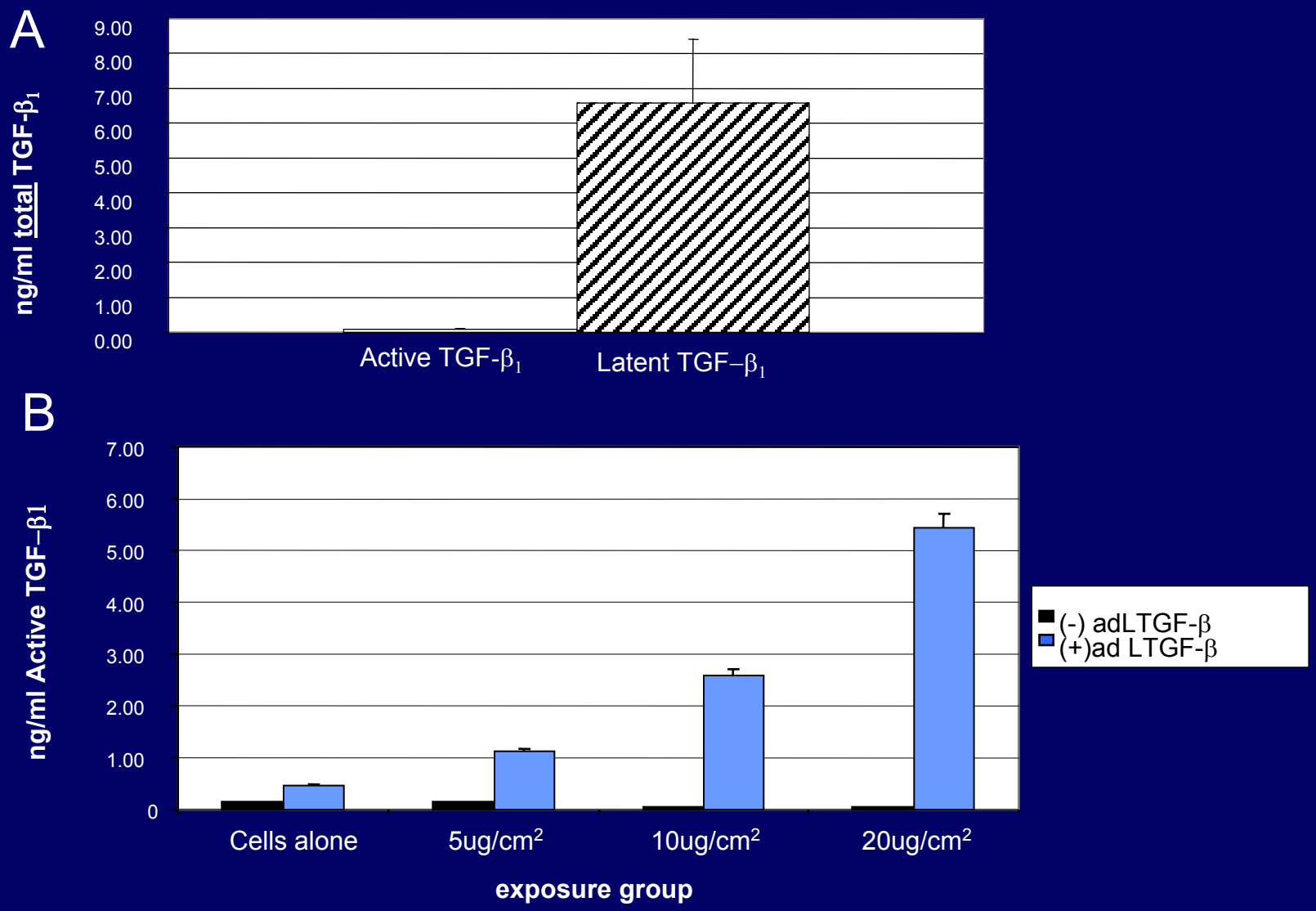


ACTIVATION OF LATENT TGFb BY ASBESTOS-DERIVED OXYGEN RADICALS

