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Modulation of Platelet-Derived Growth Factor B mRNA Abundance in Macrophages by Colchicine and Dibutyryl-cAMP

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SUMMARY

Macrophage production of growth factors for fibroblasts, in particular platelet-derived growth factor B [PDGF(B)] and transforming growth factor-β (TGF-β), is thought to be central to the pathogenesis of pulmonary fibrosis. In a search for anti-inflammatory agents that might prevent this process, we asked whether colchicine might modulate the abundance of PDGF(B) and TGF-β mRNA, as well as the mRNA of early growth response gene 2 (*EGR2*), in human macrophages. Colchicine caused a dose- and time-dependent increase in PDGF(B), but not TGF-β or *EGR2*, mRNA in human macrophages derived from culture of peripheral blood monocytes. Similarly, colchicine caused an increase in PDGF(B) mRNA in human alveolar macrophages obtained from normal volunteers. Colchicine also caused an increase in PDGF(B) protein production by macrophages, as determined by enzyme-linked immunosorbent assay. Interferon-γ further increased the PDGF(B) mRNA abundance in human alveolar but not monocyte-derived macrophages. The effect of coincubation with dibutyryl-cAMP (dBcAMP) was assessed in an attempt to prevent the colchicine-induced increase in PDGF(B) mRNA. dBcAMP alone resulted in no increase in PDGF(B) mRNA or alteration in TGF-β mRNA but resulted in a reduction in *EGR2* mRNA. When added with colchicine, dBcAMP completely abrogated the colchicine-induced increase in PDGF(B) mRNA but had little effect on TGF-β mRNA. These data, showing that colchicine increased macrophage PDGF(B) mRNA in human macrophages and that this was prevented by coincubation with dBcAMP, lead us to speculate that colchicine may not be helpful in preventing the contribution of macrophage PDGF(B) gene activation to the pathogenesis of lung fibrosis. However, this effect of colchicine may be prevented by increasing intracellular cAMP in macrophages.

Fibroblast proliferation and matrix protein production are essential steps in the pathogenesis of lung fibrotic disorders including IPF. These two fibroblast functions are, in turn, regulated by cytokines from other cell types, which are present in chronically inflamed lung tissue in IPF. Macrophages are capable of producing fibroblast mitogens including the competence factor PDGF and the progression factor insulin-like growth factor, as well as TGF-β, which stimulates collagen production by fibroblasts. There is increasing evidence of the involvement of PDGF in the pathogenesis of IPF. PDGF is a 30-kDa homo- or heterodimer of A and B chains. PDGF(B) is produced by monocytes and macrophages (1–3). In alveolar macrophages from patients with a range of human lung fibrotic disorders, including IPF, there is an increased rate of transcription of the PDGF(B) gene, an increased abundance of PDGF(B) mRNA, and increased production of PDGF(B) protein (4–6). Furthermore, visualization of PDGF(B) mRNA by *in situ* hybridization of biopsies from patients with IPF showed that this mRNA was present in macrophages and epithelial cells in increased abundance (7, 8). There are similar data implicating TGF-β in the pathogenesis of IPF, inasmuch as an increase has been observed in TGF-β protein in lung biopsies from patients with IPF (9, 10).

A number of agents have been utilized as therapy for pulmonary fibrosis, although no one drug is universally effective. Corticosteroids produce a favorable response in only one fourth to one third of patients with IPF (11). Colchicine is another anti-inflammatory agent that may reduce fibrosis (12) and has attracted attention as an agent potentially capable of interfering with the pathogenesis of lung fibrosis (13). Because growth factor gene activation in lung macrophages is believed to be important in human lung fibrosis, it seemed appropriate to ask how potential therapeutic agents might modulate this aspect of the pathogenesis. We have previously found evidence that

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ABBREVIATIONS: IPF, idiopathic pulmonary fibrosis; PDGF(B), platelet-derived growth factor B; TGF-β, transforming growth factor-β; HBSS, Hanks' balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GTC, guanidinium isothiocyanate; dBcAMP, dibutyryl-cAMP; AMDGF, alveolar macrophage-derived growth factor; PBS, phosphate-buffered saline; MNC, mononuclear cells; Kb, kilobase(s); ELISA, enzyme-linked immunosorbent assay; MOPS, 3-(*N*-morpholino)propanesulfonic acid; *EGR2*, early growth response gene 2.

