Clin Exp Immunol 1993; 94:43-50

Up-regulation of alveolar macrophage platelet-derived growth factor-B (PDGF-B) mRNA by interferon-gamma from *Mycobacterium tuberculosis* antigen (PPD)-stimulated lymphocytes

A. WANGOO, I. K. TAYLOR, A. R. HAYNES & R. J. SHAW *Department of Respiratory Medicine, St. Mary's Hospital Medical School, London, UK*

(Accepted for publication 29 April 1993)

SUMMARY

Macrophage production of PDGF-B is believed to be important in the pathogenesis of diseases where chronic lung inflammation develops into fibrosis. Since tuberculosis is characterized by chronic inflammation and tissue fibrosis, we asked if lymphokines from lymphocytes stimulated by the *Mycobacterium tuberculosis* antigen PPD, contained factors capable of increasing human alveolar macrophage PDGF-B mRNA. Supernatants from both phytohaemagglutinin (PHA)- and purified protein derivative (PPD)-stimulated lymphocytes, when added to macrophages, induced an increase in the mRNA of PDGF-B, but not transforming growth factor-beta (TGF-β). When lymphocytes from contacts of patients with tuberculosis, patients with tuberculosis, and normal subjects were compared following PPD stimulation, the lymphocytes from the contacts had the greatest proliferation response, the greatest production of interferon-gamma (IFN-γ), and their lymphokines induced the greatest increase in PDGF-B mRNA in macrophages. Recombinant human IFN-γ reproduced this ability of lymphokines to increase macrophage PDGF-B mRNA. Finally, the increase in macrophage PDGF-B mRNA following incubation with supernatants from PPD-stimulated lymphocytes was shown to be due to IFN-γ, when the increase in macrophage PDGF-B mRNA was prevented by addition of anti-human IFN-γ antibody to the lymphocyte supernatant. This study indicated that antigen-stimulated lymphocytes released IFN-γ, which in turn resulted in an increase in PDGF-B mRNA in alveolar macrophages. Such a mechanism provides a link between the DTH response and the first stages of a fibrotic reaction, and may offer an explanation for the progression of chronic inflammation to fibrosis, as occurs in the lungs of patients with untreated pulmonary tuberculosis.

Keywords alveolar macrophage interferon-gamma tuberculosis platelet-derived growth factor-B

INTRODUCTION

Untreated or chronic pulmonary tuberculosis is associated with the development of contraction and scarring of the lung. Pulmonary tuberculosis thus serves as a human disease model for lung fibrosis in which the pathogen and much of the immunology are known. In the DTH response characteristic of tuberculosis, there is recognition of *Mycobacterium tuberculosis* antigen by T cells. Interferon-gamma (IFN-γ) is thought to be a prominent product of these T cells, since purified protein derivative (PPD)-specific T cell clones produce IFN-γ [1,2]. In addition, DTH reaction can be produced in mice by transfer of Th1 cells through secretion of IFN-γ and IL-2 [3]. We have shown in bronchoalveolar lavage patients with tuberculosis, that IFN-γ mRNA is present in $CD3^+$ T cells [4]. Furthermore, IFN-γ mRNA is also present in skin biopsies of tuberculosis reactions [5], tuberculosis pleuritis [6] and at increased concentrations in tuberculous pleural effusions [7].

Recombinant IFN-γ elicits the secretion of factors with growth factor activity from lung macrophages [8]. This activity may include the potent fibroblast mitogen PDGF-B, since IFN-γ increases PDGF-B mRNA in alveolar macrophages [9]. PDGF-B in its turn may play a central role in the pathogenesis of fibrosis. In addition to stimulating fibroblast proliferation [10], PDGF-B is chemotatic for fibroblasts [11] and stimulates fibroblast-mediated tissue contraction [12]. There is already circumstantial evidence for the involvement of PDGF-B in pathological lung fibrosis. In idiopathic pulmonary fibrosis there is an increase in alveolar macrophage PDGF-B transcrip-

Correspondence: Dr R.J. Shaw, Department of Respiratory Medicine, St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG, UK.

tion [13], mRNA abundance [9,13], and PDGF protein production [14]. *In situ* hybridization studies identified increased PDGF-B mRNA in macrophages and epithelial cells, in lungs of patients with lung fibrosis [15]. Similarly, in the skin of patients with scleroderma, there is an increase in PDGF-B mRNA [16] and protein [17]. Administration of recombinant PDGF-B to human skin wounds has also been shown to accelerate healing [18].

In the present study we have tested the hypothesis that stimulation of lymphocytes from appropriately exposed individuals with the *Myco. tuberculosis* antigen PPD results in the release of an activity which in turn increases macrophage PDGF-B mRNA. Furthermore, we have asked whether this activity is due to IFN-γ.

MATERIALS AND METHODS

Lymphocyte separation

Blood was collected from tuberculosis patients, tuberculosis contacts and normal healthy volunteers for separation of mononuclear cells. Blood was diluted 50:50 with RPMI 1640 (GIBCO BRL, Paisley, UK). Mononuclear cells were isolated on a Ficoll–Paque (Pharmacia, Uppsala, Sweden) density gradient following centrifugation at 400 *g* for 35 min, at 18°C, and were washed twice in RPMI 1640 and resuspended in RPMI 1640 containing 100 U/ml penicillin and 100 μg/ml streptomycin solution (GIBCO). Cells were added to 100-mm tissue culture plate and incubated at 37°C and 5% CO_2 for 3 h to separate adherent monocytes from non-adherent lymphocytes. Following incubation the non-adherent cells were collected, centrifuged, and the cells suspended in RPMI 1640 medium containing antibiotics, 5% fetal calf serum (FCS) and 5×10^{-5} M 2-mercaptoethanol (Sigma, Poole, UK) before lymphocyte stimulation.