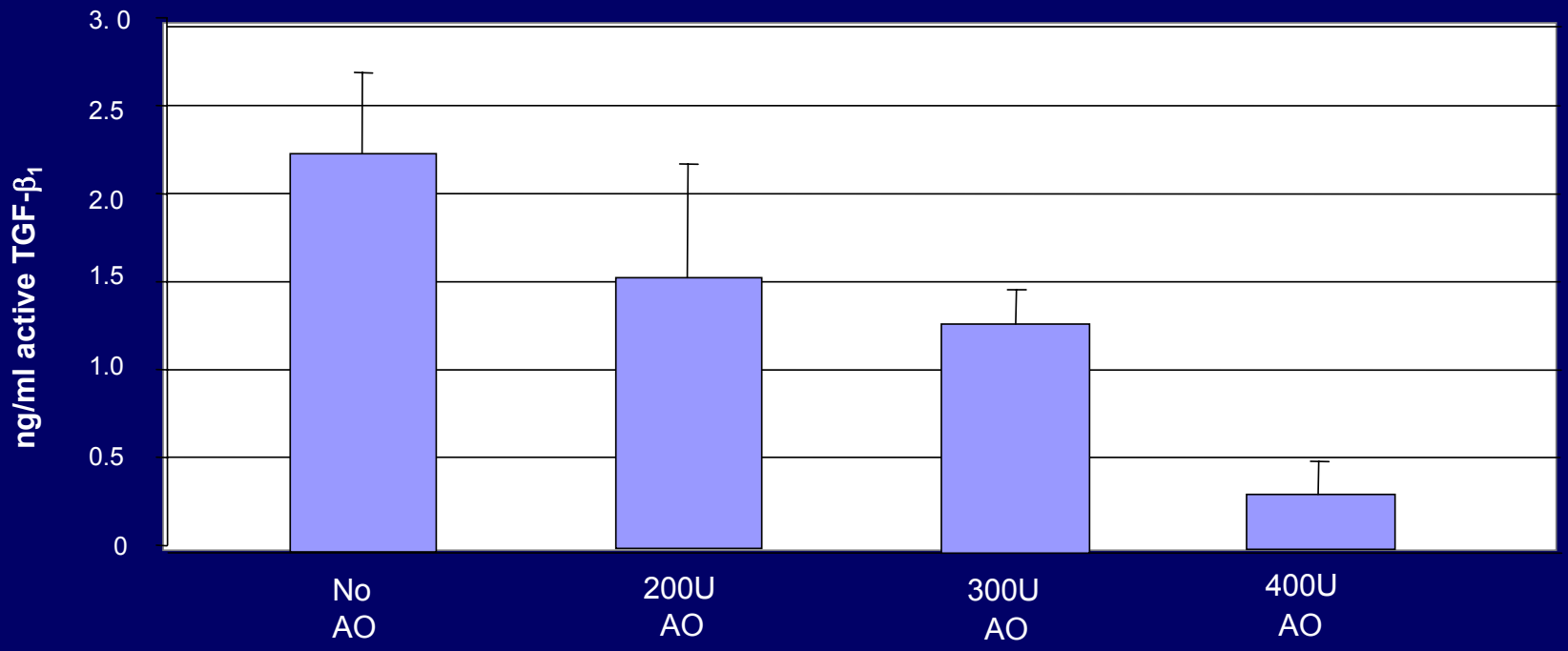
DEREK A. POCIASK AND ARNOLD R. BRODY



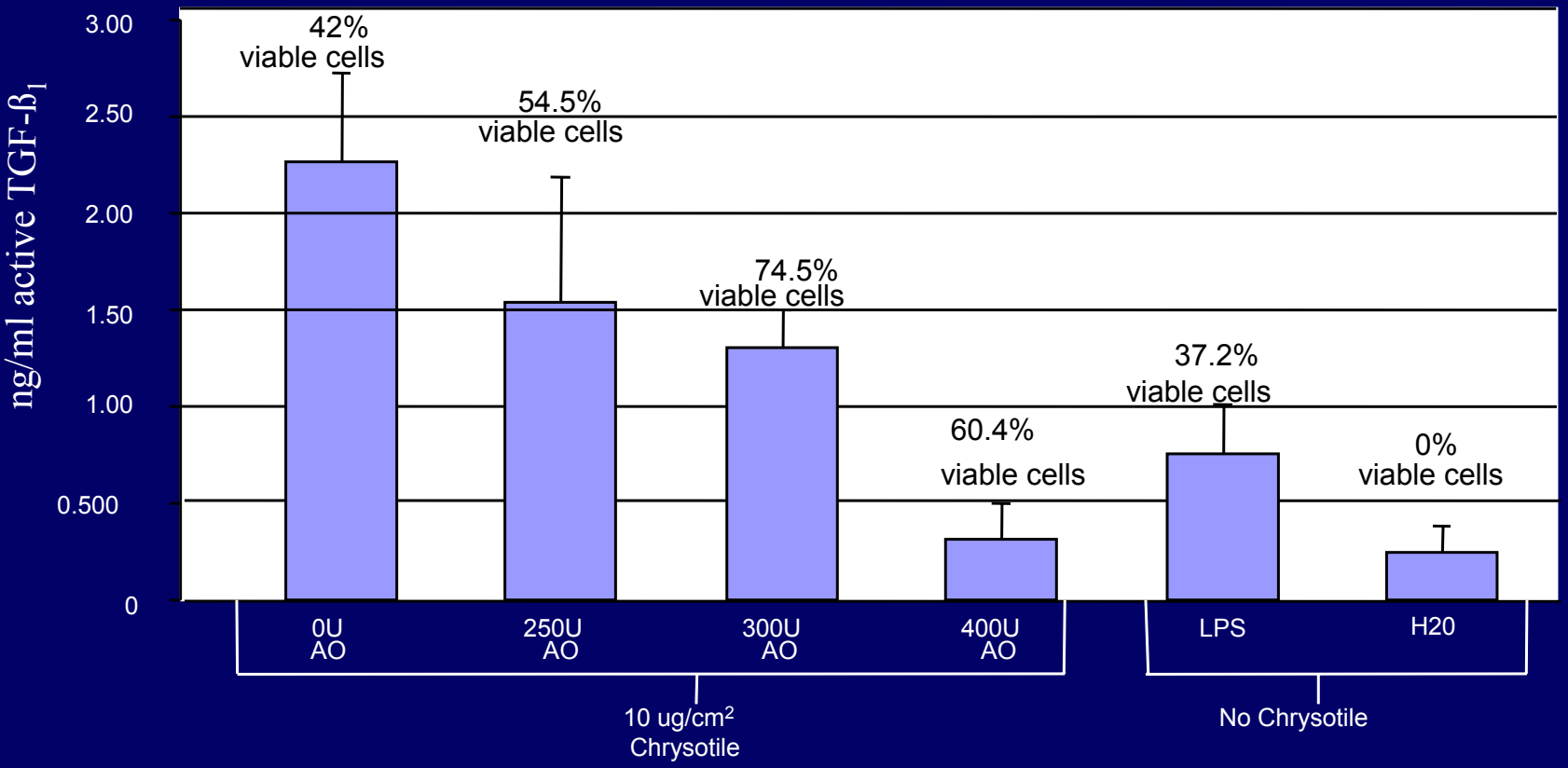
***In-vitro* Activation of Latent TGF-β_1 by Iron Mediated Reactive Oxygen Species**



