INTRODUCTION

Several cytokines are produced by IL-2 stimulated PBL (Kovacs et al., 1989a) and many of these mediators are capable of either directly or indirectly inducing fibroblast proliferation and connective tissue (collagen) deposition (Freundlich et al., 1986). It has been shown that macrophages, for example, produce several growth factors for fibroblasts, including interleukin-1 (IL-1; Schmidt et al., 1982), tumor necrosis factor-α (TNFα; Beutler et al., 1985), platelet derived growth factor (PDGF; Shimakado et al., 1985), fibroblast growth factor (FGF; Baird et al., 1985), and TGFβ (Assollant et al., 1987). Two of these cytokines, TGFβ and PDGF are highly fibrogenic (Sporn and Roberts, 1986). aberrant production of these mediators in animal models of lung fibrosis have demonstrated that activated macrophages secrete cytokines which induce the proliferation of cultured fibroblasts (Kovacs and Kelley, 1985a). An examination of the connective tissue production in a macrophage-fibroblast co-culture system revealed that the effect of macrophages on fibroblast collagen synthesis can be both qualitative, as well as quantitative, with an increase in the ratio of type III relative to type I collagen produced (Kelley et al., 1981).

The studies described herein involve a multifaceted analysis of the role of fibrogenic cytokines in the production of scar tissue. It includes 1) the induction of fibroblast proliferation and connective tissue production by mediators produced by PBL and, 2) the expression of genes which code for cytokines (PDGF and TGF-β) reported to trigger the production of connective tissue.
MATERIALS AND METHODS

Reagents. Human recombinant IL-2 was provided by Dr. Craig Reynolds (Biological Response Modifiers Program, Frederick, MD). PDGF, TGF-β and antibody against TGF-β were purchased from R & D Systems (Minneapolis, MN). Antibody against PDGF AB heterodimer was obtained from Genzyme (Boston, MA).

Isolation of subpopulations of PBL and generation of supernatant fluids. PBL obtained from normal healthy volunteers were incubated at a density of 2 x 10^6 cells/ml in AIM V or RPMI 1640 containing 1% fetal bovine serum (FBS), 100 u/ml penicillin sulfate, 100 μg/ml streptomycin, 2 mM glutamine (GIBCO, Grand Island, NY) with 100 U/ml IL-2 as described (Beckner et al, 1987). Cells and supernatants were harvested at various times for measurement of growth factor and connective tissue inducing activities and cytokine mRNA expression. In some studies adherent and non-adherent cell populations were separated as previously described (Kovacs et al, 1989a).

Purified monocytes were obtained after incubation for 2 hours, followed by removal of non-adherent cells. Monocytes were incubated overnight to allow for the induction and diminution of adherence induced expression of cytokine mRNAs previously reported by Fulhbrigge and coworkers (Fulhbrigge et al, 1987). The following morning fresh medium was added with or without IL-2 (100 U/ml) or LPS (10 μg/ml) for 5 hours (for RNA analysis) or 18 hours (for assessment of biological activity).

Fibroblast proliferation assays. NIH 3T3 cells and a rat lung fibroblast cell line (designated RL6.88, a rat lung fibroblast cell line derived in the laboratory from an adult male Sprague Dawley rat) were cultured in Dulbecco’s Modified Eagle’s Medium with 10% FBS, penicillin (100 u/ml) streptomycin (100 μg/ml) and glutamine (2 mM) in an atmosphere of 5% CO₂ in air at 37°C. Incorporation of ³H-TdR was used as an index of DNA synthesis according to the method of Glenn and Ross (Glenn and Ross, 1981) modified by Kovacs and Kelley (Kovacs and Kelley, 1985a). In brief, fibroblasts were plated and grown to confluency. Putative growth factor containing media were added in triplicate for 18 hours after which cells were pulsed for 2 hours with ³H-TdR. ³H-TdR incorporation was quantitated by liquid scintillation counting. Fresh medium containing 10% FBS served as a positive control. This level ranged from 8,000 to 29,000 cpm over the course of these studies. The amount of ³H-TdR incorporation triggered in fibroblasts by medium containing 10% FBS was always 5-fold that of cells cultured in medium without FBS.

