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Enhanced IL-1β and Tumor Necrosis Factor-α Release and Messenger RNA Expression in Macrophages from Idiopathic Pulmonary Fibrosis or after Asbestos Exposure[1]

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Abstract. Idiopathic pulmonary fibrosis (IPF) and asbestosis are fibrotic interstitial lung diseases characterized by alveolar wall fibrosis with accumulation of extracellular matrix, interstitial remodeling, and increased numbers of activated alveolar macrophages. Animal models and in vitro studies have shown that macrophage cytokines, namely IL-1β and TNF-α, play significant roles in the development of fibrosis. We found significant increases for TNF-α release in both diseases ($p < 0.01$) and a significant increase for IL-1β release in asbestosis compared to normal controls ($p < 0.01$). Also, the mRNA expression of these cytokines was increased in alveolar macrophages from patients with IPF or asbestosis compared with normals. The level of TNF-α release in macrophage supernatants correlated with the number of neutrophils per milliliter bronchoalveolar lavage fluid returned. Chrysotile, crocidolite, amosite asbestos, and silica stimulated IL-1β and TNF-α release and up-regulated their respective mRNA in macrophages or monocytes. To evaluate the role of IL-1β and TNF-α in the accumulation of extracellular matrix, we studied collagen types I and III and fibronectin gene expression in human diploid lung fibroblasts after short term (2 h) serum-free exposure to recombinant cytokines. Both cytokines up-regulated these genes 1.5- to 3.6-fold. These cytokines have the potential to influence the remodeling and fibrosis observed in the lower respiratory tract in IPF and asbestosis. *Journal of Immunology,* 1993, 150: 4188.

IPF[3] and asbestosis are characterized by thickened alveolar walls with mesenchymal cell proliferation and extracellular matrix deposition, remodeling, and alveolar accumulation of inflammatory cells, particularly AM (1, 2). BAL permits sampling of these activated cells; their short term in vitro culture demonstrates increased secretion of several mesenchymal growth factors including fibronectin, PDGF, IL-8, and AM insulin-like growth factor I (IGF-1) compared with normal control individuals (1–9). Mononuclear phagocytes exposed in vitro to immune complexes or inorganic dust release fibroblast growth factors (3–7). Silica-exposed human blood monocytes release fibroblast and thymocyte mitogenic factors that have biochemical and size chromatographic characteristics of IL-1 (8, 10–13). In a murine model of silicosis, lung TNF-α mRNA was increased between days 7 and 15 although no TNF-α was detectable in the serum of the mice at any time

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[3] Abbreviations used in this paper: IPF, idiopathic pulmonary fibrosis; PDGF, platelet-derived growth factor; BAL, bronchoalveolar lavage; HRP, horseradish peroxidase; VC, vital capacity; FEV_1 forced expired volume in one second; ANOVA, analysis of variance; IGF-I, insulin-like growth factor I; D_LCO, diffusing capacity; EGF, epidermal growth factor; AM, alveolar macrophages.

point (14). It has also been shown that intradermal administration of TNF-α stimulates a focal accumulation of fibroblasts and collagen (15), and that TNF-α added in vitro to fibroblasts stimulates DNA synthesis and increases the cell number (16).

AM in the interstitial lung diseases associated with pulmonary fibrosis release growth factors and cytokines that are responsible for chemotaxis (fibroblasts, smooth muscle cells, monocytes, neutrophils), fibroblast proliferation, and production of matrix including collagen and fibronectin (2). We have previously found that the release of oxidants, fibronectin, PDGF and AM progression-type growth activity were significantly increased in IPF and asbestosis compared with controls (5, 7, 17). We hypothesized that activated AM from patients with interstitial lung disease would spontaneously release increased amounts of IL-1β and TNF-α, and that in turn IL-1β and TNF-α could have a role in the fibrosis and remodeling observed in the lung interstitium. To test this hypothesis, we performed BAL on patients with IPF, a rapidly fatal disorder, and on individuals who had asbestos exposure with radiographic evidence of parenchymal fibrosis to obtain AM for analysis of IL-1β and TNF-α protein release and gene expression. We have also studied the effects of inorganic dust including asbestos and silica on the modulation of protein release and gene expression in vitro using specific immunoassay. Our results demonstrate that IL-1β and TNF-α play a potentially significant role in the pathogenesis of pulmonary fibrosis.