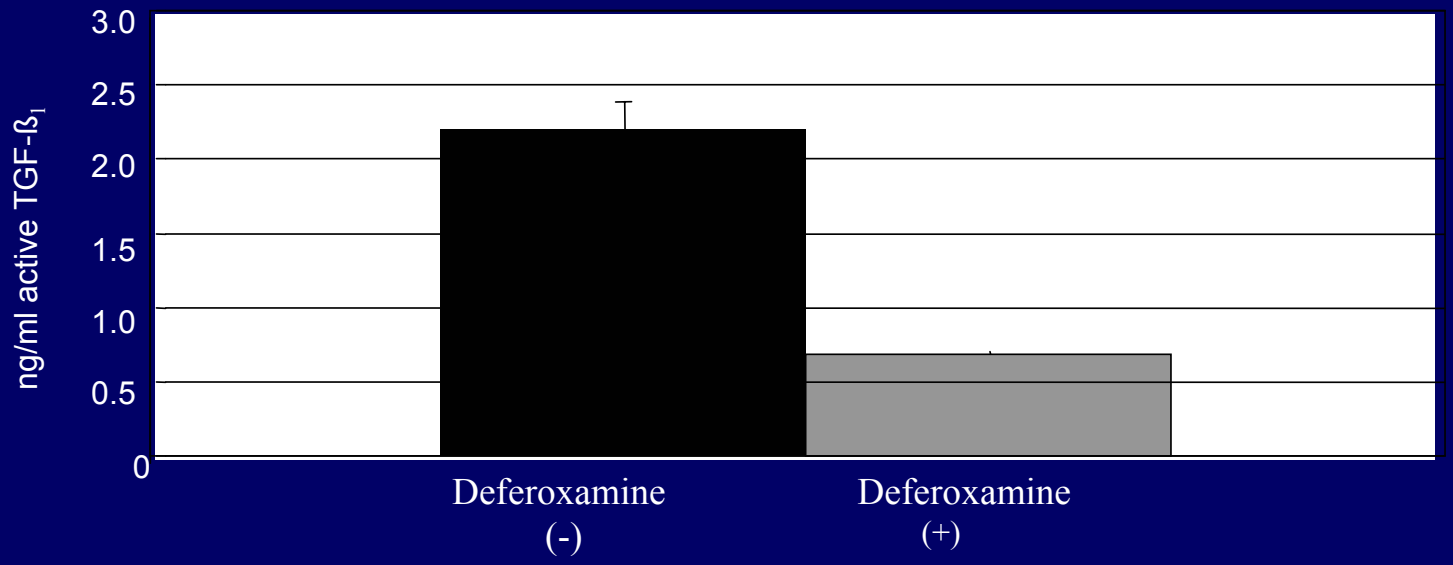
Activation of latent porcine TGF-ß$_1$ by asbestos exposure in the human epithelial cell line A549.