Production of lymphocyte supernatants

Lymphocytes were distributed in 24-well tissue culture plates at a concentration of 5×10^6 cells/ml per well. The lymphocytes were incubated at 37°C and 5% CO_2 either alone (controls) or in presence of phytohaemagglutinin (PHA) 10 μg/ml (Sigma) or concanavalin A (Con A) 20 μg/ml (Sigma) or PPD 20 μg/ml (Central Veterinary Laboratory, UK). The stimulated and non-stimulated lymphocytes were harvested after 3 days for PHA and Con A and after 5 days for PPD. To the supernatants from the non-stimulated control lymphocytes, the same final concentration of PHA, Con A or PPD was added just before terminating the culture period. Lymphocyte supernatants were centrifuged, sterile filtered and stored at −20°C before use.

Lymphocyte proliferation assay

The proliferation assay was done in 96-well tissue culture plates. Cells (2×10^5 cells/200 μl of medium) were added to each well and the cells were stimulated with 20 μg/ml of PPD for 5 days at 37°C and 5% CO_2 [7]. Cells were pulsed with 1 μCi of ^{3}H-thymidine (Amersham Life Sciences, Amersham, UK) for 16–18 h before harvesting on filter paper. Thymidine incorporation was measured by standard liquid scintillation counting methods in ct/min and results were expressed as stimulation index (SI). SI was equal to (ct/min of PPD-stimulated cells)/(ct/min of non-stimulated cells).

Assay of lymphokines for IFN-γ

IFN-γ concentrations in ng/ml were measured in PPD-stimulated and non-stimulated lymphocyte supernatants by Human IFN-gamma ELISA kit (Endogen, Boston, MA) as per manufacturer's recommendations.

Collection and separation of alveolar macrophages

A group of patients with tuberculosis and healthy volunteers who were smokers were recruited and informed consent was obtained. The study was approved by the local Ethics Committee. Bronchoscopy followed by bronchoalveolar lavage was performed by the standard technique [9]. 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 in a 500-ml polypropylene bottle and placed on ice. Following centrifugation (1000 rev/min per 10 min at room temperature) bronchoalveolar lavage cells were re-suspended in RPMI 1640 (GIBCO) medium containing 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO) and the cell count was adjusted to 1-2 million cells/ml. Seven to 10 million cells were incubated for 1 h, following which non-adherent cells were removed by washing the plate with pre-warmed RPMI 1640. The adherent cells comprised more than 90% macrophages as determined by morphology, as shown previously [9]. The cells were then incubated in RPMI 1640 containing 10% FCS (ICN/Flow Labs, High Wycombe, UK) and 2 mM glutamine with 100 U of penicillin and 100 μg/ml streptomycin.

Macrophage stimulation

Alveolar macrophages were stimulated with either 500 U/ml of human recombinant IFN-γ (Collaborative Research Inc., Bedford, MA) or 1 ml of lymphocyte supernatants produced by lymphocytes stimulated with PHA or Con A or PPD.

Neutralization assay

To demonstrate the role of IFN-γ, in some experiments PPD-derived lymphocyte supernatants were treated with specific anti-human IFN-γ neutralizing antibody (Genzyme, Boston, MA) so that 1 ng of antibody was present per 40 pg IFN-γ in the supernatant (total anti-IFN-γ antibody added was 200–2000 ng/ml). Antibody was added for 1 h before stimulation of alveolar macrophages. Anti-digoxigenin (Boehringer Mannheim, Lewes, UK) (1000 ng/ml) was used as a non-specific antibody and served as control.

Isolation of RNA from alveolar macrophages

Following incubation, the medium was removed and cells lysed in 0·5 ml guanidinium isothiocyanate (GTC) solution containing 4 M GTC (BDH Ltd, Poole, UK), 25 mM sodium citrate pH 7, 17 mM sodium-*n* lauryl sarcosine, 0·14 M 2-mercaptoethanol (Sigma). RNA was extracted by a modification of the acid-GTC-phenol chloroform method of Chomczynski [19,20]. The sample was transferred to microfuge tubes and to each tube was added 0·1 volume 2 M sodium acetate pH 4, 1 volume of water-saturated phenol (Rathburn Chemicals, Walkerburn, UK) and 0·33 volume chloroform/isoamyl alcohol (49:1) (BDH). The tubes were inverted and vortexed after each addition. The samples were incubated on ice for 20 min before centrifugation for 20 min at 10 000 *g* at 4°C. After centrifugation the aqueous

layer was aspirated and precipitated with 1 volume of Propan 2-ol (BDH) at −20°C for 1 h. Samples were re-centrifuged at 10 000 *g* at 4°C for 20 min and RNA pellets were re-suspended in GTC and reprecipitated with Propan 2-ol. After centrifugation the pellets were washed with 75% ethanol and re-suspended in sample buffer (65% deionized formamide, 15% formaldehyde and 0·05 M 3-*n*-morpholino propanesulphonic acid (MOPS) (Sigma). Samples were treated at 65°C for 10 min and stored at −20°C.

Northern(RNA Gel) analysis

For each experiment an equal amount of RNA (7–15 μg) was loaded on to a 1% agarose/formaldehyde gel, separated by electophoresis and blotted on to a nylon membrane (Hybond-N; Amersham Life Sciences, UK). In each experiment the amount of RNA loaded was shown to be the same by staining the gel with ethidium bromide and photography under u.v. light. The membranes were prehybridized at 42°C before hybridization as described previously [20], except 0·5 mg/ml herring testes DNA (Sigma) was used instead of salmon sperm DNA. Blots were hybridized at 42°C for 24 h with probes labelled with random primers using α-^{32}P-dCTP (ICN Biomedicals, Irvine, UK). After hybridization the membranes were washed. Autoradiography was performed using x-ray film (x-omat Xar-5; Eastman Kodak Co, NY) at −70°C. The relative intensities of the bands on the autoradiographs were compared by scanning densitometry using a chromoscan 3 (Joyce Loebl Ltd, UK). The results were expressed as mean (±s.e.m.) percentages of the maximum (100%) response in each experiment.