Materials and Methods

Study population

The New York University Institutional Review Board of Research Associates approved the use of human subjects for this project. We recruited nine asbestos-exposed individuals who had been exposed to asbestos for 31 $\pm$ 2 yr as insulators. All were non-smokers or ex-smokers >5 yr. Their mean age was 56 $\pm$ 2 yr. All had irregular opacities on their chest radiographs and bilateral circumscribed pleural plaques or diffuse pleural fibrosis. Pulmonary function tests performed according to American Thoracic Society recommendations revealed VC of 81 $\pm$ 5%, FEV_1 of 85 $\pm$ 6%, and mean diffusing capacity 87 $\pm$ 7%. Normal values were obtained from Morris et al. (18) for VC and FEV_1, and single-breath carbon monoxide diffusing capacity was performed according to Ogilvie (19) including calculation of breath-hold time, and predicted values were from Gaensler et al. (20). BAL recovered a mean of 302 $\pm$ 30 $\times$ 10^3 cells/ml fluid returned with a cell differential of 87 $\pm$ 5% macrophages, 11 $\pm$ 5% lymphocytes, 1 $\pm$ 0.4 neutrophils, and 0.7 $\pm$ 0.6% eosinophils.

We also recruited nine individuals (five males, four females) with idiopathic pulmonary fibrosis diagnosed with characteristic pathologic features by open lung biopsy. Their mean age was 45 $\pm$ 4 yr. All had received prior therapy and four were on maintenance prednisone at the time of the study. Five were smokers and four non- or ex-smokers. All chest radiographs demonstrated reduced lung volumes and diffuse reticulonodular opacities. Pulmonary function tests revealed mean VC 61 $\pm$ 6% predicted, mean FEV_1 62 $\pm$ 6% predicted and mean diffusing capacity 59 $\pm$ 6% predicted. BAL recovered a mean of 409 $\pm$ 129 $\times$ 10^3 cells/ml with a cell differential of 87 $\pm$ 6% macrophages, 4 $\pm$ 2% lymphocytes, 3 $\pm$ 1% neutrophils, and 6 $\pm$ 3% eosinophils. Data for IL-1β and TNF-α release were available on seven of nine individuals.

We included in this study five normal controls (all males) with normal physical examinations, chest radiographs, and pulmonary function tests. No study subject was on medication or reported exposure to inorganic dust. They were 42 $\pm$ 2 yr old. All were non-smokers or ex-smokers >5 yr. BAL recovered a mean of 208 $\pm$ 15 $\times$ 10^3 cells/ml with a cell differential of 83 $\pm$ 2% macrophages, 15 $\pm$ 2% lymphocytes, 2 $\pm$ 2% neutrophils, and <1% eosinophils.

BAL

BAL was performed with a flexible fiberoptic bronchoscope with local Xylocaine anesthesia. Normal saline (5 20-ml aliquots) was instilled and suctioned sequentially from three sites (right middle lobe, right lower lobe, lingula). The recovered fluid was filtered through sterile gauze. A total cell count was done in a hemocytometer, and cell differentials performed on cytocentrifuge slides stained with Diff-Quick and 500 cells were counted. Cell viability was determined by trypan blue exclusion, and in all cases, recovered cells were >90% viable. AM were cultured in suspension for 18 h at 10^6/ml in DMEM, and supernatants were frozen at −70°C until assay.

Isolation of human monocytes from human peripheral blood

Mononuclear cells from normal blood donors were separated by centrifugation over lymphocyte separation medium (Flow Laboratories, McLean, VA). The cells were washed, suspended in RPMI 1640 medium supplemented with 10% FCS, and seeded onto the plastic surface of a 175-cm^2 flask for 2 h at 37°C to let monocytes adhere. The flask was washed three times with RPMI 1640 to remove nonadherent cells and the monocyte-enriched population was detached by scraping with the aid of a rubber policeman. The cells were seeded in 24-well plastic tissue culture plates (Falcon, Oxnard, CA) with a density of 10^6 cells/well, and stimulated in serum-free conditions with different reagents. Stimuli included the following: UICC Rhodesian asbestos chrysotile B (50 µg/ml), UICC crocidolite asbestos (50 µg/ml), UICC amosite asbestos (50 µg/ml), silica (50

μg/ml, gift of Dr. V. Vallyathan, National Institute for Occupational Safety and Health, Morgantown, WV), beryllium (1.2 μM, Brush-Wellman, Cleveland, OH), bleomycin (0.1 U/ml, Mead Johnson, Evansville, IN). The culture supernatant was then collected and stored at −70°C.