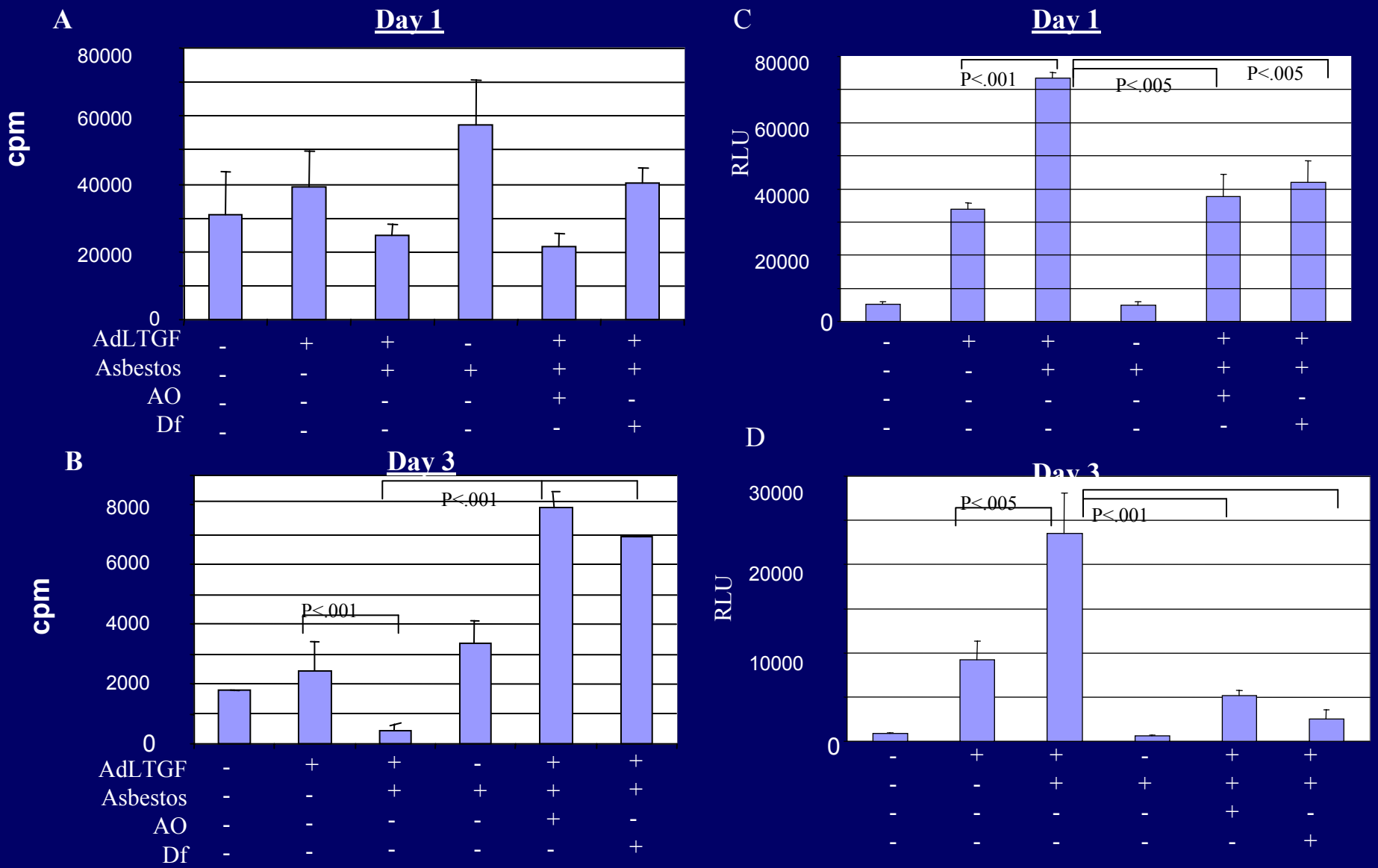
Antioxidants (AO) prevent activation of TGF-β_1.



Cell death does not increase TGF-β_1 activation.



Deferoxamine treatment decreases activation of TGF-β_1.