DNA probes

The human DNA probes used were 0·75 kb EcoR1 c-*sis* fragment (gift of M. Murray, Zymo Genetics, Seattle, WA), a 1·65-kb EcoR1 transforming growth factor-beta-1 (TGF-β1) fragment (gift of A. Purchio, Oncogene, Seattle, WA), a 3·1-kb EcoR1 HLA-DR-α fragment (gift of S. Weissman, Yale University, New Haven, CT), and a 314-bp cDNA of β-actin (see below). In all cases probe specificity was high, with the approximate sizes of the mRNA as assessed in relation to 28S (4·7 kb) and 18S (1·9 kb) ribosomal RNA of 3·8 for PDGF-B, 2·4 kb for TGF-β, and 0·76 kb for HLA-DR-α.

Reverse-transcriptase polymerase chain reaction to synthesise β-actin cDNA probe

First strand complementary DNA (cDNA) synthesis and polymerase chain reaction (PCR) from an RNA sample were carried out as described earlier [20] using primers specific for β-actin. The primers were: (i) 5′TCCTGTGGCATCCACGAAACT 3′ (sense) spanning bases 852–872, and (ii) 5′ GAAGCATTTGCGGTGGACGAT 3′ (anti-sense) spanning bases 1146–1166. The predicted size of the amplified β-actin DNA product was 314 bp [21]. After completion of PCR, the mineral oil was removed by extraction with 110 μl chloroform, and 60 μl of upper aqueous phase was electrophoresed through a low melting point agarose gel containing ethidium bromide. β-actin cDNA was excised from the gel and DNA recovered as described by Sambrook [22].

RESULTS

Characteristics of patients and volunteers

Alveolar macrophages were collected by bronchoalveolar lavage from 26 subjects. Ten of these were patients with pulmonary tuberculosis. The remaining 16 subjects were normal, and included two non-smokers. These individuals were either normal volunteers or had bronchoscopy for other reasons and were found to have no abnormality. Peripheral blood lymphocytes were collected from eight patients with pulmonary tuberculosis, 11 contacts of whom four had a grade 4 Heaf test, five a grade 2/3 Heaf test and two a grade 1/2 Heaf test, as well as 10 normal volunteers, all of whom had had a previous BCG.

Supernatants from PHA- and Con A-stimulated lymphokines induced an increase in macrophage PDGF-B mRNA

Supernatants from lymphocytes stimulated by PHA caused a 50% increase in PDGF-B mRNA in alveolar macrophages when compared with supernatants from unstimulated lymphocytes (Fig.1). A similar but smaller increase in macrophage PDGF-B mRNA abundance was observed when macrophages were incubated with supernatant derived from Con A-stimulated lymphocytes. By contrast, the abundance of TGF-β and β-actin mRNA was similar in macrophages incubated in medium alone, or supernatant from PHA- or Con A-stimulated lymphocytes, or unstimulated lymphocytes. Compared with an abundance of 100% for β-actin mRNA in macrophages incubated with supernatants from lymphocytes stimulated by PHA, the abundance was 90·3% and 88% in cells incubated in medium alone and lymphokines from unstimulated lymphocytes, respectively. Similarly, in comparison to β-actin mRNA abundance of 100% in macrophages incubated with supernatants from lymphocytes stimulated by Con A, the abundance of β-actin in macrophages incubated with medium alone was 88·9%, and with lymphokines from unstimulated lymphocytes β-actin mRNA abundance was 95·6% (Fig. 2). When HLA-DR-α mRNA was measured, there was a modest increase in HLA-DR-α mRNA abundance in macrophages treated with supernatants obtained from either stimulated or unstimulated lymphocytes, compared with macrophages incubated in medium alone.

Comparison of the magnitude of the increase in macrophage PDGF-B mRNA following stimulation by either supernatants from PHA-stimulated lymphocytes or recombinant IFN-γ

The abundance of PDGF-B mRNA in alveolar macrophages was similar following incubation with either supernatants from PHA-stimulated lymphocytes or when incubated with recombinant IFN-γ (Fig. 3). The abundance of PDGF-B mRNA following either of these conditions was greater than that in macrophages incubated in medium alone. β-actin mRNA abundance in macrophages was constant. An abundance of 93·7% following incubation with medium alone and 92·5% following incubation with lymphokines from PHA-stimulated lymphocytes was observed in comparison with 100% following IFN-γ stimulation (Fig. 2).

Increase in macrophage PDGF-B mRNA following incubation in supernatants from PPD antigen-stimulated lymphocytes

To examine the ability of supernatants from PPD-stimulated lymphocytes to increase macrophage PDGF-B mRNA, lym-