corticosteroids increase PDGF(B) mRNA in aveolar macrophages (14). In this study, we provide evidence that colchicine also caused an increase in PDGF(B) mRNA, but not TGF-β mRNA, in macrophages derived from human blood monocytes and in human alveolar macrophages obtained by broncho-alveolar lavage. Furthermore, the addition of the cAMP analogue dBcAMP was able to prevent the colchicine-induced increase in PDGF(B) mRNA abundance. These data lead to the speculation that colchicine, by increasing macrophage PDGF(B)mRNA, may not be helpful in treating this aspect of the pathogenesis of lung fibrosis. However, agents that increase intracellular cAMP may play a role in preventing this increase in macrophage PDGF(B) mRNA.

Materials and Methods

Monocyte isolation and differentiation to macrophages. Blood was collected from normal healthy volunteers for separation of MNC and autologus serum. Blood was diluted 50:50 with HBSS (Sigma, Poole, UK) containing 25 mM HEPES, pH 7.4 (Sigma). MNC were isolated on a Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient after centrifugation at 400 × *g* for 35 min at 18° and were washed twice in HBSS. The MNC were finally resuspended in 60 ml of M199 (GIBCO BRL, Paisley, Renfrewshire, UK), containing 5% heat-inactivated autologous serum, 25 mM HEPES, pH 7.4, 100 units/ml penicillin, and 100 μg/ml streptomycin solution (GIBCO BRL). Fifteen milliliters of this were added to each of four 100-mm tissue culture plates and incubated at 37° and 5% CO_2 for 3 hr, to separate adherent monocytes from nonadherent cells. After incubation the plates were gently washed three times with HBSS to remove nonadherent cells; to the adherent cells, 15 ml of M199 containing 5% autologous serum and antibiotics were added. The monocytes were allowed to differentiate to macrophages over the next 6 days. Medium was changed on days 4 and 6 by the removal and replacement of 10 ml/plate. The cells were stimulated with appropriate stimulant on day 6 for 24 hr.

Collection and separation of alveolar macrophages. A group of healthy volunteers who were smokers were recruited, and informed consent was obtained. This study has been approved by the local Ethics Committee. Bronchoscopy followed by broncho-alveolar lavage was performed by the standard technique (5). Sterile saline at room temperature was instilled into a segment of the right middle lobe in 60-ml aliquots, to a total of 240 ml. The aspirated fluid was collected into a 500-ml polypropylene bottle and placed on ice. After centrifugation (1000 rpm for 10 min at room temperature) broncho-alveolar lavage cells were resuspended in RPMI 1640 (GIBCO BRL) medium containing 100 units/ml penicillin, and 100 μg/ml streptomycin, and the cell count was adjusted to 1–2 × 10^6 cells/ml. Seven to 10 × 10^6 cells were incubated for 1 hr, after which nonadherent cells were removed by washing of the plate with prewarmed RPMI 1640. The adherent cells comprised >90% macrophages, as determined by morphology. The cells were then incubated in 5 ml of RPMI 1640 containing 10% fetal calf serum (ICN/Flow Laboratories, High Wycombe, UK), 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin.

Cell stimulation. The monocyte-derived macrophages and alveolar macrophages were stimulated with colchicine (Sigma) at a dose of 0.5 μg/ml unless otherwise stated, human recombinant interferon-γ (Collaborative Research Inc., Bedford, MA) at 500 units/ml, or dBcAMP (Sigma) at a dose of 1 mM. Cell cultures were incubated for 24 hr with these stimulants unless otherwise stated.