Isolation of RNA and Northern blot analysis

Human peripheral blood monocytes and AM were lysed by addition of 5.5 M guanidinium isothiocyanate buffer. Cytoplasmic RNA was isolated through $CsCl_2$ gradient ultracentrifugation.

Human adult lung fibroblasts (American Type Culture Collection CCL 135, Bethesda, MD) were grown to confluence in 7 to 10 days, washed in DMEM, cultured 24 h in serum-free medium, and stimulated with TNF-α (10 ng/ml), IL-1α (1 ng/ml), or IL-1β (1 ng/ml) for 2 h. The cells were broken in lysis buffer containing 0.1 M NaCl, 0.01 M Tris (pH 8.6), 0.002 M EDTA (pH 7.5), 1% Triton X-100, 0.5% deoxycholate, and 1% β-mercaptoethanol. The cell lysates underwent oligo(dT)cellulose column chromatography to isolate polyadenylated mRNA that was subsequently fractionated by electrophoresis through a 1% agarose-6% formaldehyde denaturing gel, and transferred onto a nitrocellulose filter (BA 85, Schleicher and Schuell Keene NH). The baked filter was incubated in 40 ml of prehybridization solution (50% formamide, 0.5% SDS, 10X Denhardt's solution, 2.5% herring sperm DNA, and 4X SSPE) at 42°C for 6 to 12 h. IL-1β cDNA (provided by S. Gillis, Immunex, Seattle, WA), TNF-α cDNA (Genentech, So. San Francisco, CA), collagen type I, αI cDNA (American Type Culture Collection 61322) collagen type III, αI cDNA (American Type Culture Collection 61324) and fibronectin cDNA (provided by Dr. Francisco E. Baralle, Trieste, Italy) were nick-translated with [α-^{32}P]dCTP (sp. act. 3000 Ci/mmol, New England Nuclear, Boston, MA) with a nick translation kit (Boehringer Mannheim, Indianapolis, IN). The hybridizations were carried out at 42°C for 10 to 40 h. The filter was then washed in a solution containing 2X SSC and 0.5% SDS at room temperature for 20 min followed by 0.1X SSC plus 0.5% SDS at 65°C for 30 min. To ascertain that equal amounts of RNA were loaded in individual lanes, the filters were reprobed with ^{32}P-labeled pHe7 cDNA (housekeeping gene) whose mRNA level is non-cell-cycle dependent (21). Autoradiography was performed at −70°C. Laser densitometry was used to quantitate mRNA levels.

ELISA assay for TNF-α and IL-1β

The TNF-α ELISA kit was purchased from Biokine (Minneapolis, MN); the IL-1β ELISA kit was kindly supplied by Dr. R. Dondero, Cistron Biotech, Pine Brook, NJ. For the assay, the frozen supernatants prepared from unstimulated or stimulated peripheral blood monocytes, or AM, were thawed at room temperature and added to wells of rigid flat bottom microtiter plates coated with murine mAb to human TNF-α or to human IL-1β. After incubation of the samples and thorough washing of the wells, HRP-conjugated anti-TNF-α antibody or anti-IL-1β antibody, respectively, was added to the test wells. After a second incubation, the excess HRP-conjugated antibody was removed by washing. The HRP substrate was then added, and the color intensity measured with a microtiter plate reader.

Statistics

Data were assessed using one-way Kruskal-Wallis Analysis of Variance (ANOVA). For both IL-1β and TNF-α the ANOVA was significant leading to Dunnett's test. Dunnett's test follows a significant ANOVA when one wants to compare disease or exposure groups with normal subjects. The Spearman rank correlation test was used to evaluate associations between TNF-α or IL-1β and pulmonary function or BAL parameters. The Student's *t*-test was used to compare results of BAL between disease groups and normal controls.