For cell proliferation studies, NIH 3T3 cells were seeded at 2,000 cells/well (in 0.2 ml RPMI 1640 medium with 1% FBS) in 96
well plates and test media were added in triplicate. After 4 days, media were removed, cells were washed with phosphate buffered saline, fixed, and stained with 0.2% crystal violet in 2% ethanol. Bound dye was eluted in 0.2 ml of 1% sodium dodecyl sulfate and absorbance determined at 570 nm using a Dynatech (Chantilly, VA) ELISA plate reader.

For most studies we elected to utilize the \(^3\)H-TdR incorporation assay, because it permits the analysis of direct acting growth. The cell proliferation assay was used to confirm that mediators produced by activated PBL allow fibroblasts to complete the cell cycle and replicate.

Quantitation of connective tissue production. Collagen production was measured in WI38-VA13 (an immortalized human lung fibroblast cell line, ATCC, Rockville, MD). WI38-VA13 cells were cultured with \(^8\)-aminopropionitrile and ascorbic acid in the presence or absence of supernatants from IL-2-treated PBL for 24 hours. \(^3\)H-proline was then added to the cultures for an additional 18 hours. The incorporation of labelled proline into collagen was quantitated by the release of tritium into the supernatant following collagenase treatment (Peterkovsky and Diegeman, 1971). In brief, cell layer and supernatant were collected, precipitated with trichloroacetic acid, and washed. The precipitates were treated with collagenase (Form III; American Biofactures, Lynbrook, NY) and the counts liberated by enzyme digestion were measured by liquid scintillation counting.

Immunoblot analysis of cytokine protein levels. Recombinant cytokines and supernatants from cultured cells were blotted on nitrocellulose filters. The filters were blocked and sequentially incubated with primary and secondary antibodies. Primary antibodies included antibodies specific for human PDGF AB (Genzyme) and porcine TGF\(\beta^1\) (R & D Systems). The anti-TGF\(\beta^1\) antibody reacts with TGF\(\beta^1\) and TGF\(\beta^2\). The second antibody is biotin-conjugated anti-rabbit IgG. The filters were then incubated with avidin-conjugated alkaline phosphatase followed by a substrate for color development.

RNA isolation and hybridization. Fibroblasts were cultured for 18 hours with supernatants from IL-2-treated PBL or recombinant cytokines after which cells were scraped with a rubber scraper into guanidine-isothiocyanate (Chirgwin et al, 1979) and centrifuged on cesium chloride density gradients (Glisin et al, 1974). Monocytes and macrophages were also scraped into guanidine-isothiocyanate. Northern blot analysis was performed using cDNA probes labelled by random priming with \(^3\)P-dCTP as previously described (Kovacs et al, 1986). cDNA probes used in these studies were obtained from the following sources: procollagen \(\alpha1(I)\) from Dr. D. W. Rowe (Univ. of
Connecticut, Farmington, CT); rat fibronectin from Dr. R. O. Hynes (MIT, Cambridge, MA); murine TGF-β was obtained from Dr. R. Derynk (Genentech, S. San Francisco, CA); c-sis (PDGF B chain) from ATCC (Rockville, MD); and histone H4 from N. Heinz (Rockefeller Univ, New York, NY).
RESULTS

Induction of fibroblast proliferation by cytokines secreted by activated PBL. Supernatant fluids from IL-2 treated PBL stimulate the incorporation of $^3$H-TdR into quiescent monolayers of NIH 3T3 fibroblasts (Table 1). A comparison of the growth factor activity in supernatant fluids from PBL cultured for 4 days with IL-2 in serum-free, defined medium (AIM V) reveals that the level of growth factor activity is comparable to the growth promoting activity in medium containing 10% FBS (which serves as a positive control). This amount of growth factor triggers a 3-fold induction of $^3$H-TdR incorporation over the level of growth factor activity in the defined medium. IL-2 alone failed to induce $^3$H-TdR incorporation in confluent monolayers of fibroblasts (data not shown).

A kinetic analysis reveals that supernatants collected after 24 hours of culture of PBL with IL-2 exhibit a 2-fold enhancement of fibroblast $^3$H-TdR incorporation over the level induced by PBL cultured in the absence of IL-2 (Figure 1a). The IL-2-induced growth factor activity reached a peak at 3-4 days of culture that was 4-fold higher than supernatants collected from untreated PBL. NIH 3T3 fibroblasts exhibit a dose dependent incorporation of $^3$H-TdR in response to supernatants from IL-2 treated PBL (Figure 1b).