Isolation of mRNA. After incubation, the medium was removed and cells were lysed in 0.5 ml of GTC solution containing 4 M GTC (BDH Ltd., Poole, UK), 55 mM sodium citrate, pH 7, 17 mM sodium *n*-lauryl sarcosine, and 0.14 M 2-mercaptoethanol (Sigma). RNA was extracted by a modification of the acid-GTC-phenol-chloroform method of Chomczynski and Sacchi (15). The sample was transferred to microfuge tubes, and to each tube was added 0.1 volume of 2 M sodium acetate, pH 4, 1 volume of water-saturated phenol (Rathburn Chemicals, Walkerburn, Peebleshire, UK), and 0.33 volume of chloroform/isoamyl alcohol (49:1) (BDH Ltd.). The tubes were inverted and vortexed after each addition. The samples were incubated on ice for 20 min before centrifrigation for 20 min at 10,000 × *g* at 4°. After centrifrigation the aqueous layer was aspirated and precipitated with 1 volume of propan-2-ol (BDH Ltd.) at −20° for 1 hr. Samples were recentrifuged at 10,000 × *g* at 4° for 20 min, and RNA pellets were resuspended in GTC and reprecipitated with propan-2-ol. After centrifugation the pellets were washed with 75% ethanol and resuspended in sample buffer (65% deionized formamide, 15% formaldehyde, 0.05 M MOPS (Sigma). Samples were treated at 65° for 10 min and stored at −20°.

Northern (RNA gel) analysis. For all experiments equal amounts of RNA (7–15 μg) were loaded onto a 1% agarose/formaldehyde gel (16), separated by electrophoresis, and blotted onto a nylon membrane (Hybond-N; Amersham Life Sciences, UK). In all experiments the amounts of RNA loaded were shown to be the same by staining of the gel with ethidium bromide and photography under UV light. The membranes were prehybridized at 42° before hybridization, as described previously (5), except 0.5 mg/ml herring testes DNA (Sigma) was used instead of salmon sperm DNA. Blots were hybridized at 42° for 24 hr with probes labeled with random primers, using [α-^{32}P]dCTP (ICN Biomedicals, Irving, UK). After hybridization the membranes were washed. Autoradiography was performed using X-ray film (X-Omat XAR-5; Eastman Kodak Co., Rochester, NY) at −70°. The relative intensities of the bands on the autoradiographs were compared by scanning densitometry, using a Chromoscan 3 (Joyce Loebl Ltd., Tyne and Wear, UK). The results were expressed as mean ± standard error of percentages of the maximum (100%) response in each experiment.

DNA probes. The human DNA probes used were a 0.75-kb *Eco*RI c-*sis* fragment (gift of M. Murray, Zymo Genetics, Seattle, WA), a 1.65-kb *Eco*RI TGF-β_1 fragment (gift of A. Purchio, Oncogene, Seattle, WA), a 0.6-kb *Hind*III-*Eco*RI *EGR2* fragment (gift of V. P. Sukhatme, University of Chicago, Chicago, IL), and a 3.1-kb *Eco*RI HLA-DR-α fragment (gift of S. Weissman, Yale University, New Haven CT). In all cases probe specificity was high, with the approximate sizes of the mRNA, as assessed in relation to 28 S (4.7-kb) and 18 S (1.9-kb) rRNA, being 3.8 kb for PDGF(B), 2.4 kb for TGF-β, 3.5 kb for *EGR2*, and 0.76 kb for HLA-DR-α.

Cell Viability. Cell viability was determined by the trypan blue (Sigma) exclusion test, as per the manufacturer's recommendations.

ELISA for detecting human PDGF(B) protein. The methods were modified from those of Gersuk *et al.* (17). Wells of a 96-well flat-bottomed SolidPhase 2 microplate (Alpha Laboratories, Eastleigh, Hampshire, UK) were incubated for 18 hr at 4° with 35 μl of supernatants from alveolar or monocyte-derived macrophages, made up to 100 μl with coating buffer (carbonate buffer, pH 9.6). Unincorporated binding was blocked with 100 μl of PBS containing 2% bovine serum albumin (Sigma), for 1 hr at 37°, and the wells were washed with PBS-Tween 20 (0.05%). One hundred microliters of anti-PDGF(BB) antibody (R&D Systems, Minneapolis, MN), diluted 1/500 in PBS containing 0.5% bovine serum albumin, were added and incubated for 1.5 hr at 37°. The wells were washed three times and incubated for 1.5 hr at 37° with 100 μl of horseradish peroxidase-labeled rabbit anti-goat IgG antibody (DAKO, High Wycombe, Buckinghamshire, UK), which was diluted 1/1000. After three washes, 100 μl of substrate [5-mg tablet of *o*-phenylenediamine (Sigma) dissolved in 12.5 ml of phosphate citrate buffer with sodium perborate (Sigma)] were added for 30 min. The reaction was stopped by adding 100 μl of 1 M HCl and absorbances were measured at 490 nm using a Dynatech MR5000 ELISA reader (Dynatech Laboratories, Billinghurst, W. Sussex, UK.). The data were analyzed in comparison to known concentrations of recombinant PDGF(BB) (Boehringer Mannheim, Lewes, E. Sussex, UK), which were assayed along with the samples. Controls consisted of wells

containing only the coating buffer, wells without anti-PDGF(BB) antibody, and wells without horseradish peroxidase conjugate.