Results

Spontaneous release of cytokines from AM

BAL recovered significantly more cells in asbestos-exposed ($302 \pm 30 \times 10^3$ cells/ml) and IPF patients ($409 \pm 129 \times 10^3$ cells/ml) than in normal controls ($208 \pm 15 \times 10^3$ cells/ml, $p < 0.05$, both instances). The spontaneous release of IL-1β and TNF-α into culture medium over 18 h was higher in both patient groups than in normal controls (Figs. 1 and 2). Both patient groups released significantly more TNF-α than controls (TNF-α: asbestos-exposed 953 ± 192 pg/ml, $p < 0.005$ compared with normal subjects; IPF 791 ± 190 pg/ml, $p < 0.05$ compared with normal subjects; normal controls 290 ± 38 pg/ml). Only the asbestos-exposed released significantly more IL-1β than normal subjects (IL-1β: asbestos-exposed 760 ± 120 pg/ml, $p < 0.001$ compared with normal subjects; IPF 541 ± 193 pg/ml, NS, and normal subjects 138 ± 24 pg/ml).

There was a significant correlation between the number of neutrophils per milliliter of BAL fluid recovered and the spontaneous release of TNF-α for both groups combined ($r = 0.55$, $p < 0.05$). There were no other significant correlations between clinical parameters including pulmonary function values or BAL cells and cytokine release.

In addition to spontaneous protein release, fresh AM lavaged from individuals studied with asbestos exposure and IPF were evaluated for IL-1β and TNF-α gene expression by Northern analysis. Compared with a normal control, AM from diseased individuals expressed increased mRNA levels for both IL-1β and TNF-α, whereas levels of the housekeeping gene pHe 7 revealed similar amounts of RNA in each lane (Fig. 3).

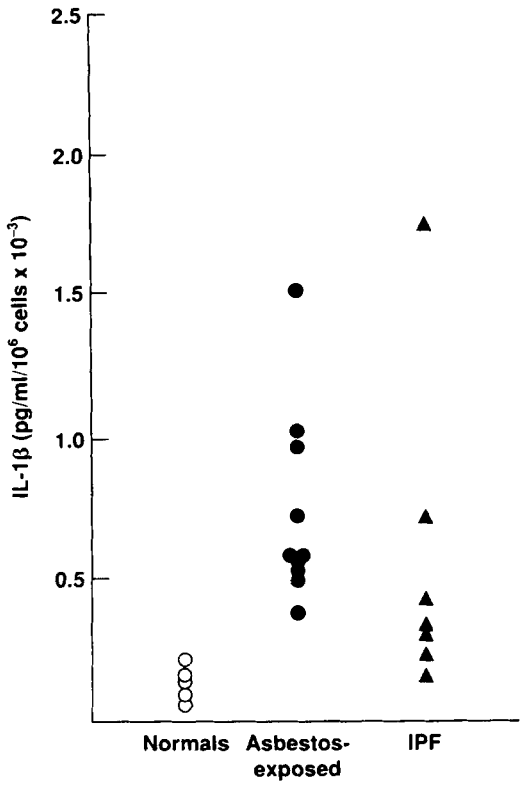

FIGURE 1. Spontaneous release of IL-1β from AM from normal persons and patients with IPF and asbestos exposure. Human AM recovered by BAL were cultured for 18 h and the supernatants assayed for IL-1β by ELISA.

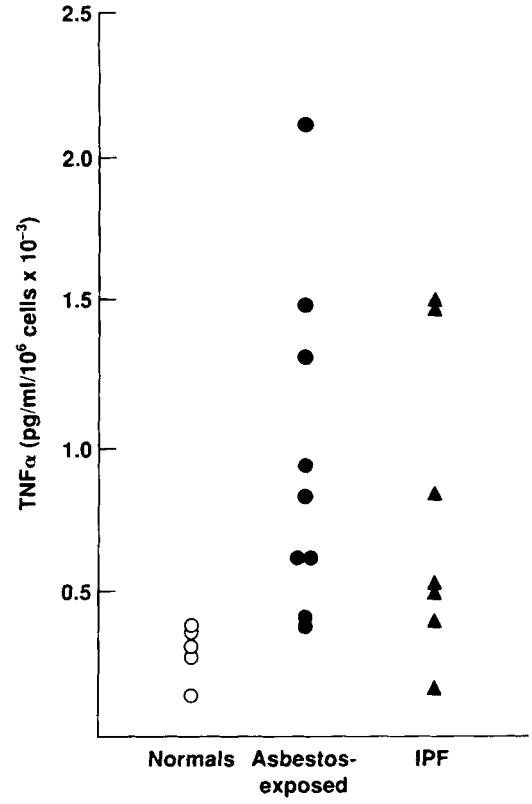

FIGURE 2. Spontaneous release of TNF-α from AM from normal persons and patients with IPF and asbestos exposure. Human AM were cultured for 18 h and supernatants were evaluated for TNF-α by ELISA. Both patient groups released significantly greater amounts of TNF-α than controls ($p < 0.05$).

