Type I and Type III collagen mRNA expression in human lung fibroblasts.

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The predominant histological features of pulmonary fibrosis are an accumulation of collagen in the alveolar interstitium, and an increase in the number of fibroblasts [1]. There is an alteration in collagen production with an increase in Type I collagen relative to Type III. The mechanisms involved in this regulation of collagen production at transcriptional and translational level are poorly understood. Levels of many cytokines are elevated in the lungs of patients with interstitial lung disease and recent reports indicate that they may play an important role in the alteration of collagen production observed in pulmonary fibrosis[2].

Most studies to date have examined the effect of a single cytokine on Type I collagen and the aim of this study is to investigate the effect of various cytokines on Type I and Type III collagen production at transcriptional/translational level in both normal and fibrotic lung fibroblast cell lines.

Normal human lung fibroblast cell line (LU 11, American Type Culture Collection, Rockville, MD) and fibrotic cell line (LL 29, ATCC) were cultivated in Dulbecco's minimal medium (DMEM) containing 10% fetal calf serum, 2mM glutamine, 2.3 mM amphotericin, 0.2mg/ml Penicillin/Streptomycin and 50µg/ml ascorbic acid. Confluent cell layers were incubated in serum-free DMEM containing 50µg/ml ascorbic acid for 24h to remove most serum proteins. The medium was then replaced by fresh serum free DMEM containing 50µg/ml ascorbic acid and the cytokines were added as follows; transforming growth factor β (TGFβ) 2.5ng/ml; interferon γ (Inf γ) 100U/ml; platelet derived growth factor (PDGF) 5ng/ml; or interleukin 1(IL-1) 100U/ml.

Total RNA was extracted from the lung fibroblast monolayers with phenol and precipitated with ethanol. The concentration of the RNA was determined by measuring its OD at 260nm. The cDNA for human α1(I) collagen and α1(III) collagen and for phosphoglyceraldhyde (pGAD) which was used as a control, were purified and radiolabelled with 32P NTP by the random primer synthesis method of Feinberg and Goldstein [3]. RNA was denatured, serially diluted and applied to Hybond filters in a dot blot apparatus, these were hybridised with 106CPM/ml of labelled cDNA. The dot blots were washed and exposed to X-Ray film at -70°C with an intensifying screen, and were quantified by densitometry. Total cellular DNA was measured using a micro-assay technique in a Hoefer TKO 100 mini Fluorometer.

The quantities of RNA extracted from normal and fibrotic cell lines were similar (0.27-0.37mgRNA per mgDNA) and were not affected by the cytokines, TGFβ, but the other cytokines had no effect. Preliminary results on the normal cell line LU11 indicate that α1(I) mRNA may be increased by Inf γ. Further studies are being undertaken to confirm these results.

Total cellular RNA was isolated from fibroblasts. Specific mRNAs were measured by hybridising the dots with cDNA probes representing α1(I) collagen and pGAD. After autoradiography, the bands were quantitated by densitometry. Results are expressed as peak area.