In addition to time dependence, the production of fibroblast growth inducing mediators by PBL is dependent on the concentration of IL-2 in the medium (Figure 2). At day 3, maximal levels of mediators are released by PBL when cultured with 100 U/ml of IL-2. Minimal detectable levels of growth factor activity is present at 3 days of culture with 33 U/ml of IL-2.

To confirm that growth factor activities secreted by IL-2-treated PBL were capable of triggering an increase in cell number (and not merely the induction of $^3$H-TdR incorporation), preconfluent cultures of NIH 3T3 cells were incubated in the absence or presence of supernatants for 3 days. Supernatants from IL-2 treated PBL from 18 patients and volunteers prepared on different days over a 5 month period of time all triggered the proliferation of fibroblasts. The stimulation of fibroblast proliferation from individual samples ranged from 2- to 5-fold that of fibroblasts cultured with medium alone (Table 2).

Since NIH 3T3 cells are a transformed fibroblast-like cell line, we wanted to confirm that the growth factor activity (or activities) in supernatants from IL-2-treated PBL were capable of triggering the proliferation of non-transformed cells. Supernatants were tested for growth factor activity on rat lung fibroblasts. All supernatants tested induced proliferation; the
induction ranged from 36 to 114% of the proliferation triggered by medium containing 10% FBS.

**Induction of fibroblast proliferation by recombinant cytokines.** An analysis of $^3$H-TdR incorporation by NIH 3T3 cells revealed that PDGF and TGF-β at doses of 5 ng/ml and 2 ng/ml, respectively, were able to trigger cells to enter the cell cycle (Figure 3). In contrast, IL-1β and TNF-α failed to stimulate quiescent NIH 3T3 cells. Studies using preconfluent cells confirmed that PDGF and TGF-β induce the proliferation of NIH 3T3 cells (Table 2). IL-2 alone had no effect in either proliferation assay (data not shown).

TGF-β, PDGF, IL-1β and TNF-α were tested in pairs for possible synergistic actions on fibroblast proliferation. These studies revealed that pairs of cytokines tested at suboptimal doses acted additively, not synergistically, on the proliferation of fibroblasts (data not shown).

Since it was apparent that TGF-β and PDGF were the cytokines best able to trigger fibroblast $^3$H-TdR incorporation and cell proliferation and that these activities might be present in the supernatant fluids from IL-2 treated PBL, we attempted to block growth factor activities with polyclonal antibodies against TGF-β, and PDGF AB heterodimer. In several attempts with different supernatant preparations antibodies failed to inhibit the induction of fibroblast $^3$H-TdR incorporation by fibroblasts (data not shown). These negative results suggest 1) that TGF-β and PDGF are not the only cytokines responsible for the proliferation of fibroblasts induced by supernatants from IL-2 treated PBL, 2) that other forms of these cytokines (namely PDGF AA homodimer, PDGF BB homodimer, TGF-β₁, and TGF-β₂) are the mediators involved in the induction of fibroblast proliferation, or 3) that the levels of antibodies tested were not adequate to inhibit the growth factor activities. In support of the third possibility, Raines and coworkers (Raines et al, 1989) stated that it is difficult to inhibit PDGF activity with antibodies against PDGF.

**Induction of collagen and fibronectin gene expression in fibroblasts following treatment with cytokines derived from IL-2 treated PBL.** Initially, co-culture studies were performed, in which PBL were incubated with confluent monolayers of human lung fibroblasts, WI38-VA13, in the presence or absence of IL-2. These investigations detected in a 3-fold increase in expression of the α chain of type I procollagen mRNA in fibroblasts treated with PBL and IL-2 over that of cells treated with IL-2 alone or PBL without IL-2 (data not shown). Subsequent experiments revealed that cell contact was not required for the stimulation of procollagen gene expression. Mediators are elaborated by IL-2 treated PBL which induce fibroblasts to express the α chain of type I procollagen mRNA, as well as fibronectin mRNA (Figure 4).
Scanning densitometric analysis of the expression of the α chain of type I procollagen mRNA showed that supernatants from 3 day cultures of PBL with IL-2 induced 3-fold the level of procollagen mRNA as did cells treated with medium alone (Table 3).