Stimulated release of cytokines from mononuclear phagocytes

To further evaluate the release of these cytokines from mononuclear phagocytes, we evaluated a variety of potentially fibrogenic stimuli for their ability to cause release of IL-1β and TNF-α from pulmonary lavaged macrophages and human peripheral blood monocytes from normal volunteers. After a 24-h incubation, LPS (positive control, not shown), chrysotile asbestos, and silica stimulated >1500 pg/ml IL-1β release; amosite and crocidolite asbestos, and bleomycin-stimulated 500 to 1000 pg/ml. Beryllium was not different from control (Fig. 4). After a 24-h incubation, the stimulated release of IL-1β from normal AM was amosite 640 pg/ml, crocidolite 410 pg/ml, chrysotile 800 pg/ml, silica 1300 pg/ml, and untreated cells 15 pg/ml.

TNF-α release was increased for all types of asbestos, and silica (400 to 600 pg/ml), but less than the positive control, LPS (850 pg/ml, not shown) (Fig. 5). Beryllium was a weaker stimulus. Similar to IL-1β, the stimulated release of TNF-α from normal AM was amosite 380 pg/ml, crocidolite 420 pg/ml, chrysotile 410 pg/ml, silica 575 pg/ml, and untreated cells 10 pg/ml.

Northern analysis was utilized to determine if the stimulated protein release was accompanied by up-regulation of the mRNA for IL-1β and TNF-α in human blood monocytes over the first three hours of culture. The resting level of IL-1β mRNA was nondetectable in control samples. In marked contrast, chrysotile asbestos and silica and to a lesser extent crocidolite and amosite up-regulated IL-1β mRNA expression (Fig. 6*A*). Consistent with the protein data, bleomycin and beryllium were weaker stimuli. A housekeeping control gene (pHe 7) demonstrated that equal amounts of RNA were placed in each lane and that the up-regulation was specific.

The resting level of TNF-α mRNA also was barely detectable; amosite and chrysotile were the strongest stimuli for TNF-α mRNA up-regulation (Fig. 6*B*). Bleomycin and crocidolite asbestos were weaker stimuli for TNF-α mRNA up-regulation and protein release. Beryllium was not different from control at this time point. The control housekeeping gene (pHe 7) detected equal amounts of RNA in each lane.

TNF-α and IL-1β effect on fibroblast collagen and fibronectin gene expression

To study the role of mononuclear phagocyte production of TNF-α and IL-1β on extracellular matrix production, we

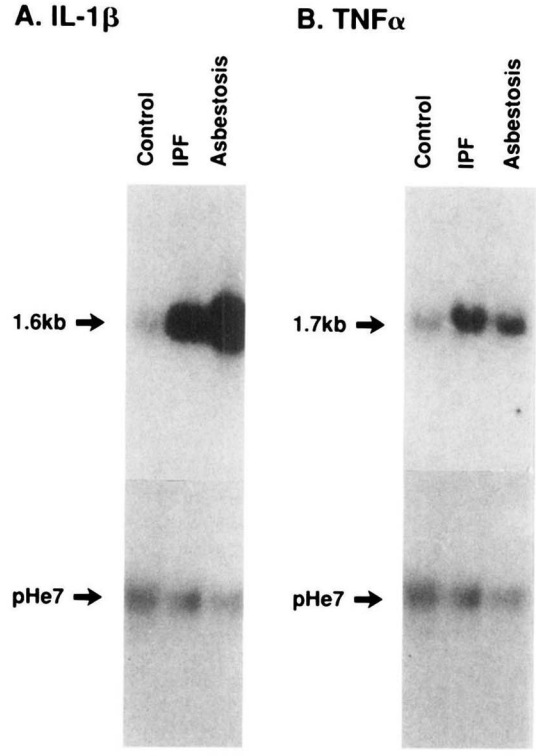

FIGURE 3. Northern analysis of IL-1β and TNF-α mRNA in AM from individuals with asbestos exposure, IPF, or control. Fresh AM were lysed in GITC buffer and 50 μg total RNA electrophoresed through a 1% denaturing gel containing 6% formaldehyde. *A*, expression of IL-1β mRNA was greater in AM from individuals with asbestos exposure or IPF than from a normal volunteer. *B*, expression of TNF-α mRNA in IPF and asbestos exposure is greater than in a normal control. Equal amounts of RNA were loaded into each lane as evaluated by hybridization with the housekeeping gene pHe 7 cDNA probe.

