

Platelet-derived growth factor AA and AB dimers are present in normal human epithelial lining fluid

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Platelet-derived growth factor AA and AB dimers are present in normal human epithelial lining fluid. M. Allam, N. Martinet, H. Gallati, P. Vaillant, M. Hosang, Y. Martinet. ©ERS Journals Ltd 1993.

ABSTRACT: Normal lung architecture is related to the presence of mesenchymal cells (fibroblasts and smooth muscle cells), and to the production by these cells of extracellular matrix. The turnover of mesenchymal cells is under a fine regulation due, at least in part, to the local presence of different mediators acting on their cell cycle.

Since normal human alveolar macrophages obtained by bronchoalveolar lavage (BAL) spontaneously release platelet-derived growth factor (PDGF), a cytokine with chemotactic and growth activity on mesenchymal cells, we evaluated normal human epithelial lining fluid (ELF) for the presence of PDGF. Active only as a dimer, PDGF is a glycoprotein composed of two chains (A and B) and, thus, can be present in three forms: AA, AB, and BB dimers.

Interestingly, normal ELF contains PDGF AA dimers, and to a lesser extent AB dimers, while no significant level of BB dimers is detected. Furthermore, ELF PDGF is biologically active and responsible for a significant part of the chemotactic activity and the "competence" growth activity for mesenchymal cells present in normal ELF. These findings suggest that ELF PDGF has a role in normal lung structure maintenance and tissue repair.

Eur Respir J., 1993, 6, 1162-1168.

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Keywords: Bronchoalveolar lavage; lung architecture; lung epithelial lining fluid; mesenchymal cells; platelet-derived growth factor; tissue repair

Received: July 23 1992
Accepted after revision February 28 1993

This work was supported in part by grants from Fonds Special des Comités Départementaux Contre les Maladies Respiratoires et la Tuberculose (88 D/4, 89 G/5) and Ligue Française Contre le Cancer, Comités de la Haute-Saône et de Meuse.

Normal lung structure is maintained by the presence of mesenchymal cells and of their extracellular matrix, that contribute to lung architecture [1, 2]. In the normal lung, mesenchymal cells (fibroblasts, myofibroblasts and smooth muscle cells (SMC)) are characterized by a slow turnover, and their replication is currently thought to be controlled by the local presence of mediators with growth activity such as platelet-derived growth factor, (PDGF) [3-7]. In this respect, PDGF is a potent growth factor, as well as a chemotactic factor for fibroblasts and SMC, and PDGF has been shown to be produced by several cell types present in normal lung [8-12].

PDGF is composed of two chains (A and B) sharing about 60% homology [5-7]. Three types of dimers are possible: AA, AB and BB [13]. Recently, two types of PDGF receptor subunits (α and β) have been characterized [14-18]. The α subunit binds PDGF A and B chain, whilst the β subunit binds only PDGF B chain, this specificity modulating PDGF biological activity [19, 20].

Current concepts suggest that normal alveolar macrophages release PDGF, which influences, in a paracrine fashion, normal mesenchymal cell turnover, and the repair processes involving the lung [9, 10, 21-23]. In order to evaluate whether PDGF is present in normal lung, we have tested normal epithelial lining fluid (ELF), the fluid bordering the alveoli and containing alveolar macrophages, for the presence of PDGF.

Materials and methods

Epithelial lining fluid sampling

ELF from 15 normal subjects (10 men, 5 women, mean age 35 ± 7 years, all nonsmokers, with no current or past history of lung disorder and with normal lung function tests and chest X-ray) was obtained by bronchoalveolar lavage (BAL), using a standard procedure [24]. Briefly, after local anaesthesia with xylocaine, successive 50 ml aliquots of sterile saline solution were infused, and subsequently recovered by gentle aspiration. For each subject, three different lobes were lavaged, with a total maximum of 250 ml of saline solution instilled per subject. The recovered samples were pooled and, after removing three aliquots (for total cell count, cell differential by cyto-centrifuge, and urea level measurement), the total BAL fluid was supplemented with bovine serum albumin (BSA, 1 mg·ml⁻¹, Sigma, St Louis, MO, USA), in order to avoid PDGF loss due to its "stickiness" to the vessels, before centrifugation (800 \times g, 10 min, 4°C) to remove cells and insoluble material. BAL fluids were then dialysed (48 h, 4°C, 6-8 dialysis solution changes), either against 1 M acetic acid (pH 2.5), or against 50 mM ammonium acetate (pH 7.4), lyophilized, dissolved in Dulbecco's modified Eagle's medium (DMEM, Sigma), and kept frozen (-70°C) until

use. In order to eliminate the dilution factor induced by BAL, ELF *in situ* concentration was evaluated by the urea method [25] and restored, by dilution, after lyophilization.