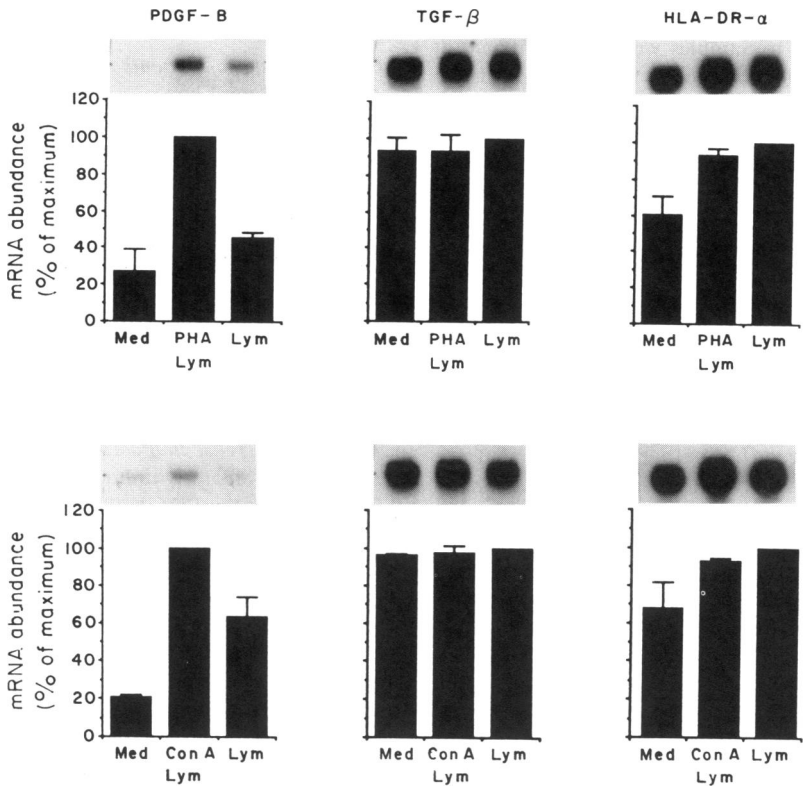

Fig. 1. Abundance of PDGF-B, transforming growth factor-beta (TGF-β) and HLA-DR-α mRNA in alveolar macrophages incubated with supernatants from phytohaemagglutinin (PHA Lym) and concanavalin A (Con A)-stimulated lymphocytes (Con A Lym) and non-stimulated lymphocytes (Lym), expressed as a percentage of maximum (mean $\pm$ s.e.m.). Insets, representative autoradiographs of Northern blots. Med, Medium.

phocytes from patients with tuberculosis were compared with cells from normal volunteers. First, lymphocytes were obtained from patients with tuberculosis and macrophages from normal volunteers. Under these conditions, the supernatants from the PPD-stimulated lymphocytes caused an increase in macrophage PDGF-B mRNA, compared with macrophages incubated in medium alone or with supernatants from unstimulated lymphocytes (Fig. 4a). There were no appreciable changes in TGF-β or HLA-DR-α mRNA under these conditions. β-actin mRNA was also unchanged. Compared with a value of 100% for β-actin mRNA in macrophages stimulated by lymphokines from PPD-stimulated lymphocytes, the β-actin mRNA abundance was 99·9% in macrophages incubated in medium alone, and 88·6% in macrophages incubated in lymphokines from unstimulated lymphocytes (Fig. 2).

Second, both lymphocytes and macrophages were obtained from subjects who did not have tuberculosis. Under these conditions, there was an increase in the abundance of PDGF-B mRNA in macrophages incubated in supernatants from PPD-stimulated lymphocytes, compared with supernatants from unstimulated lymphocytes (Fig. 4b). There was no appreciable change in TGF-β or HLA-DR-α mRNA abundance in the macrophages following incubation with supernatants from lymphocytes or media alone. β-actin mRNA abundance in macrophages was 96·7% following incubation in medium alone, 90·6% when incubated in lymphokines from unstimulated lymphocytes, compared with 100% when incubated in supernatants from lymphocytes stimulated with PPD (Fig. 2).

Relationship between the ability of supernatants from PPD-stimulated lymphocytes to increase macrophage PDGF-B mRNA, the concentration of IFN-γ in the supernatants and the proliferation of lymphocytes in response to PPD

Lymphocytes from patients with tuberculosis, from contacts of patients with tuberculosis and from normal volunteers were compared. The ability of the supernatants from stimulated lymphocytes from different subject groups to increase macrophage PDGF-B was compared. These experiments were carried out on four separate occasions when greater than 55 million macrophages were recovered from bronchoalveolar lavage of one subject. These bronchoalveolar lavage cells came from four normal volunteers, who were life-long smokers. This part of the study allowed the effect of different stimuli on macrophages from the same normal individual to be compared. Compared with incubation in medium alone, incubation with IFN-γ or supernatants from PPD-stimulated lymphocytes was associated with an increase in macrophage PDGF-B mRNA (Fig. 5a). There was a greater abundance of PDGF-B mRNA in macrophages incubated in supernatants from PPD-stimulated lymphocytes, compared with those incubated in supernatants from unstimulated lymphocytes. In addition, the increase in PDGF-B mRNA in macrophages was greater following incubation with supernatants from PPD-stimulated lymphocytes obtained from contacts of cases of tuberculosis, than from incubation with supernatants from PPD-stimulated lymphocytes obtained from cases of active tuberculosis or normal volunteers. It was noted that the increase in macrophage PDGF-B mRNA following

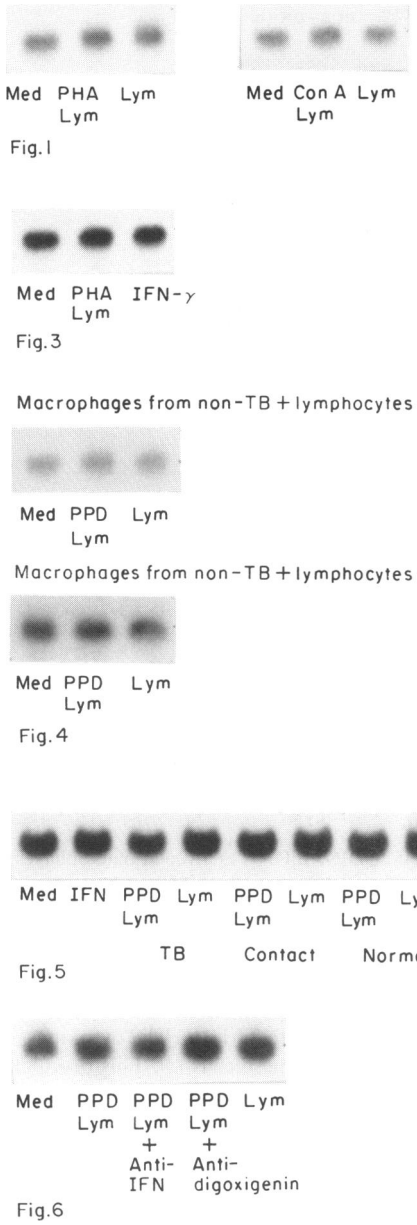

Fig. 2. Representative autoradiographs of Northern blots for β-actin. These have been labelled to show corresponding figures of autoradiographs containing PDGF-B Northern blots (Figs 1, 3–6). Med, Medium; Lym, supernatants from non-stimulated lymphocytes; PHA, Con A, PPD Lym, lymphokines from phytohaemagglutinin, concanavalin A and purified protein derivative-stimulated lymphocytes.