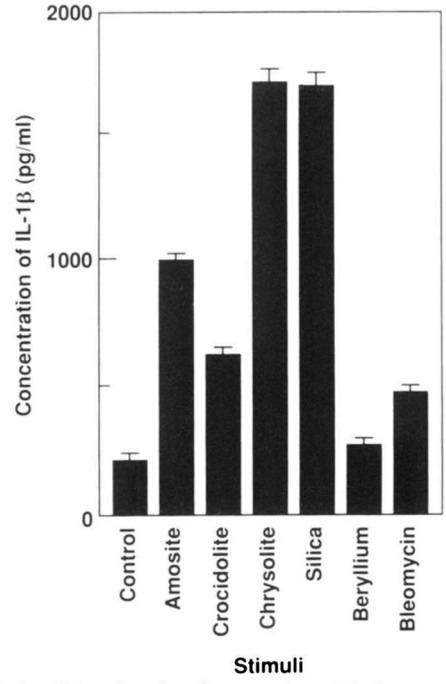

FIGURE 4. Stimulated release of IL-1β from peripheral blood monocytes. Human peripheral blood monocytes were isolated and cultured in the presence of various stimuli with the supernatants analyzed for IL-1β with ELISA. All three types of asbestos and silica were strong stimuli of IL-1β release.

chose the three most dominant components of intercellular matrix to test including collagens I and III and fibronectin. We stimulated human diploid lung fibroblasts with recombinant cytokines over a 2-h period in serum-free medium and performed Northern blot analysis. The collagen type I and type III probes detected a doublet band; and the fibronectin probe detected a single mRNA species. All three extracellular matrix genes were up-regulated after 2 h exposure to the recombinant cytokines (Fig. 7). After TNF-α, IL-1α, and IL-1β stimulation collagen type I mRNA expression increased 2.0-fold, 2.7-fold, and 2.3-fold, respectively; collagen type III mRNA expression increased 1.5-fold, 2.0-fold, and 1.8-fold, respectively; and fibronectin gene expression increased 2.1-fold, 1.7-fold, and 3.6-fold, respectively (Fig. 7).

Discussion

In the early course of IPF and asbestosis there is a striking accumulation of AM in alveolar spaces and ducts (22). These AM are activated and release peptide growth factors including PDGF, IGF-I, and various forms of transforming growth factor-β (6–8, 23–25). Past studies demonstrate significant macrophage-derived growth factors for fibroblast proliferation include PDGF and IGF-I (6, 7, 26). Morphometric studies of biopsies from patients with IPF and lungs from asbestos-exposed rats demonstrate increased numbers of cells in the interstitium including mononuclear phagocytes and fibroblasts (27–29). However, the role of TGF-β is more complex because it inhibits fibroblast proliferation in vitro and stimulates extracellular matrix accumulation including collagen and fibronectin both in vitro and in vivo (23–25).

Role of IL-1β and TNF-α in asbestosis and idiopathic pulmonary fibrosis

The increased production of IL-1β and TNF-α that we have observed in mononuclear phagocytes is caused by enhanced expression of the mRNA for the cytokines. These cytokines are well known to play an important role in the inflammatory response (30, 31). Activated AM release other inflammatory mediators such as PGE_2 and leukotriene B_4 after inorganic dust exposure (32–34). Leukotriene B_4 is chemotactic for neutrophils and can stimulate macrophage release of TNF-α, which stimulates the potent chemotaxin IL-8 (33, 35). In this regard, it is of interest to note that IL-8 release is increased in IPF and correlates with

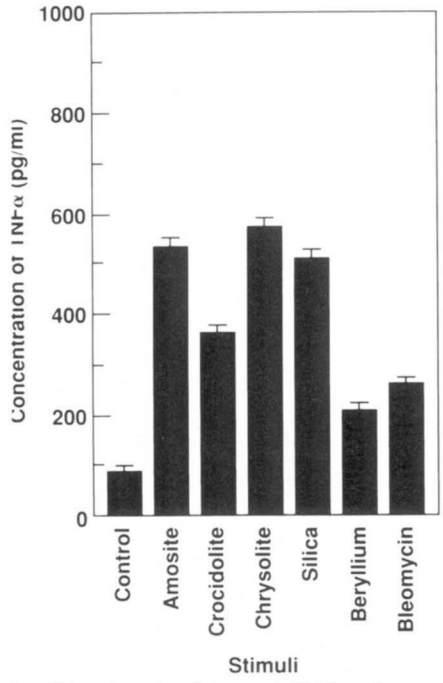

FIGURE 5. Stimulated release of TNF-α from peripheral blood monocytes. Human peripheral blood monocytes were isolated, cultured for 24 h in the presence of fibrogenic stimuli, and the supernatants analyzed for TNF-α with ELISA. All three types of asbestos and silica were strong stimuli of TNF-α release.