Platelet-derived growth factor biological activities

With regard to PDGF biological activities, ELF samples were tested for the presence of chemotactic and growth activity for mesenchymal cells. The chemotactic activity was detected using modified Boyden chambers (Neuro Probe, Cabin John, MD, USA) and, as target cells, smooth muscle cells (SMC) obtained by enzymatic digestion of normal human trachea [8, 26]. In some control experiments, Balb/c3T3 fibroblasts (ATCC, Rockville, MD, USA, provided by O. Blanchard (INSERM U284, Nancy, France)) were used as target cells. The lower chambers were filled with dilutions of ELF (in DMEM), or of PDGF as positive control (human B-chain homodimer, Bissendorf Biochemicals, Hannover, West Germany), or of DMEM alone, as negative control. The lower chambers were separated from the upper chambers filled with target cells (3×10^5 cells·ml⁻¹, in DMEM) by polycarbonate filters (8 µm diameter pore size, Neuro Probe) previously coated with human type IV collagen (Sigma). After incubation (4 h, 37°C, 5% CO₂) the filters were removed, stained with Diff-Quik (American Scientific Products, McGraw Park, IL, USA), and non-migrating cells were removed by wiping with a rubber policeman. The migratory response was observed by light microscope examination and quantified, after stain extraction (0.1 N HCl, 15 min), by absorbance at 600 nm (Beckman Instruments, Palo Alto, CA, USA) [26]. Each ELF sample was tested in duplicate, and the results were expressed as chemotactic units present in 1 ml of ELF. This biological activity was further characterized in a checkerboard analysis, to clarify whether it was merely chemokinetic or chemotactic [27]. Sequential 1:2 dilutions of ELF samples were placed above and below filters, in all possible combinations, and SMC migration was quantified. ELF samples from six different normal subjects were tested, and the results express the mean values.

Since several mediators with chemotactic activity for mesenchymal cells are known to be present in ELF [28, 29], we further characterized this activity to determine PDGF relative involvement. Several aliquots of ELF were tested, with or without biochemical treatments related to PDGF properties: 1) heat stability was evaluated by boiling (10 min) ELF samples, and cooling them to room temperature before testing for chemotactic activity; 2) acid stability was tested by dialysis of ELF samples against acetic acid (1 M, pH 2.5, 48 h), lyophilizing and redissolving them in HCl (10 mM) and DMEM before testing; 3) to evaluate trypsin sensitivity, 100 µg of trypsin (Sigma) was added to 1 ml of ELF before incubation (30 min, 37°C); then 200 µg of soybean trypsin inhibitor (Sigma) was added before testing (soybean trypsin inhibitor at the concentration used, with or without trypsin, did not alter ELF chemotactic activity); 4) 2-mercaptoethanol was added (1 h, 37°C) to ELF sam-

ples to evaluate the sensitivity to reduction; the samples were then dialysed against DMEM before testing; and 5) the effects of the addition of anti-PDGF antibody, (rabbit anti-human PDGF antibody, kindly provided by C-H. Heldin, Ludwig Institute for Cancer Research, Uppsala, Sweden) on ELF chemotactic activity was tested by incubation (2 h, 22°C) of ELF samples with optimal concentrations of antibody before testing (the antibody alone, at the concentration used, had no significant chemotactic activity). As controls, normal samples of ELF were tested before and after incubation with anti-fibronectin and anti-transforming growth factor (TGF)-β antibodies (rabbit anti-human fibronectin antibody and rabbit anti-human TGF-β antibody kindly provided by C. Rivat, INSERM U284, Nancy, France).