Results

Dose dependence of colchicine-induced increase in PDGF(B) mRNA. Colchicine caused an increase in PDGF(B) mRNA in monocyte-derived macrophages in a dose-dependent manner (Fig. 1). Maximum abundance of PDGF(B) mRNA was observed after 0.5 μg/ml colchicine treatment. A smaller increase in PDGF(B) mRNA abundance was observed after treatment with the lower dose, 0.05 μg/ml, of colchicine. After 0.005 μg/ml colchicine treatment the PDGF(B) mRNA abundance was similar to that in cells incubated in medium alone. In contrast, the abundance of TGF-β mRNA was similar in cells treated with medium alone or a dose range of colchicine. When *EGR2* mRNA was measured, there was a small increase in *EGR2* mRNA abundance with 0.005 μg/ml colchicine. At higher drug concentrations *EGR2* mRNA abundance was similar to that in cells incubated in medium alone.

Duration of colchicine exposure required to increase PDGF(B) mRNA abundance. To determine the optimum time required to increase PDGF(B) mRNA, monocyte-derived macrophages were treated with 0.5 μg/ml colchicine and the cells were harvested at 0, 2, 8, and 24 hr. There was an increase in PDGF(B) mRNA abundance in a time-dependent manner (Fig. 2). The maximum increase in PDGF(B) mRNA was observed when cells were exposed to colchicine for 24 hr. TGF-β mRNA abundance was unaltered over this time course. When *EGR2* mRNA was measured there was an initial decrease when cells were exposed to colchicine for 2 hr, followed by an increase in *EGR2* mRNA to an abundance slightly below that observed in cells incubated in medium at the 24-hr time point.

Modulation of colchicine-dependent increase in PDGF(B) mRNA by interferon-γ in monocyte-derived macrophages. To investigate whether interferon-γ further increased the colchicine-dependent increase in PDGF(B) mRNA, colchicine and interferon-γ were added together to the monocyte-derived macrophages. There was no increase in PDGF(B) mRNA abundance when interferon-γ and colchicine were combined, compared with stimulation by colchicine alone (Fig. 3). The abundance of TGF-β mRNA was also relatively unaffected. Both the cells stimulated by colchicine plus interferon-γ and those stimulated by colchicine alone were similar to cells incubated in medium alone, with respect to TGF-β mRNA abundance. Interferon-γ was shown to be active in conjunction with colchicine when HLA-DR-α mRNA was measured. The combination of interferon-γ and colchicine resulted in a greater abundance of HLA-DR-α mRNA, compared with cells stimulated with colchicine or medium alone.

Effect of colchicine on PDGF(B) mRNA abundance in alveolar macrophages. To determine whether the colchicine-induced increase in PDGF(B) mRNA in monocyte-derived macrophages also occurred in normal human lung macrophages, alveolar macrophages were studied. Colchicine caused an increase in the PDGF(B) mRNA abundance of approximately 8-fold in alveolar macrophages (Fig. 4). Moreover, in contrast to monocyte-derived macrophages, the increase in PDGF(B) mRNA was further increased when alveolar macrophages were stimulated with both interferon-γ and colchicine in combination.

Increase in PDGF(B) protein production after colchicine. To determine whether colchicine increased PDGF(B) protein levels in the culture supernatants, we developed an ELISA to detect PDGF(B). Using recombinant PDGF(BB), the sensitivity of the ELISA was 0.3–20 ng/ml. When supernatants from stimulated cells were examined, there was an increase in PDGF(B) protein in supernatants from macrophages stimulated with colchicine, compared with cells incubated in medium alone ($p < 0.05$) (Fig. 5). There was a similar but less marked increase in PDGF(B) protein in the supernatants of monocyte-derived macrophages after colchicine stimulation.