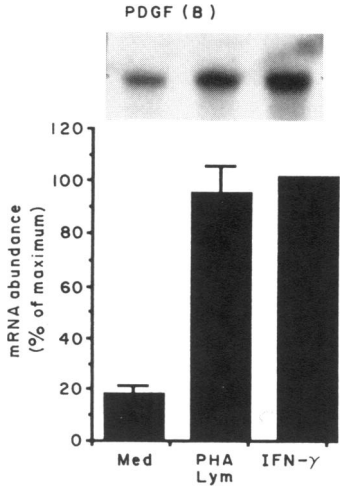

Fig. 3. Relative increase in macrophage PDGF-B mRNA following stimulation by either supernatants from phytohaemagglutinin (PHA)-stimulated lymphocytes (PHA Lym) or recombinant IFN-γ (mean ± s.e.m.). Inset, representative autoradiograph of Northern blots of mRNA from alveolar macrophages stimulated as indicated. Med, Medium.

incubation in supernatants from PPD-stimulated lymphocytes from these normal volunteers was greater than that when a different group of donors was used in Fig. 4. In all these groups, mRNA abundance of β-actin was constant. Compared with an abundance of 100% for β-actin mRNA in macrophages stimulated with IFN-γ, the abundance was 91·2% following medium alone, 88·8% with lymphokines from PPD-stimulated lymphocytes, 95·9% with lymphokines from unstimulated lymphocytes from TB patients, 94·3% with lymphokines from PPD-stimulated lymphocytes, 102·8% with lymphocytes from unstimulated lymphocytes from the contact group, 90·6% with lymphokines from PPD-stimulated lymphocytes from normal controls, and 96·9% with lymphokines from unstimulated lymphocytes from normal controls (Fig 2).

When the IFN-γ concentration in the lymphocyte supernatants was measured using the same donors, PPD stimulation of lymphoctyes irrespective of the clinical status of the donor was associated with a marked increase in IFN-γ concentration in supernatants (Fig. 5b). PPD stimulation of lymphocytes from contacts resulted in higher concentrations of IFN-γ compared with PPD stimulation of lymphocytes from cases of active tuberculosis or normal volunteers.

Finally, the relationship was sustained when proliferation in response to PPD was measured using the same lymphocyte donors. Lymphoctyes from all groups proliferated in response to PPD (Fig. 5c). However, the SI in response to PPD was greater for cells from contacts than for cells from patients with active tuberculosis or the control group.

Role of IFN-γ from PPD-stimulated lymphocytes in causing the increase in macrophage PDGF-B mRNA

When alveolar macrophages were incubated with supernatants from PPD-stimulated lymphocytes obtained from donors previously screened and shown to have a large lymphocyte proliferation response to PPD, there was a marked increase in the macrophage PDGF-B mRNA compared with that in macrophages incubated in medium alone (Fig. 6). This increase in macrophage PDGF-B mRNA following incubation with supernatants from PPD-stimulated lymphocytes was abrogated by the addition to the supernatants of neutralizing antibodies against IFN-γ. Under these conditions the PDGF-B mRNA abundance was similar to that in macrophages incubated in medium alone, or incubated with supernatants from stimulated lymphocytes. Addition of an irrelevant antibody (anti-digoxigenin) did not prevent the supernatants from PPD-stimulated lymphocytes from causing an increase in macrophage PDGF-B mRNA. In this experiment, β-actin mRNA abundance was similar in macrophages in all the groups. Compared with 100%