ELF growth activity for fibroblast was measured using ³H-thymidine incorporation by Balb/c3T3 fibroblasts. Balb/c3T3 were seeded in 24-well Falcon plates (Becton Dickinson Labware, Lincoln Park, NJ, USA, 10⁴ cells·ml⁻¹ per well) in DMEM supplemented with 10% fetal calf serum (FCS), (Biosystems, Strasbourg, France), and allowed to grow (48 h, 37°C, 5% CO₂). Then, after several vigorous washes with DMEM, 0.8 ml of serum-free DMEM was added to each well with 0.1 ml of ELF sample to be tested. After incubation (3 h, 37°C, 5% CO₂), platelet poor plasma (PPP) was added to a final concentration of 5%. PPP was prepared from human plasma (obtained from Centre Regional de Transfusion Sanguine, CHRU, Vandoeuvre-les-Nancy), clarified by centrifugation (28,000 ×g, 45 min, 4°C), defibrinogenized by heat (56°C, 30 min), and treated by CM-Sephadex (Whatman Biosystems, Maidstone, UK) to remove residual PDGF, before sterilization by filtration (0.2 µm filter, Sartorius, Göttingen, West Germany). Labelling was carried out by addition of ³H-thymidine (100 µl·well⁻¹ of a 1 µCi·ml⁻¹ solution, Centre d'Etudes Nucleaires de Saclay, Gif-sur-Yvette, France). After labelling (6 h, 37°C, 5% CO₂), the cells were extensively washed with 5% cold trichloroacetic acid solution (Sigma) and solubilized in NaOH (0.25 N, 0.8 ml·well⁻¹). Finally, incorporated ³H-thymidine was quantified with a liquid scintillation counter (Beckman). Each sample was tested in triplicate, and the results correspond to the mean expressed as counts per minute (cpm) incorporated by 4 × 10⁴ cells in culture. In some control experiments, ELF growth activity was evaluated by cell counting in parallel with ³H-thymidine incorporation to confirm that ³H-thymidine incorporation was directly related to fibroblast replication.

Since PDGF is a "competence" growth factor, *i.e.* a mediator acting early in the G1 phase of fibroblast cell cycle, the nature of fibroblast growth activity present in ELF was characterized in a "complementation" test, as described previously [3, 4, 21]. Briefly, Balb/c3T3 were cultured in DMEM with serum for two days at seeding concentration of 4 × 10⁴ cells·ml⁻¹ per well in 24-well Falcon plates (Becton Dickinson). Then, after several vigorous washes, PDGF, PPP containing a "progression" growth activity, *i.e.* acting later in the G1 phase of fibroblast cell cycle, and ELF samples were added in different combinations, and all cultures were processed for two days. Fibroblast replication was evaluated in parallel

by ^3H -thymidine incorporation and cell counting. In some experiments, ELF samples were incubated with PDGF antibody before being tested in the "complementation" test.

Characterization and quantification of PDGF dimers present in ELF

Since PDGF can be present in three forms (AA, AB and BB dimers) and since these three types of dimers share, but to different extents, the same biological activities, the specific amount of each dimer in BAL samples was quantified after reconstitution of BAL samples to ELF *in situ* concentration. Enzyme-linked immunosorbent assays (ELISAs) specific for each type of human PDGF (AA, AB and BB) were carried out using mouse monoclonal antibodies raised against PDGF AA and BB dimers, and, by using two antibodies for each ELISA, it was possible to specifically quantify each type of dimer.

For determination of PDGF AA dimers, 96-well flat-bottomed polystyrene microtitre plates were coated with $200\ \mu\text{l-well}^{-1}$ of a mouse monoclonal antibody against human PDGF AA, at $5\ \mu\text{g}\cdot\text{ml}^{-1}$ in $100\ \text{mM}\ \text{NaHCO}_3$, pH 9.5 at room temperature for 16–24 h. Plates were washed three times with bidest water and then blocked with $300\ \text{mM}\ \text{Tris}/\text{HCl}$, pH 7.5, 1% bovine serum albumin and 0.025% thimerosal for 24 h at room temperature. Following removal of the blocking buffer, samples and human PDGF AA as a standard were titrated in doubling dilutions in a final volume of $200\ \mu\text{l-well}^{-1}$ in $250\ \text{mM}$ phosphate buffer pH 8.0 containing 0.5% bovine serum albumin. To each well $50\ \mu\text{l}$ of the mouse monoclonal antibody (MoAb) against human PDGF AA conjugated with horseradish peroxidase were added and incubated for 16–24 h at room temperature, followed by washing with sterile water and peroxidase activity was determined by