Effect of dBcAMP on colchicine-mediated PDGF(B) mRNA increase in monocyte-derived macrophages. We sought to determine whether the increase in PDGF(B) mRNA after colchicine treatment was prevented by the addition of dBcAMP. Treatment with dBcAMP alone did not result in an increase in PDGF(B) mRNA (Fig. 6). However, coincubation of dBcAMP with colchicine resulted in a reduction in PDGF(B) mRNA abundance, compared with cells incubated with colchicine alone. Neither colchicine, dBcAMP, nor the combination resulted in an appreciable alteration in the abundance of TGF-β mRNA. There was a modest decrease in *EGR2* mRNA abundance in cells incubated with colchicine, compared with cells incubated in medium alone. When dBcAMP was added alone or in conjunction with colchicine, there was a marked reduction in *EGR2* mRNA. Cell viability was slightly reduced by the addition of colchicine (Table 1). There was no further loss of

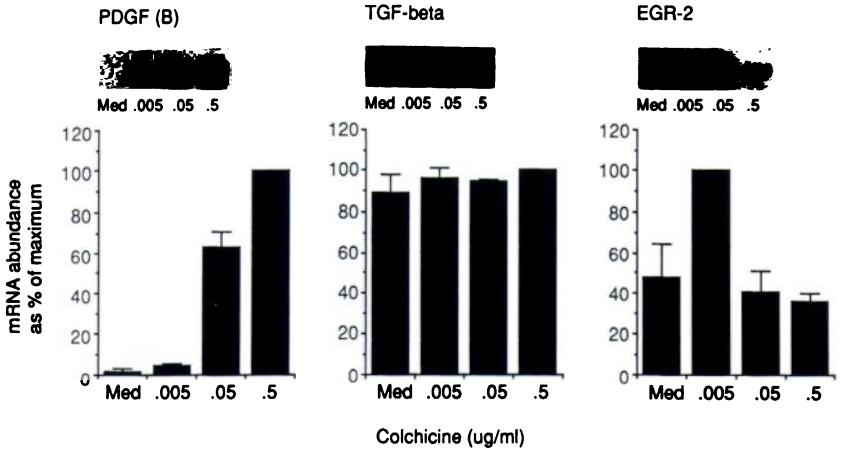

Fig. 1. Dose response. PDGF(B), TGF-β, and *EGR2* mRNA abundance in monocyte-derived macrophages after stimulation with increasing concentrations of colchicine, to a maximum of 0.5 μg/ml. Relative mRNA abundance was expressed as a percentage of the maximum (100%) response in each experiment. *Insets*, representative autoradiographs of Northern blots. *Med*, medium.

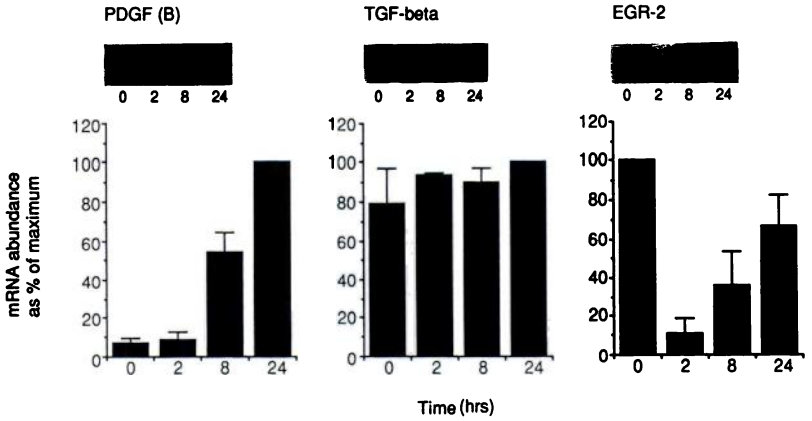

Fig. 2. Time course. Time of exposure to colchicine required for up-regulation of PDGF(B) and relative abundance of TGF-β and *EGR2* mRNA expression, in monocyte-derived macrophages. Cells were harvested 0, 2, 8, and 24 hr after treatment with colchicine. *Insets*, representative autoradiographs of Northern blots.