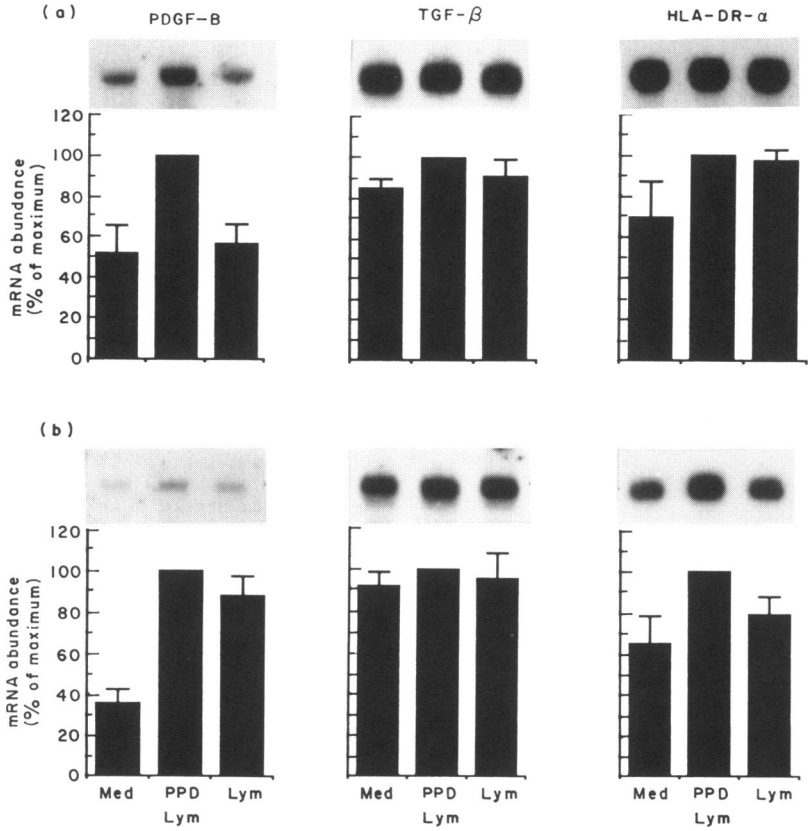

Fig. 4. Relative abundance of PDGF-B, transforming growth factor-beta (TGF-β) and HLA-DR-α mRNA in alveolar macrophages incubated with supernatants from purified protein derivative (PPD)-stimulated lymphocytes (PPD Lym). (a) Macrophages from subjects without tuberculosis, lymphocytes from patients with tuberculosis. (b) Macrophages and lymphocytes from subjects without tuberculosis (mean $\pm$ s.e.m.). Insets, representative autoradiographs of Northern blots. Med, Medium; Lym, supernatants from non-stimulated lymphocytes.

for β-actin mRNA expression in macrophages stimulated with lymphokines from PPD-stimulated lymphocytes, the abundance was 101·8% following medium alone, 95·2% following lymphokines from PPD-stimulated lymphocytes with anti-IFN-γ, 96·1% following lymphokines from PPD-stimulated lymphocytes with anti-digoxygenin antibody, and 92·7% with lymphocytes from unstimulated lymphocytes (Fig. 2).

DISCUSSION

This study provides evidence of a link between antigen-driven DTH and the first stages of the fibrotic response. The results suggest that in response to the *Myco. tuberculosis* antigen PPD, lymphocytes from sensitized individuals have the ability to make IFN-γ, which in turn causes macrophages to increase the abundance of mRNA of the potent fibroblast growth factor PDGF-B. These data offer one possible mechanism by which chronic inflammation in response to persistent antigen can over time progress to tissue fibrosis.

Both proliferation and IFN-γ production by PPD-stimulated lymphocytes from subjects exposed to tuberculosis are well recognised [7,23], and it is thought to be a function of cells of the Th1 subtype [1]. Although others have suggested that mycobacterium-reactive human T cell clones bearing $\alpha\beta$ or $\gamma\delta$ T cell receptors have a more complex phenotype resembling murine Th0 cells, with synthesis of both Th1 and Th2 cytokines, IFN-γ was a prominent product of these cells [2]. This production of IFN-γ may be an essential component of the host defence against mycobacteria, since MoAbs against IFN-γ block the development of DTH lesions when Th1 clones are introduced into recipient mice [24]. In this study, the greatest production of IFN-γ occurred in lymphocytes from contacts of patients with tuberculosis. The contacts were healthy individuals with strongly positive Heaf skin tests to PPD, whereas patients with active disease included a number of very sick individuals who might be expected to have diminished immunity. The large proliferation response and IFN-γ production by lymphocytes from normal subjects and the variable ability of supernatants from these cells to increase macrophage PDGF-B mRNA, may reflect the policy in this country of giving BCG to all children, as well as the high prevalence of tuberculosis in this community, with the consequent possibility of repeated exposure. Nevertheless, when donors were compared, there was an association between the lymphocyte proliferation response, IFN-γ release, and the ability of lymphocyte supernatants to increase macrophage PDGF-B mRNA.

IFN-γ has previously been shown to increase macrophage PDGF-B mRNA [9]. In this study, a similar increase in macrophage PDGF-B mRNA occcurred following addition of supernatants from stimulated lymphocytes. There was also complete abrogation, by antibodies against IFN-γ, of the

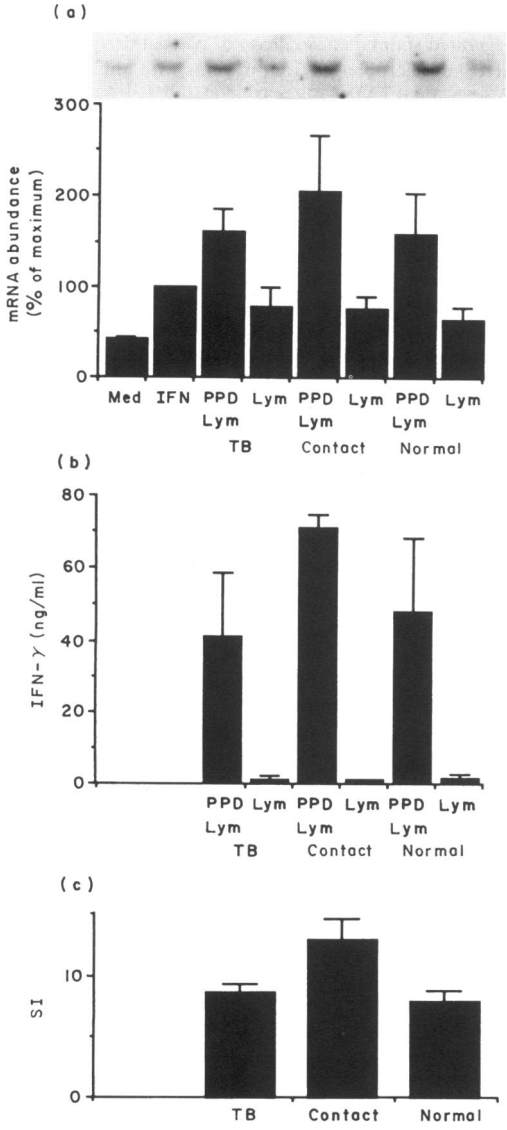

Fig. 5. Comparative relationship between PDGF-B mRNA abundance in alveolar macrophages incubated with supernatants from purified protein derivative (PPD)-stimulated lymphocytes (PPD Lym) and non-stimulated lymphocytes (Lym) (a), concentration of IFN-γ in the supernatants as determined by ELISA (b), and the proliferation of lymphocytes in response to PPD as determined by ^{3}H-thymidine uptake (c) in tuberculosis patients (TB), contacts of tuberculosis patients (Contact) and healthy volunteers (Normal) (mean ± s.e.m.). Inset (a), representative autoradiograph of mRNA abundance of Northern blots.