incubation with substrate buffer ($200\ \mu\text{l}$, $2.5\ \text{mM}\ \text{H}_2\text{O}_2$ with $1\ \text{mM}$ 3, 3', 5, 5'-tetramethylbenzidine in $30\ \text{mM}$ citrate buffer, pH 4.1) for 10 min before the reaction was stopped with $100\ \mu\text{l}\ 1\ \text{M}\ \text{H}_2\text{SO}_4$. Extinction was measured at 450 nm.

For determination of PDGF AB dimers, microtitre plates were coated with a mouse MoAb against human PDGF BB and a second antibody peroxidase conjugated anti-PDGF AA was used. For determination of PDGF BB dimers, first and second antibodies were anti-PDGF BB mouse MoAbs. The results are expressed as ng of each dimer present in 1 ml of ELF.

In order to evaluate whether acid or neutral extraction would modulate the quantification of PDGF dimers detected by these ELISAs, samples of BAL fluid were tested by ELISA after acetic acid dialysis or ammonium acetate dialysis before lyophilization.

Statistical analysis

All data are presented as mean \pm standard error of the mean (SEM). All statistical comparisons were made using a two-tailed Student's t-test.

Results

Mesenchymal chemotactic activity in ELF

Since PDGF is a strong chemotactic factor for mesenchymal cells, samples of ELF obtained by BAL were tested in a chemotactic assay for SMC, to evaluate the presence of PDGF. SMC migrated in response to the presence of acid-extracted ELF, in a dose-dependent manner (fig. 1a) with a maximum for 1:2 dilution of ELF samples previously

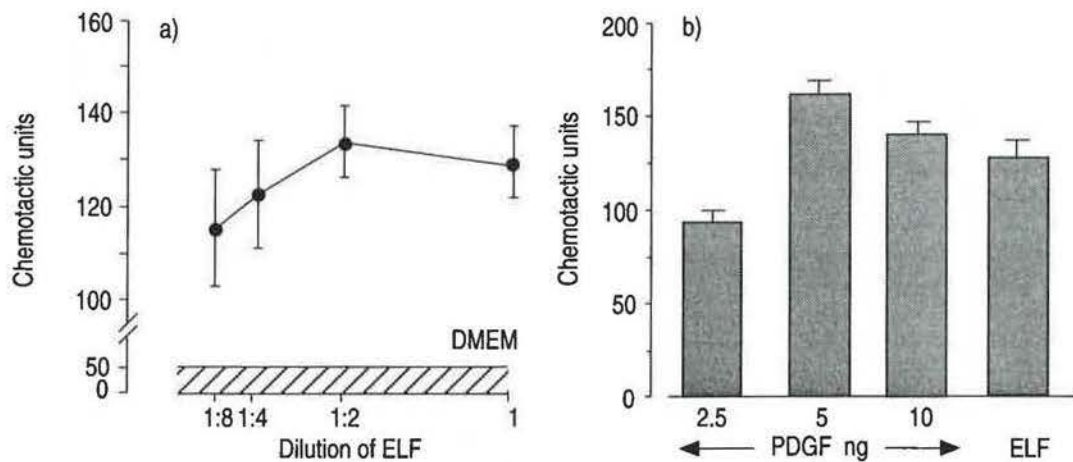


Fig. 1. — Chemotactic activity for mesenchymal cells in normal human epithelial lining fluid (ELF). Acid-extracted samples of ELF obtained by bronchoalveolar lavage were tested for the presence of chemotactic activity using modified Boyden chambers and smooth muscle cells as target cells. a) Dilution curve of the chemotactic activity present in a sample of ELF (mean \pm SEM). The shaded area corresponds to the chemotactic activity measured in the presence of DMEM alone. Each dilution was tested in three different experiments. Shown in one representative example (mean \pm SEM of triplicate measurement for one subject) obtained from four different subjects tested. b) Chemotactic activity present in undiluted ELF samples from 8 subjects. Compared to purified human platelet PDGF standards (2.5, 5 and $10\ \text{ng}\cdot\text{ml}^{-1}$) alone. DMEM: Dulbecco's modified Eagle's medium; PDGF: platelet-derived growth factor.

reconstituted to ELF *in vivo* concentration. Then, ELF samples from several other subjects were tested for the presence of a chemotactic activity before, and after dilution (data not shown), and compared to the chemotactic activity of standard PDGF solutions alone (fig. 1b). The mean value of the chemotactic activity present in undiluted ELF (129±8 chemotactic units·ml⁻¹ of ELF) was lower than for 5 ng·ml⁻¹ of PDGF (163±6 chemotactic units, p<0.01).