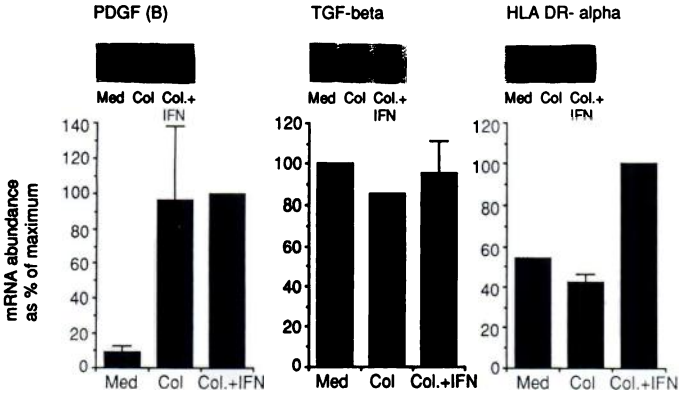

Fig. 3. Modulation of colchicine-dependent increase by interferon-γ. Relative abundance of PDGF(B), TGF-β, and HLA-DR-α mRNA expression in monocyte-derived macrophages, after stimulation with colchicine (*Col*) and interferon-γ (*IFN*). *Insets*, representative autoradiographs of Northern blots. *Med*, medium.

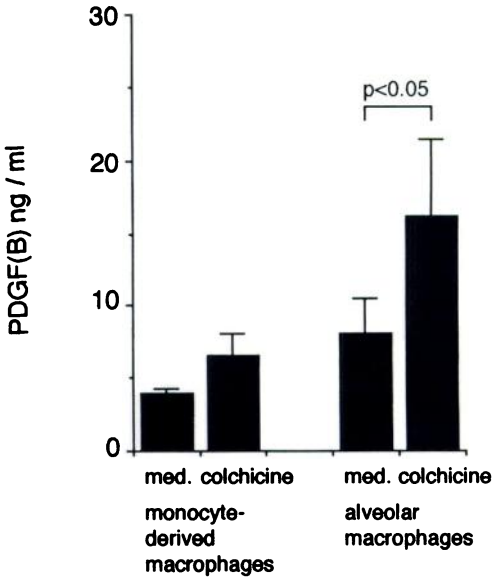

Fig. 5. Concentration of PDGF(B) protein in supernatants from monocyte-derived macrophages and alveolar macrophages incubated with colchicine or medium alone (*med.*) for 24 hr. The results are expressed as the mean ± standard error (monocytes, six experiments; alveolar macrophages, seven experiments).

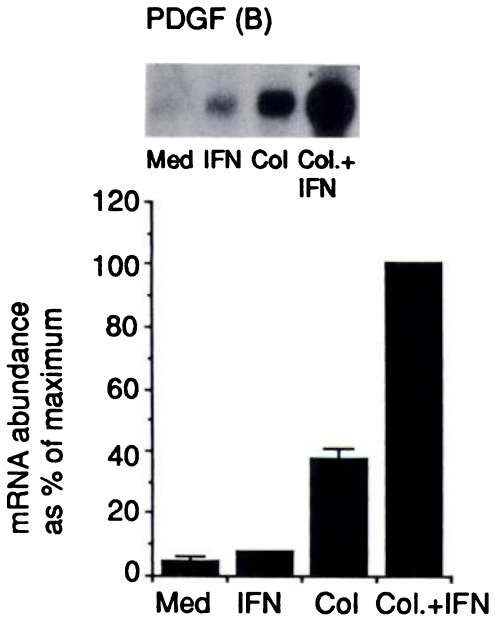

Fig. 4. Colchicine-induced PDGF(B) mRNA increase in human alveolar macrophages. PDGF(B) mRNA abundance in alveolar macrophages stimulated with medium alone (*Med*), interferon-γ (*IFN*), colchicine (*Col*), or both in combination, expressed as a percentage of maximum. *Insets*, representative autoradiographs of Northern blots of RNA from alveolar macrophages stimulated as indicated.

viability when dBcAMP was added in conjunction with colchicine.

