had no effect. PD98059 had no additional effect in the presence of anti-α5 integrin antibodies, suggesting an action through similar regulatory pathways. Although the drug rapidly reduced ERK phosphorylation, it had little effect on the expression of ERK-1 or ERK-2 proteins. These data suggest that the regulation of Cx expression, and thus of GJ intercellular communication in AECs is exerted through integrin-mediated cell-ECM interactions at the plasma membrane, which are linked to the activation of intracellular kinase-signaling cascades.

**Identifying Fibrosis Susceptibility Genes in Two Strains of Inbred Mice***

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*(CHEST 2002; 121:315S)*

The 129-J (129) and C57BL/6 (C57) strains of mice are commonly used in models of lung disease. These mice also are used to generate gene knockouts and transgenic mouse models. We have previously demonstrated that the 129 strain exhibits a reduced fibroproliferative response to inhaled asbestos, compared to the C57 mice that develop clear fibrogenic foci at sites of lung injury. Primary lung fibroblasts obtained from the 129 strain also exhibit reduced growth responses to platelet-derived growth factor and serum, and the cells have reduced expression of transforming growth factor-β and platelet-derived growth factor-α receptor in culture. We report here our experiments designed to identify a gene or complex of genes that mediate these two clearly identified phenotypes in vivo. The experiments were carried out on the two inbred founder strains (129, C57), on an F1 intercross between these two strains and on backcross progeny between the F1s and either the 129 or C57 inbred founders. The study progressed in two phases: (1) blinded histopathologic scoring of asbestos-exposed (48 h after two 5-h exposures) and unexposed control mice, and (2) microarray analysis of whole-lung messenger RNA from selected exposed and control mice. The histopathology scores ranged from 0 (normal) to 4 (diffuse interstitial fibrogenesis). The average (± SD) score for all strains of unexposed mice was 0.5 ± 1.0 (n = 16). The score for asbestos-exposed 129 mice was 1.5 ± 0.7 (n = 5), and the exposed C57 mice were significantly greater (p < 0.001) at 2.7 ± 0.8 (n = 10). The F2 generation yielded highly significant differences (p < 0.0005) between the F1 × 129 (score, 1.5 ± 0.75; n = 19) vs F1 × C57 (score, 2.4 ± 0.65; n = 21) backcrosses. Histopathologic analysis of the F1 generation of progeny yielded a bell-shaped curve of scores from 0 to 3. Messenger RNAs from the lungs of the founders, F1 generation, and F2 backcrosses (exposed and unexposed) were subjected to hybridization by microarray. RNAs from low and high responders in each group are currently being compared. Results from the microarray analysis are just now becoming available and will be analyzed by appropriate statistical tests. The first data set is anticipated by the end of February, 2001, and repeat analyses should be available by April or May.

**Use of Oligonucleotide Microarrays to Analyze Gene Expression Patterns in Pulmonary Fibrosis Reveals Distinct Patterns of Gene Expression in Mice and Humans***

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**Reference**


**Abbreviation:** UIP = usual interstitial pneumonia

In order to determine transcriptional programs that are active in human pulmonary fibrosis, we analyzed gene expression patterns using GeneChip microarrays (Affymetrix; Santa Clara, CA) in samples from patients with histologic diagnoses of usual interstitial pneumonia (UIP), samples obtained from lung resections for cancer that were determined normal by histologic examination, and

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pooled normal-lung RNA obtained commercially. Surprisingly, gene expression patterns were quite homogenous among pulmonary fibrosis and control samples, despite the expected variability in genetic background and condition of subjects. Using the total number of misclassifications score, the information-content score, and Gaussian-error score, we determined that 100 genes were significantly differentially expressed between UIP and control lungs. Groups of genes that were significantly overexpressed included metalloproteases, extracellular matrix-related genes, markers of smooth-muscle differentiation, and antioxidants. Interestingly, we observed little evidence for overexpression of inflammatory-related genes (mainly immunoglobulins and complement components). To assess whether similar gene programs were activated in mice and humans, we compared gene expression patterns in the lungs of patients with pulmonary fibrosis with gene expression patterns observed in lungs of C57BL/6 mice after bleomycin. Notably, most genes that were over expressed in human UIP were also induced by bleomycin in our mice, suggesting that the proteins encoded by these genes play an important role in the continuous destruction/remodeling process typical of pulmonary fibrosis. The global transcriptional patterns as well as the information about individual genes provided by our experiments should prove useful in the design of new interventions to halt disease progression.

**Induction of Cyclo-oxygenase-2 in Non-small Cell Lung Cancer cells by Adenovirus Vector Infection**

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**Key words:** adenovirus; cyclo-oxygenase-2; gene therapy; non-small cell lung cancer

**Abbreviations:** COX = cyclo-oxygenase; ERK = extracellular signal-related kinase; NF = nuclear factor; PGE = prostaglandin

Induction of cyclo-oxygenase (COX)-2 protein and prostaglandin (PGE)-2 production by non-small cell lung cancer cells following infection with a first generation (ΔE1, ΔE3) Ad vector. Notably, PGE-2 has been implicated in tumor-induced immunosuppression. ΔE1, ΔE3-Ad vector infection of non-small cell lung cancer cell line NCI-820 induced dose-dependent increases in PGE-2 production (moi10–50). Induction was related to the transgene expressed. Detectable increases in COX-2 protein were seen by Western blot 36 h after infection; these were paralleled by increases in phosphorylation of extracellular signal-related kinase (ERK)-1/2. Selective blockade of ERK with PD98029 reduced constitutive COX-2 expression and prevented induction of PGE-2 following Ad infection. An inhibitor of nuclear factor (NF)-κB translocation to the nucleus, SN50, had no effect on constitutive nor inducible PGE-2 levels. In contrast, Ad vector infection did induce NF-κB activity as measured by an NF-κB luciferase reporter plasmid transfected into cell line NCI-820. Further, UV/psoralen-inactivated vector did not have similar effects on PGE-2, indicating the importance of gene expression. Ad vector infection leads to ERK phosphorylation with parallel increases in COX-2 protein and PGE-2 production. These effects appear unrelated to NF-κB and are dependent on gene expression by the vector. In this context, induction of PGE-2 by Ad vectors may need to be considered when defining targets for immunogene therapy

**Towards an Effective Gene Therapy for Idiopathic Pulmonary Fibrosis**

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**Abbreviations:** AdCMV.DC = cytomegalovirus promoter; IPF = idiopathic pulmonary fibrosis

An effective gene therapy is required for idiopathic pulmonary fibrosis (IPF) because: (1) IPF is a chronic but progressive lung disease for which current therapy is minimally effective, (2) the molecular mechanism of complicated