The production of total collagen protein was measured by collagenase digestion of $^3$H-proline labelled protein as described in Methods. Table 4 shows that there was a 55% increase in the amount of collagen synthesis in WI38-VA13 fibroblasts cultured with 3 day a supernatant over that of untreated cells. Treatment of WI38-VA13 cells with supernatants from PBL cultured for 3 days in the absence of IL-2 caused a modest 13% increase in collagen synthesis, which may be attributed to the growth factor activities in AIM V medium. Collagen production was not enhanced by treatment of fibroblasts with IL-2 alone (data not shown).

Expression of TGF-β and PDGF B chain mRNAs in IL-2 treated PBL. An analysis of the expression of cytokine genes reveals that mRNAs coding for TGF-β (Figure 5a) and PDGF B chain (Figure 5b) are expressed in PBL treated with IL-2. TGF-β is not spontaneously expressed by non-adherent PBL (Figure 5a), but can be induced as soon as 2 hours after the addition of IL-2. TGF-β mRNA is expressed in both non-adherent and adherent cells after IL-2 treatment (data not shown), suggesting either that 1) multiple cell types contained within in the mixed population of PBL may be capable of expressing TGF-β mRNA in response to IL-2 stimulation, or 2) a single cell type, such as monocytes or LGL, exhibiting both adherent and non-adherent characteristics expresses TGF-β. The kinetics of expression of TGF-β mRNA in non-adherent cells reveals that TGF-β mRNA reaches a peak at 2 hours after treatment with IL-2 and remains elevated for several days. Whether the sustained expression of TGF-β mRNA is a result of continuous transcription or an altered half life of the message is unclear.

In contrast to TGF-β mRNA, only the adherent population of PBL expressed PDGF genes. Non-adherent PBL do not express appreciable levels of PDGF A chain and B chain mRNAs in the presence or absence of IL-2 stimulation (data not shown). PDGF B chain mRNA is not spontaneously expressed by adherent PBL (Figure 5b), but, like TGF-β, can be induced following treatment with IL-2. The kinetics of expression of PDGF B chain mRNA differ from that of TGF-β. PDGF B chain mRNA is detectable at 2 hours of stimulation with IL-2, reaches a peak at 18 hours, and gradually returns to baseline levels.

To confirm that cytokine proteins were being elaborated by IL-2 treated PBL supernatants were collected after 18 or 72 hours for immunoblot analysis. Data shown in Figures 6a and 6b reveal that both TGFβ and PDGF B chain proteins are produced and secreted by PBL following IL-2 treatment.
DISCUSSION

Our data demonstrate that mediators produced by IL-2-treated PBL are capable of inducing the proliferation of fibroblasts and the production of connective tissue. Furthermore, Northern blot analysis reveals that genes which code for TGF-β and PDGF B chain are expressed in IL-2-treated PBL.

The role of cytokines in the production of connective tissue is not unique to this system. Aberrant production of cytokines has been reported in animal models of lung fibrosis (Kovacs and Kelley, 1985a). For example, mediators elaborated by interferon-gamma treated mononuclear cells stimulate the proliferation of cultured lung fibroblasts (Kovacs and Kelley, 1985b). In addition, evidence suggests that a PDGF-like protein, probably derived from monocytes (Shimakado et al, 1985), is responsible for the pathogenesis of atherosclerosis (Ross, 1986). Furthermore, several groups have reported on the role(s) of PDGF and TGF-β in wound healing (Grotendorst et al, 1981; Cormack et al, 1987; Lynch et al, 1987).

The cell type within the mixed population of PBL which expressed TGF-β and PDGF B chain mRNAs in this study has not been determined. Reports have shown that several cell types are capable of producing TGF-β, including T lymphocytes (Kehrl et al, 1986) and monocytes (Assoian et al 1987). In contrast, the monocyte is the only immune cell which has been reported to produce PDGF-like proteins (Shimakado et al 1985) and express the genes which code for PDGF A chain (Sariban and Kufe, 1988) and B chain mRNAs (Martinet et al, 1986). These reports are supported by the fact that the monocyte-enriched adherent (and not the non-adherent) fraction of the PBL express PDGF B chain mRNA (Figure 5b).