Discussion

The role of colchicine in the treatment of fibrotic disorders is unclear. Colchicine is known to have anti-inflammatory actions and has a proven therapeutic role in disorders such as gout and familial Mediterranean fever. Colchicine prevents the migration of inflammatory cells into damaged tissues (18) and down-regulates tumor necrosis factor receptors on monocytes (19). However, colchicine may have proinflammatory actions, because it can increase IL-1 production (20). Colchicine has traditionally been thought to have antifibrotic effects. Thus, fibroblast stimulation, by supernatants from lipopolysaccharide-stimulated, monocyte-enriched, peripheral blood MNC, was reduced if the blood was collected from patients taking colchicine (21). Colchicine also causes macrophages to reduce production of fibronectin and AMDGF (13, 20). Because AMDGF comprises a number of growth factors, among which PDGF(B) is thought to be prominent (22), we were surprised that colchicine markedly increased PDGF(B) mRNA and

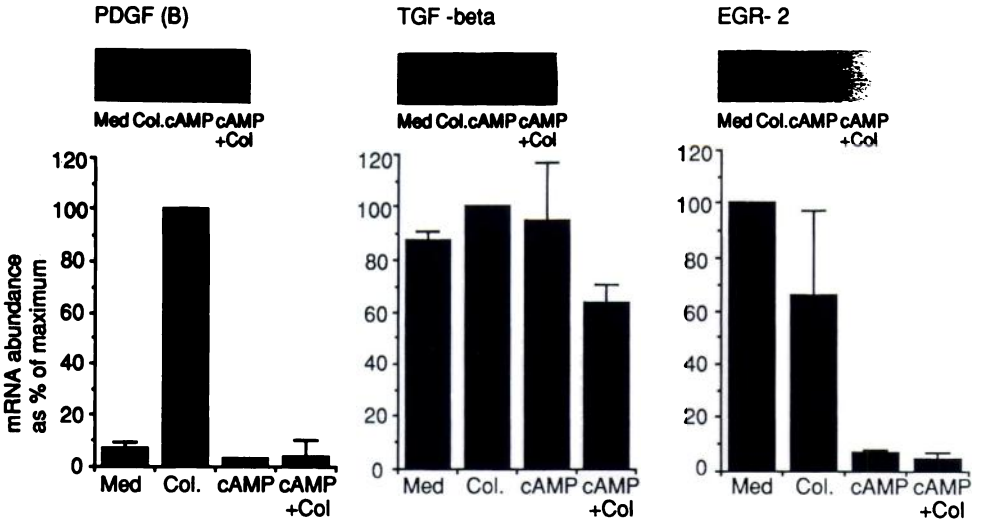

Fig. 6. Effect of dBcAMP on colchicine-mediated PDGF(B) mRNA increase. PDGF(B), TGF-β, and *EGR2* mRNA expression in monocyte-derived macrophages stimulated with colchicine (*Col*), dBcAMP (*cAMP*), or the combination. Results are expressed as a percentage of the maximum (100%). *Insets*, representative autoradiographs of Northern blots. *Med*, medium.

TABLE 1

Viability of monocyte-derived macrophages after treatment for 24 hr with medium alone, colchicine, dBcAMP, or the combination

Values are mean ± standard error (three experiments).

Viability			
Medium	Colchicine	dBcAMP	Colchicine + dBcAMP
%			
99.3 ± 0.57	93.3 ± 7.6	96.0 ± 1.0	92.6 ± 2.5

PDGF(B) protein production. This may reflect the fact that the original studies on AMDGF were performed before the ability existed to measure PDGF(B) mRNA or protein; thus, an isolated increase in PDGF(B) may have been masked by a reduction in other growth factors. Not only are the *in vitro* effects of colchicine complex, but a clear understanding of the role of colchicine has not been provided by clinical studies. Colchicine has not found a place in the treatment of lung fibrosis. It is thus possible that the drug affects many cells and has diverse actions on different aspects of the pathogenetic pathway that leads to tissue fibrosis.

Colchicine acts by depolymerizing microtubules in the cell. The mechanism by which alterations in microtubule polymerization affect gene activation are unknown. The present study supports the view that there is communication between cytoskeletal elements and the PDGF(B) gene. Such communication was suggested when cytocholasin D, an agent that disrupts cytoskeletal integrity, reduced PDGF(B) mRNA during monocyte adherence (23). Studies to define the exact mechanism by which colchicine interacts with transcriptional and post-transcriptional mechanisms are currently in progress.