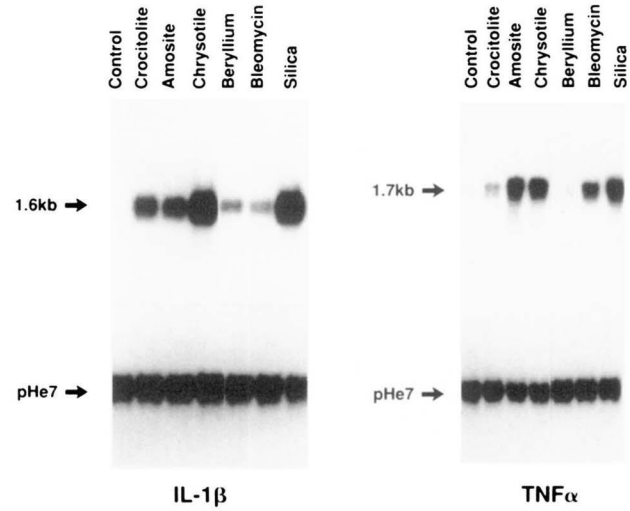

FIGURE 6. Northern analysis of in vitro stimulation of peripheral blood monocytes for IL-1β and TNF-α. Human blood monocytes were isolated, cultured in the presence of stimuli for 3 h, and total RNA was extracted and analyzed by Northern blot hybridization. *A*, striking up-regulation of IL-1β was noted for all three types of asbestos and for silica. *B*, similarly, TNF-α was up-regulated by the three asbestos types and silica, and bleomycin was not different from the control. The housekeeping gene pHe 7 was used as a control demonstrating an equal amount of RNA in each lane.

BAL neutrophils (9). Despite the low percentage of neutrophils in our patients we found that AM TNF-α correlated with the number of neutrophils recovered in BAL fluid. Furthermore, TNF-α directly stimulates neutrophils to release superoxide anion (36). Increased amounts of reactive oxidant species are released by BAL cells from patients with IPF and asbestosis (1, 5, 17). The mediators IL-1β and TNF-α are considered important mediators in an animal model of silicosis and AM release of TNF-α and IL-1β into cell supernatants correlated with histopathology (14, 37). Asbestos and silica but not other mineral dusts such as the innocuous titanium dioxide (TiO_2) can activate rat AM in vitro to release TNF-α and LTB_4 (38). Other inflammatory interstitial lung diseases such as sarcoidosis, hypersensitivity pneumonitis, and HIV-infected individuals spontaneously release increased amounts of IL-1β or TNF-α into macrophage supernatants, or are primed to do so after LPS stimulation (39–43).

Bleomycin, a potent fibrogenic agent, when injected intratracheally in mice induces pulmonary fibrosis with increased lung mRNA levels for TNF-α that can be significantly reduced by pretreatment with anti-TNF-α antibody (44). TNF-α stimulates the growth of diploid human fibroblasts at concentrations of 10^{-12} to 10^{-13} M, and acts synergistically with insulin (16). TNF-α increases the number of EGF receptors by 40 to 80% in those cells without affecting receptor-binding affinity after 2 to 4 h of in vitro treatment (45). TNF-α synergizes with either EGF or PDGF by stimulating fibroblast DNA synthesis and cell growth (46). IL-1β is a potent stimulus for PDGF AA release in fibroblasts and the mitogenic effect of IL-1β can be blocked by antibodies to PDGF-AA (47). TNF-α or IL-1 can rapidly increase cAMP levels and protein kinases, and elevate mRNA levels for early response proto-oncogenes c-*fos* and c-*myc* that are associated with early G_1 events of the cell cycle (48, 49). Finally, IL-1β and TNF-α induce c-*jun* in cultured fibroblasts that is followed by induction of the procollagenase gene, an important protease involved in the matrix remodeling observed in advanced IPF and asbestosis (50, 51). Both TNF-α and IL-1β are capable of inducing the interstitial collagenase type I gene and protein in fibroblasts; increased collagenase in lavage fluid of IPF patients has been previously reported and probably contributes to the extensive remodeling seen in biopsies of moderate to advanced idiopathic pulmonary fibrosis (52–55).