Although other cytokines, including IL-1α, IL-1β, and TNF-α, are produced by IL-2 treated PBL in vivo (Kasid et al, 1989; Gremlo et al, 1988) and in vitro (Kovacs et al, 1989a), in our hands, these cytokines do not directly stimulate fibroblast proliferation or connective tissue production in vitro. This suggests that either IL-1 and TNF-α are not involved in the activation of collagen gene expression or, alternatively, that they act indirectly. It is possible that their actions are mediated by triggering the production of secondary cytokines or arachidonic acid metabolites. Recent evidence suggests that, at least for IL-1, the induction of fibroblast proliferation, which was initially reported by Schmidt and coworkers (Schmidt et al, 1982), involves secondary cytokines. IL-1 apparently acts on fibroblast growth indirectly by inducing the fibroblasts to secrete PDGF A chain homodimer, which in turn stimulates cells to enter the cell cycle (Raines et al, 1989).

Investigation of the control of expression of fibrogenic
cytokines produced by IL-2-stimulated PBL and their role in the production of scar tissue is of immediate clinical relevance. Information about the induction of expression of fibrogenic cytokines by IL-2 (and other immune mediators), as well as the response of cells to those factors, will enable us to develop methods by which their effects can be suppressed and/or eliminated. In addition, the results of these studies are also directly relevant to the control of the aberrant wound healing which is the hallmark of a variety of diseases including atherosclerosis and pulmonary fibrosis.
REFERENCES


TABLE 1. FIBROBLAST $^3$H-TdR INCORPORATION IN RESPONSE TO CYTOKINES DERIVED FROM IL-2 TREATED PBL.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$^3$H-TdR INCORPORATION (CPM)</th>
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<tr>
<td>none$^b$</td>
<td>3,215</td>
</tr>
<tr>
<td>medium with 10% FBS$^b$</td>
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<td>AIM V medium alone</td>
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<tr>
<td>supernatant from IL-2 treated PBL$^c$</td>
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'Mean of triplicate determinations.
$^b$negative and positive controls for assay.
$^c$PBL were cultured in AIM V medium + IL-2 (100 U/ml) for 4 days. The supernatant was tested at a dilution of 1:12. Standard error $\leq 5\%$. 
TABLE 2. FIBROBLAST PROLIFERATION IN RESPONSE TO CYTOKINES ELABORATED BY IL-2 TREATED PBL.

<table>
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<th>TREATMENT</th>
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<tr>
<td>medium with 10% FBS</td>
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<tr>
<td>supernatant #1(^b)</td>
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<tr>
<td>supernatant #2</td>
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</tr>
<tr>
<td>supernatant #3</td>
<td>0.46</td>
</tr>
<tr>
<td>supernatant #4</td>
<td>1.00</td>
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<tr>
<td>PDGF (5 ng/ml)</td>
<td>0.40</td>
</tr>
<tr>
<td>TGF-β (5 ng/ml)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

\(^a\)Mean of samples tested in triplicate on NIH 3T3 cells (see Methods for details).

\(^b\)PBL from four individuals were cultured in AIM V medium + IL-2 for 4 days. Supernatants were tested at a 1:8 dilution.

Standard error ≤ 5\%.
### TABLE 3. DENSITOMETRIC SCAN OF SLOT BLOT SHOWING PROCOLLAGEN RNA EXPRESSION BY FIBROBLASTS TREATED WITH CYTOKINES FROM IL-2 TREATED PBL.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>OD UNITS^b</th>
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</thead>
<tbody>
<tr>
<td>none</td>
<td>1.0</td>
</tr>
<tr>
<td>medium</td>
<td>1.4</td>
</tr>
<tr>
<td>PBL + IL-2</td>
<td>4.2</td>
</tr>
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</table>

^aConfluent monolayers of WI38-VA13 fibroblasts were cultured overnight in the absence or presence of fresh medium or supernatants from a 3 day culture of PBL treated with IL-2. Slot blots were prepared with total cellular RNA (5, 2.5, 1.25 ug) and hybridized with a probe for the α chain of type I procollagen.