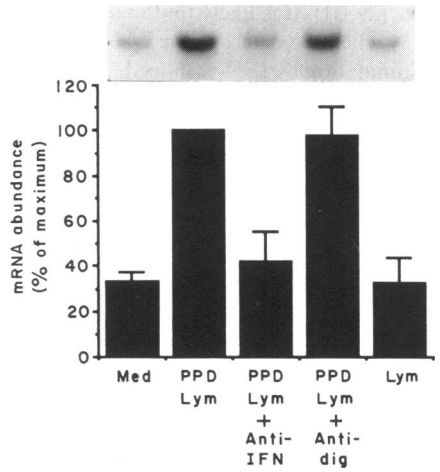

Fig. 6. PDGF-B abundance in alveolar macrophages stimulated with medium (Med), supernatants from purified protein derivative-(PPD)-stimulated lymphocytes (PPD Lym), supernatants from PPD-stimulated lymphocytes treated with anti-IFN-γ (anti-IFN) antibody, PPD-derived supernatants treated with the irrelevant antibody anti-digoxigenin (anti-dig), and non-stimulated lymphocytes (Lym). Results expressed as a percent of the PDGF-B mRNA abundance of macrophages stimulated with supernatants from PPD-stimulated lymphocytes (mean ± s.e.m.). Inset, representative autoradiograph of Northern blot.

increase in macrophage PDGF-B mRNA. This suggests that with respect to increasing macrophage PDGF-B mRNA, IFN-γ is the predominant lymphokine from these antigen-stimulated cells.

An increase in macrophage PDGF-B mRNA is only the first of many steps in the initiation of a fibrotic reaction. It will be important to know if there is an increase in PDGF protein, and if there is evidence of increased PDGF-B mRNA or protein in lungs of patients with tuberculosis. The suggestion that antigen stimulation of T cells can promote the pathogenesis of fibrosis via increases of PDGF-B mRNA in macrophages may offer a hypothesis to explain other forms of lung fibrosis such as idiopathic pulmonary fibrosis, where there is evidence of increased PDGF-B mRNA [9,13] and of increased IFN-γ [25]. A second growth factor, TGF-β, has been shown to be involved in the development of human lung fibrosis [26,27]. TGF-β is a product of activated macrophages [28]. We have not previously found TGF-β mRNA to be regulated in response to IFN-γ [9], and in the present study there was no evidence of alteration in TGF-β mRNA abundance in macrophages incubated with supernatants from stimulated lymphocytes. Thus, it seems unlikely that antigen-dependent lymphocyte activation controls the TGF-β-mediated component of the fibrotic response. In the present study, the absence of any change in TGF-β mRNA or β-actin mRNA (which served as a 'house keeping' gene) indicated that there was no overall change in mRNA abundance following the different stimuli.

The abundance of HLA-DR-α mRNA was included as a control in this study. IFN-γ is known to increase HLA-DR-α mRNA [9]. The failure to demonstrate a large increase in HLA-DR-α mRNA in alveolar macrophages incubated with supernatants from stimulated lymphocytes in the present study is likely to be due to the great abundance of HLA-DR-α mRNA already present in the unstimulated cells. Alternatively, it may imply that PDGF-B mRNA abundance is more sensitive than HLA-DR-α mRNA to *in vitro* stimulation by IFN-γ, or other *in vitro* derived lymphokines may have an inhibitory effect on HLA-DR-α mRNA expression in macrophages in this *in vitro* system.

This study adds one further small piece of information to our understanding of the intimate relationship between lymphocytes and macrophages in the setting of chronic inflammation in granulomatous disease. IFN-γ is already known to stimulate many macrophage/monocyte functions such as IL-1, TNF,

GM-CSF, etc. [29–31]. It is likely that many additional factors come into play when the macrophage is selecting its functional response to stimulation by IFN-γ. Indeed, the PDGF-B mRNA increase in macrophages in response to IFN-γ is not maximal, since it can be further increased by certain drugs [20,32]. Thus, it may be that macrophages can take into account an array of chemical and drug stimuli when determining the type and magnitude of their ultimate response.

ACKNOWLEDGMENTS

This work was supported by The Medical Research Council, British Lung Foundation and Lilly Industries. This work was presented at the American Thoracic Society Meeting, Miami FL, 1992.

REFERENCES

1 Maggi E, Parronchi P, Manetti R *et al.* Reciprocal regulatory effect of IFN-gamma and IL-4 on the *in vitro* development of human Th-1 and Th-2 clones. J Immunol 1992; **148**:2142–7.

2 Barnes PF, Abrams JS, Lu S *et al.* Patterns of cytokine production by mycobacterium-reactive human T cell clones. Infect Immun 1993; **61**:197–203.

3 Cher DJ, Mosmann TR. Two types of murine T cell clone. II. Delayed Type Hypersensitivity is mediated by Th1 clones. J Immunol 1987; **138**:3688–94.

4 Robinson DS, Ying S, Taylor IK, Wangoo A, Kay AB, Hamid Q, Shaw RJ. Evidence of Th-1 T cell cytokine mRNA expression by bronchoalveolar cells in pulmonary tuberculosis. Am Rev Respir Dis 1993 (Abstr.); **147**: A467.

5 Tsicopoulos A, Hamid Q, Varney V, Ying S, Moqbel R, Durham SR, Kay AB. Preferential messenger RNA expression of Th1-type cells (IFN-gamma$^+$, IL-2$^+$) in classical delayed type (tuberculin) hypersensitivity reactions in human skin. J Immunol 1992; **148**:2058–61.

