pecific therapy (ie, IV augmentation therapy with purified α1-antitrypsin) and by emerging data suggesting the biochemical and clinical efficacy of augmentation therapy.

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In Vivo Adenovirus-Mediated Expression of Human Pre-Elafin, a Potent Neutrophil Elastase Inhibitor*

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Human neutrophil elastase is thought to be involved in lung diseases such as emphysema or cystic fibrosis. We have recently characterized an elastase-specific inhibitor (elafin) as a potent human neutrophil elastase inhibitor in the lung. The gene codes for a secreted active 10 kd protein, pre-elafin (PE), which can be cleaved in secretions, giving rise to a 6 kd product, elafin. A 538-base pair (bp) fragment containing the endogenous leader and polA sequences of the PE gene were inserted into the E1 deleted region (replication-deficient) of adenovirus (Ad)5 under the control of either the human cytomegalovirus (HCMV) promoter ($30$ bp), the Ad2-major late promoter (MLP), or the mouse cytomegalovirus (MCMV) promoter.

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We have previously shown in vitro that MCMV drives the synthesis of PE more efficiently than HCMV or MLP, both in human and rodent epithelial cells and fibroblasts. The aim of this study was to assess in vivo the comparative strength of these promoters.

Three replication-deficient recombinant Ad5 viruses (HCMV-PE, MLP-PE, MCMV-PE) were isolated and titrated. Sprague-Dawley rats were injected intratracheally with 0.3 mL of phosphate-buffered saline containing either $3 \times 10^8$ (n=2), $1.5 \times 10^9$ (n=2), or $3 \times 10^9$ (n=2) plaque-forming units. After either 2 or 5 days, the lungs were lavaged with phosphate-buffered saline. The BAL fluids were centrifuged and supernatant content of PE was assessed by enzyme-linked immunosorbent assay (ELISA). The left lung was snap-frozen in liquid nitrogen for Northern blot analysis of PE messenger RNA (mRNA).

Northern blot analysis of PE mRNA from lungs infected with HCMV-PE and MLP-PE (days 2 and 5) was negative. In contrast, MCMV-PE-infected lungs showed significant levels at day 5. These data were confirmed at the protein level by ELISA whereby BAL recovered from MCMV-PE-infected lungs (increasing doses) showed very high levels of PE at day 5 (71.1, 54.3, and 66.6 ng/mL, respectively). Interestingly, levels at day 2 were much lower (3.1, 13.7, and 6.9 ng/mL, respectively). No detectable levels (<0.5 ng/mL) of PE protein were present in BAL from lungs infected with HCMV-PE and MLP-PE (day 2 or day 5).

Our data show that, compared to HCM and MLP, MCMV is a very potent promoter in rat lung tissue. We were able to detect significant levels of both PE mRNA and protein. We believe that our findings have important implications for the efficiency of gene transfer of anti-inflammatory molecules such as PE in rodent models of lung inflammation.

Rat Prostacyclin Synthase*

Cloning and Regulation of Gene Expression in the Lung

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Prostacyclin synthase (PGIS) is the final committed enzymatic step in the pathway of prostacyclin (PGI$_2$) production, occurring at a branch point where substrate (PGH$_2$) can be directed either toward prostacyclin, which affords protection, or thromboxane, which augments injury. The imbalance of prostacyclin and thromboxane is involved in the pathogenesis of several diseases, such as pulmonary hypertension, acute lung injury, and atherosclerosis. These studies were designed to pursue the molecular cloning and characterization of rat prostacyclin synthase.

An 1153-base pair fragment of the rat PGIS gene was cloned and sequenced in its entirety. This fragment represents 77% of the predicted full complementary DNA sequence and contains a complete open reading frame. To study regulation of PGIS gene expression, this fragment was used as a probe for Northern blot analysis under a variety of conditions. Studies were performed to determine rat tissue-specific expression and expression during rat lung development. The in vitro effects of steroid hormones on lung PGIS expression were examined in animals treated with corticosteroids, testosterone, and estrogen. The effects of antioxidant and lipopolysaccharide (LPS) administration on lung PGIS expression were evaluated. The isolated-perfused rat lung (a model of shear-stress) was used to elucidate the outcome of differing flow rates on lung PGIS expression.

The results of these studies indicate that the PGIS sequence across species is highly conserved. The rat gene is most strongly expressed in heart and lung, and recognizes a major messenger RNA species of 2.0 kilobase (kb) with two minor bands of 2.9 and 5.6 kb. Skeletal muscle shows a unique band of 4.4 kb. In lung development, PGI$_2$ synthase is upregulated late in gestation at day 21. PGIS gene expression is induced by corticosteroids, antioxidants, LPS, and shear stress. Testosterone increases gene expression, while estrogen has the opposite effect. These studies demonstrate the following: (1) rat PGI$_2$ synthase is closely homologous to the bovine and human genes; (2) multiple forms of transcripts exist and may represent alternative splicing or distinct isoforms; (3) the gene is expressed in rat lung late in gestation; (4) PGIS expression is induced by shear stress; (5) unlike cycloxygeynase, PGIS is induced by corticosteroids and antioxidants; and (6) testosterone increases gene expression while estrogen decreases expression. Using the rat model will enable examination of gene expression under a variety of conditions and offers the potential for gene therapy trials.

Surfactant Protein-B Deficiency*

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Pulmonary surfactant is the mixture of lipids and proteins needed to reduce alveolar surface tension at the air-liquid interface and to prevent end-expiratory atelectasis. An inability to produce sufficient quantities of surfactant due to immaturity is the primary cause of the respiratory distress syndrome (RDS) observed in infants born prematurely, and diminished surfactant function contributes to the pathophysiologic condition of ARDS.

While the phospholipid components of pulmonary surfactant are important in its surface tension-lowering properties, specific surfactant proteins have been identified that...