Effects of IL-1β and TNF-α on Collagen and Fibronectin Gene Expression

Heppleston and Styles demonstrated in 1967 that macrophages released a fibrogenic factor that stimulated enhanced collagen production after exposure to silica (56). In our studies, we demonstrated that IL-1β and TNF-α up-regulated mRNA expression of collagen types I and III, and fibronectin from cultured adult lung fibroblasts after a 2-h

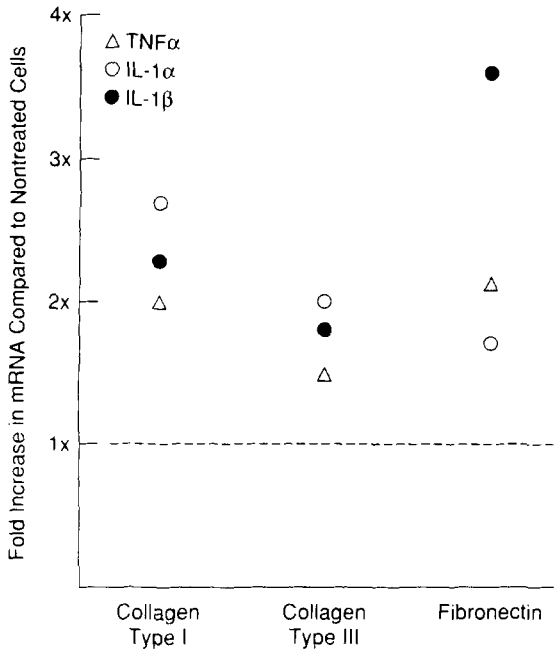

FIGURE 7. *Northern analysis of human lung fibroblasts for collagen types I and III and fibronectin gene expression after IL-1 or TNF-α stimulation. Human lung fibroblasts were cultured and stimulated with IL-1α (1 ng/ml), IL-1β (1 ng/ml) or TNF-α (10 ng/ml) and RNA was extracted as described in Materials and Methods. The -fold increase was A, collagen type I: TNF-α 2.0×, IL-1α 2.7×, IL-1β 2.3×; B, collagen type III: TNF-α 1.5×, IL-1α 2.0×, IL-1β 1.8×; and C, fibronectin: TNF-α 2.1×, IL-1α 1.7×, and IL-1β 3.6×.*

stimulation in serum-free conditions. Our results are consistent with the reports that rIL-1α and rIL-1β increased proα1 (I) and proα2 (I) collagen mRNA in several human diploid fibroblast cell lines including rheumatoid arthritis synovial fibroblasts (57–60). The hypothesis that IL-1β and TNF-α play a significant role in the accumulation of extracellular matrix and generation of fibrosis is supported by evidence that 1) s.c. low dose perfusion of TNF-α (35 ng/h, 7 days) in mice showed a local proliferation of fibroblasts, capillaries, and epidermal cells with a significant increase in hydroxyproline (15); 2) IL-1β- and TNF-α-stimulated extracellular matrix production (57–60); and 3) IL-1β and TNF-α are mitogenic, directly or indirectly, in vitro for fibroblast proliferation (13, 16, 47). In vitro experiments of skin fibroblasts treated with IL-1β or TNF-α in the presence of 10% FCS for up to 72 h have reported down-regulation of collagen gene expression and protein release (60). The decrease was dependent on de novo protein synthesis, as the TNF-α-dependent decrease in collagen I mRNA expression was blocked by cycloheximide. Thus the effect of IL-1β and TNF-α on the expression of collagen mRNA may be influenced by experimental conditions such as co-factors, e.g., PGE_2 and IFN-γ, that inhibit collagen synthesis at the gene level (61, 62), and variations among fibroblast cell lines tested (63, 64).

The enhanced release of IL-1β and TNF-α and growth factors in IPF and asbestosis provides a potential therapeutic opportunity in inhibiting their release. In this regard, augmentation of PGE in the lower respiratory tract may inhibit mesenchymal cell proliferation and have other therapeutic effects on growth factor release, oxidants, and neutrophil chemotaxis (65).

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