6 Barnes PF, Fong SJ, Brenan PJ, Twomey PE, Mazundar A, Modlin RL. Local production of tumor necrosis factor and IFN-gamma in tuberculos pleuritis. J Immunol 1990; **145**:149–54.

7 Ribera E, Espanol T, Martinez-Vazquez JM, Ocana I, Encabo G. Lymphocyte proliferation and gamma- interferon production after *in vitro* stimulation with PPD. Differences between tuberculous and nontuberculous pleurisy in patients with positive tuberculin skin test. Chest 1990; **97**:1381–5.

8 Kovacs EJ, Kelley J. Lymphokine regulation of macrophage-derived growth factor secretion following pulmonary injury. Am J Pathol 1985; **121**:261–8.

9 Shaw RJ, Benedict SH, Clark RAF, King TE Jr. Pathogenesis of pulmonary fibrosis in interstitial lung disease. Alveolar macrophage PDGF(B) gene activation and up-regulation by interferon gamma. Am Rev Respir Dis 1991; **143**:167–73.

10 Beckman MP, Belsholtz C, Heldin CH *et al.* Comparison of biological properties and transforming potential of human PDGF-A and PDGF-B chains. Science 1988; **241**:1346–9.

11 Nister M, Hammacher K, Mellstrom K *et al.* A glioma-derived PDGF-A chain homodimer has different functional activities from PDGF AB heterodimer purified from human platelets. Cell 1988; **52**:791–9.

12 Clark RAF, Folkvord JM, Hart CE, Murray MJ, McPherson JM . Platelet isoforms of platelet-derived growth factor stimulate fibroblasts to contract collagen matrices. J Clin Invest 1988; **84**:1036–40.

13 Nagaoka I, Trapnell BC, Crystal RG. Upregulation of platelet-derived growth factor-A and -B gene expression in alveolar macrophages of individuals with idiopathic pulmonary fibrosis. J Clin Invest 1990; **85**:2023–37.

14 Martinet Y, Rom WN, Grotendorst GR, Martin GR, Crystal RG. Exaggerated spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. N Engl J Med 1987; **317**:202–9.

15 Antoniades HN, Bravo MA, Avila RE, Galanopoulos T, Neville-Golden J, Maxwell M, Selman M. Platelet-derived growth factor in idiopathic pulmonary fibrosis. J Clin Invest 1990; **86**:1055–64.

16 Olsen DR, Uitto J. Differentiation expression of type IV procollagen and laminin genes by fetal *vs.* adult skin fibroblasts in culture; determination of subunit mRNA steady-state levels. J Invest Dermatol 1989; **93**:127–31.

17 Gay S, Jones RE Jr, Huang GQ, Gay RE. Immunohistologic demonstration of platelet-derived growth factor (PDGF) and sis-oncogene expression in scleroderma. J Invest Dermatol 1989; **92**:301–3.

18 Pierce GF, Mustoe TA, Senior RM *et al.* In vivo incisional wound healing augmented by platelet derived growth factor and recombinant *c-sis* gene homodimeric protein. J Exp Med 1988; **167**:974–87.

19 Chomczynski P, and Sacchi N. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; **162**:156–9.

20 Haynes AR, Shaw RJ. Dexamethasone-induced increase in platelet-derived growth factor (B) mRNA in human alveolar macrophages and myelomonocytic HL60 macrophage-like cells. Am J Resp Cell Mol Biol 1992; **7**:198–206.

21 Ponte P, Ng Sun-Yu NJ, Engel J, Gunning P, Kedes L. Evolutionary conservation in the untranslated region of actin mRNA:DNA sequence of a human beta actin cDNA. Nucleic Acid Res 1984; **12**:1687–96.

22 Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbour: Cold Spring Harbor Laboratory Press, 1989:6.30.

23 Huygen K, Van Vooren JP, Turneer M, Bosmans R, Dierckx P, Bruyn JD. Specific lymphoproliferation, gamma interferon production, and serum immunoglobulin G directed against a purified 32kDa mycobacterial protein antigen (P32) in patients with active tuberculosis. Scand J Immunol 1988; **27**:187–94.

24 Fong TAT, Mosman TR. The role of IFN-gamma in delayed type hypersensitivity mediated by Th1 clones. J Immunol 1989; **143**:2887–93.

25 Robinson BWS, Rose AH. Pulmonary gamma interferon production in patients with fibrosing alveolitis. Thorax 1990; **45**:105–8.

26 Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci USA 1991; **88**:6642–6.

27 Khalil N, O'Connor RN, Unruh HW *et al.* Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 1991; **5**:155–62.

28 Assoian RK, Fleurdelys BE, Stevenson HC *et al.* Expression and secretion of type beta transforming growth factor by activated human macrophages. Proc Natl Acad Sci USA 1987; **84**:6020–4.

29 Philip R, Epstein LB. Tumour necrosis factor as immunomodulators and mediator of monocyte cytotoxicity induced by itself, gamma interferon and interleukin 1. Nature 1986; **323**:86–9.

30 Piacibello W, Lu L, Wachter M, Rubin B, Broxmeyer HE. Release of granulocyte macrophage colony stimulating factors from major histocompatibility complex class II antigen positive monocytes is enhanced by human gamma interferon. Blood 1985; **66**:1343–51.

31 Newton RC. Effect of interferon on the induction of human monocyte secretion of interleukin 1 activity. Immunology 1985; **56**:441–9.

32 Wangoo A, Haynes AR, Sutcliffe SP, Sorooshian M, Shaw RJ. Modulation of PDGF(B) mRNA abundance in macrophages by colchicine and dibutyril cAMP. Mol Pharmacol 1992; **42**:584–9.