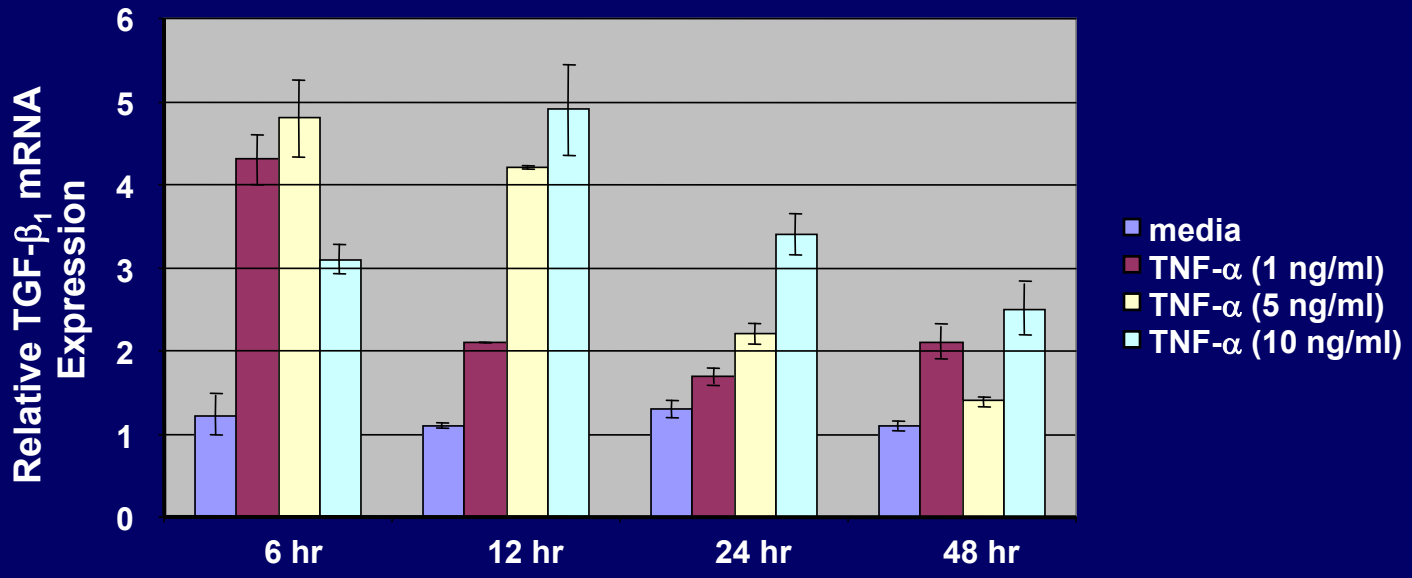


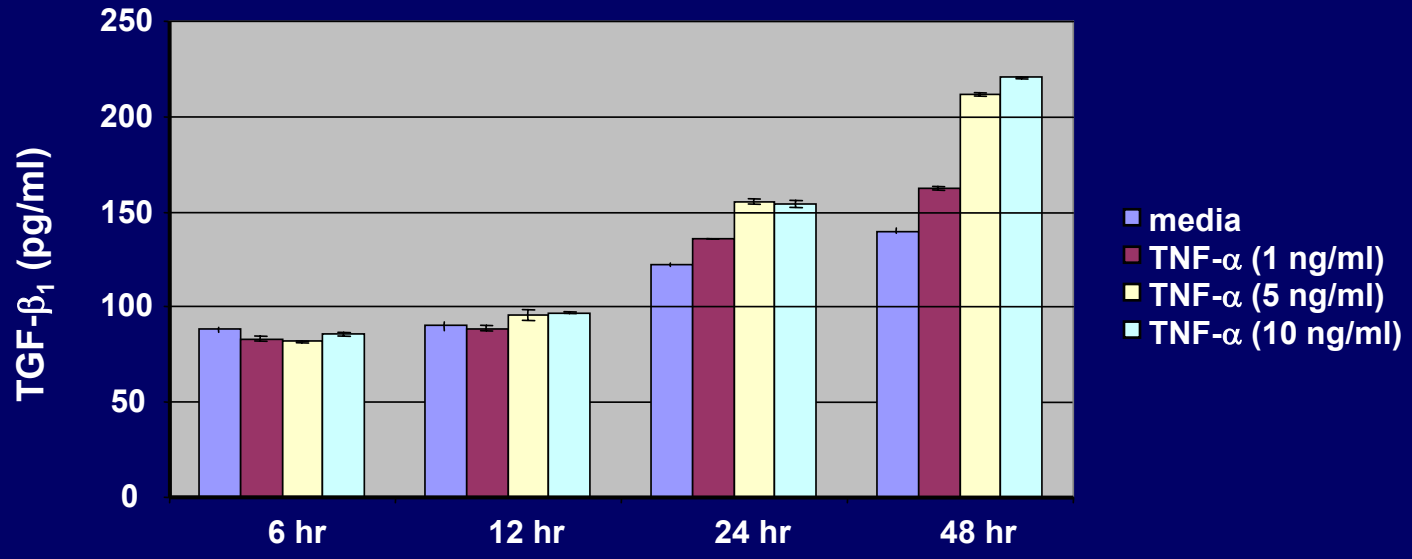
Activation of TGF-β_1 through asbestos mediated ROS is biologically significant in mink lung cells as measured by 3H thymidine incorporation (A,B) and PAI-luciferase induction (C,D).