Because in human lung fibrotic disease one potential therapeutic goal is the reduction of macrophage PDGF(B), the action of dBcAMP was of great interest. In the monocyte-derived macrophages, there was a low abundance of PDGF(B) mRNA before stimulation. dBcAMP had little effect on PDGF-B mRNA in these unstimulated cells but prevented the increase in PDGF(B) mRNA in cells after colchicine stimulation. These data raise the possibility that increases in intracellular cAMP concentrations prevent PDGF(B) gene activation in macrophages, as has been suggested for some tumor cell lines (24). The observation that dBcAMP has opposing actions, compared with colchicine and dexamethasone, on PDGF(B) mRNA abundance in monocytic cells is in keeping with the effects of these agents on collagenase synthesis. Dexamethasone and colchicine inhibit collagenase synthesis in cultured monocytes, whereas this is restored by dBcAMP (25). The ability of cAMP analogues to modulate the expression of mRNA of a number of inflammatory cytokines in monocytes and macrophages (26, 27), as well as PDGF(B), may offer an avenue for future therapeutic intervention.

Interferon-γ alone or in combination with dexamethasone is known to increase macrophage PDGF(B) mRNA (5, 13). Interferon-γ had little effect on the colchicine-induced increase in PDGF-B in monocytes but caused a further increase in the colchicine-induced increase in PDGF(B) mRNA in alveolar macrophages. The increase in HLA-DR-α mRNA after interferon-γ treatment served to confirm the activity of interferon-γ.

There is increasing evidence for the importance of TGF-β in the pathogenesis of IPF (9, 10). TGF-β mRNA abundance in macrophages appears to be regulated differently from PDGF(B) mRNA abundance. There appears to be a less clear-cut relationship between alveolar macrophage TGF-β mRNA and the clinical status of the patient (5). TGF-β mRNA abundance appeared to be little affected by corticosteroids (14) or, in this study, by colchicine alone or in combination with dBcAMP. These results suggest that reduction of TGF-β production may not be best achieved by modulating TGF-β gene activation.

The *EGR2* gene encodes a zinc finger-containing protein, which has DNA-binding capability and, thus, may modulate subsequent gene activation (28). Previous studies have suggested that *EGR2* gene activation is involved in monocyte to macrophage differentiation (23, 29). For the purposes of the present study, *EGR2* mRNA abundance served as a control for the action of colchicine and dBcAMP.

Human monocyte-derived macrophages serve as a model for tissue macrophages. Such monocyte-derived macrophages are recognized to express PDGF(B) mRNA (3). However, there are clearly differences between *in vitro* derived macrophages and alveolar macrophages, as illustrated when surface phenotype analysis suggested that monocyte-derived macrophages were not identical to alveolar or peritoneal macrophages (30). Of interest in the context of lung fibrosis, alveolar macrophages

in IPF have been found to exhibit a more monocyte-like immunophenotype (31). The relevance of observations made in the present study using *in vitro* derived cells was confirmed when alveolar macrophages from normal human volunteers were studied. Cells from smokers were utilized to provide sufficient cell numbers for all experimental conditions.

Cell viability is an important consideration in studies using pharmaceuticals agents. In the present study colchicine caused a small decrease in cell viability, from 99 to 93%. Despite this slight loss of viability with colchicine, there was an increase in PDGF(B) mRNA with modest changes in *EGR2* mRNA and no change in TGF-β mRNA. The reduction in PDGF(B) and *EGR2* mRNA after the subsequent addition of dBcAMP was thought to be of therapeutic importance because addition of dBcAMP had little effect on viability and did not affect TGF-β mRNA.

The present study provided evidence that colchicine had different effects on PDGF(B) and TGF-β mRNA abundance in macrophages. In particular, colchicine caused a marked increase in PDGF(B) mRNA. Furthermore, the cAMP analogue dBcAMP reduced the colchicine-induced increase in macrophage PDGF(B) mRNA. This appreciation of the ability to therapeutically manipulate macrophage PDGF(B) mRNA may provide initial insights into possible therapeutic strategies for diseases such as IPF, where macrophage PDGF(B) production is believed to be important. The present study also suggests that it will be important to consider the concurrent therapy when the involvement of PDGF(B) in the pathogenesis of disease is inferred on the basis mRNA measurement or mRNA *in situ* hybridization data.

Acknowledgments

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