^bArbitrary units obtained from scanning densitometric analysis of xray filters.
<table>
<thead>
<tr>
<th>TREATMENT</th>
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<th>NET CHANGE</th>
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<tbody>
<tr>
<td>none</td>
<td>8832</td>
<td></td>
</tr>
<tr>
<td>PBL</td>
<td>9964</td>
<td>+ 13%</td>
</tr>
<tr>
<td>PBL + IL-2</td>
<td>13674</td>
<td>+ 55%</td>
</tr>
</tbody>
</table>

*CPM \(^3\)H-proline incorporation into collagen in fibroblasts treated with supernatants from PBL cultured with or without IL-2. Standard deviation ≤ 10%.
FIGURE LEGENDS

FIGURE 1. Kinetics and dose response of growth factor activity elaborated by PBL following treatment with IL-2. Confluent monolayers of NIH 3T3 fibroblasts were incubated with test media as described in Methods. A) Fibroblasts were treated with supernatants from PBL cultured in the presence or absence of IL-2 (1000 u/ml) for various periods of time. Supernatants were tested at a 1:8 dilution. B) Dose response of \(^3\)H-TdR incorporation in NIH 3T3 fibroblast following culture treatment with a 3 day supernatant from IL-2 treated PBL. Arrows indicate the level of \(^3\)H-TdR incorporation by fibroblasts in response to treatment with medium containing 10% FBS. Standard error ≤5%.

FIGURE 2. Dose response of IL-2 on growth factor production by PBL. PBL were treatment with varying doses of IL-2 for 3 days after which supernatants were collected. The supernatants were assayed for growth factor activity on confluent monolayers of NIH 3T3 fibroblasts as described above. Arrows indicate the level of \(^3\)H-TdR incorporation by fibroblasts in response to treatment with medium containing 10% FBS. Standard error ≤5%.

FIGURE 3. Induction of \(^3\)H-TdR incorporation in NIH 3T3 fibroblasts cultured with recombinant cytokines. Confluent monolayers of fibroblasts were incubated with various doses of TGF-β, PDGF, IL-1β, and TNF-α. The arrow indicates the level of \(^3\)H-TdR incorporation by fibroblasts in response to treatment with medium containing 10% FBS. Standard error ≤5%.

FIGURE 4. Northern blot analysis of expression of the α chain of type 1 procollagen and fibronectin mRNAs in WI38-VA13 fibroblasts. Fibroblasts were cultured for 18 hours in the presence of fresh medium or a supernatant from IL-2 treated PBL. RNA was isolated and Northern blot analysis performed. The blot was first hybridized with a probe for the α chain of type I procollagen, then stripped and rehybridized with a probe for fibronectin. Finally the filter was rehybridized with a probe for histone H4.

FIGURE 5. Expression of TGF-β and PDGF B chain mRNAs by PBL. PBL were cultured with IL-2 for various periods of time and separated into adherent and non-adherent cells. A) Total cellular RNA was extracted from non-adherent PBL and Northern blot analysis was performed. The blot was hybridized with a probe for TGF-β. B) RNA was prepared from adherent PBL cultured
with IL-2. The blot was hybridized with a probe for PDGF B chain.

FIGURE 6. Immunoblot analysis of TGFβ and PDGF protein production by PBL treated with IL-2. Filters were prepared with supernatant fluids from cells treated with IL-1 for 18 hours. The filters were incubated sequentially with primary antibodies against TGFβ (Panel A) or PDGF AB (Panel B), secondary antibody, which is biotin-conjugated, avidin-conjugated alkaline phosphatase and a substrate.
Figure 2

[Graph showing the relationship between Log Reciprocal Dilution and CPM x 10^-3 for 10% FBS]
Figure 3

The graph shows the relationship between CPM (counts per minute) and cytokine concentration (ng/ml). The cytokines plotted are PDGF, TGF-beta, TNF alpha, and IL-1 beta. The x-axis represents the cytokine concentration ranging from 0 to 25 ng/ml, and the y-axis represents CPM from $10^{-3}$ to 40.
Figure 4

- Type 1 procollagen
- Fibronectin
- Histone
Figure 5b

0h, 2h, 18h, 48h

PDGF B chain
<table>
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<tr>
<th></th>
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<tbody>
<tr>
<td>a</td>
<td>-</td>
<td>-</td>
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<td>pTGFβ (10 ng)</td>
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<td>b</td>
<td>-</td>
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</tr>
<tr>
<td>c</td>
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<tr>
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<td></td>
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<td>medium with 10% FBS</td>
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Figure 5a

supernatants from IL-2 treated PBL

b
d

e

PDGF (20 ng)

buffer