Conclusions

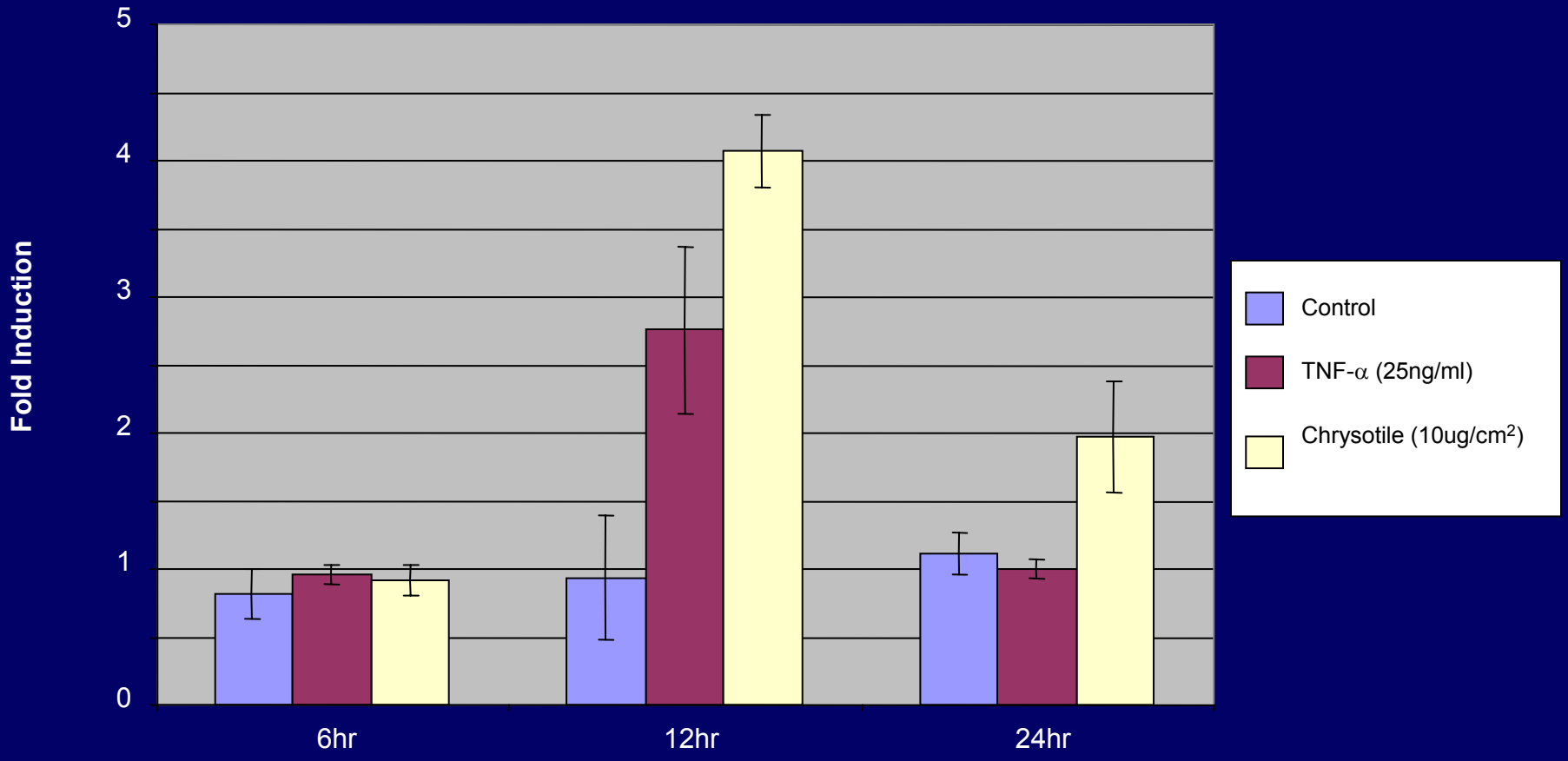
- TGF-β can be activated through iron catalyzed oxidative mechanisms
- TGF-β can be activated in cell culture in the presence of asbestos
  - increasing asbestos concentrations show increasing amounts of active TGF-β
- The use of SOD and catalase decrease the activation of TGF-β in cell culture
  - Increasing 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-β_1 mRNA and protein in Swiss 3T3 fibroblasts in a dose and time dependent manner





TNF-α and Chrysotile Asbestos Induce the Expression of TGF-ß$_1$ mRNA in A549 Lung Epithelial Cells



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