In order to specify whether the chemotactic activity measured was truly chemotactic or only chemokinetic, different combinations of serial dilutions of ELF were placed on both sides of filters in Boyden chambers in a checkerboard analysis (table 1). The results suggest the presence of a limited chemokinetic activity, but that most of the SMC migration observed was due to the presence of a chemotactic activity.

Since at least 2 other chemotactic mediators are known to be present in ELF, fibronectin and TGF-β [28, 29], tests were performed to evaluate the chemotactic activity of PDGF in the ELF samples. Samples of ELF were tested before and after different biological treatments (fig. 2). While both heat and acid treatments resulted in a limited diminution of the initial chemotactic activity, trypsin digestion and reduction resulted in a marked decrease of the chemotactic activity. These observations were compatible with the presence of PDGF. However, at least one chemotactic factor known to be present in normal ELF, TGF-β, reacts to these biological treatments in a very similar pattern. Thus, several ELF samples were tested before or after incubation with anti-PDGF antibody. This incubation resulted in a decrease of ELF chemotactic activity suggesting that this activity was partly due to the presence of PDGF. As controls, some samples of ELF were tested before or after incubation with anti-fibronectin or anti-TGF-β antibodies, again resulting in a significant decrease of the chemotactic activity, 18±2%, p<0.05; and 22±2%, p<0.01, respectively.

Acid versus Neutral extraction

Furthermore, since the first experiments were carried out with ELF treated by acid dialysis, and since acid extraction might play a role in biological activation, in subsequent experiments, aliquots of ELF from the same subjects were dialysed in parallel against acetic

Table 1. - Checkerboard analysis of SMC migration with several ELF dilutions above and below filters

		ELF dilutions above filter		
		1:8	1:4	1:2
ELF dilutions below filter	1:8	61±2	82±8	117±4
	1:4	68±2	68±2	96±3
	1:2	71±2	75±3	70±2

Results, in chemotactic units, correspond to the mean of five different experiments. ELF: epithelial lining fluid; SMC: smooth muscle cell.

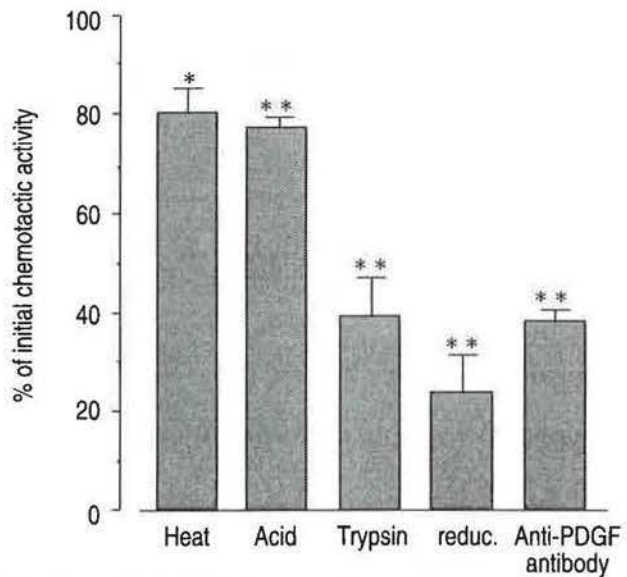


Fig. 2. - Biochemical characterization of the chemotactic activity for mesenchymal cells present in normal human epithelial lining fluid (ELF) (mean±SEM) of 5 subjects tested. Heat: 100°C, 10 min; acid: pH 2.5; reduc: reduction (2-mercaptoethanol); Anti-PDGF antibody: incubation with anti-PDGF antibody; PDGF: platelet-derived growth factor. *, **: p<0.05, p<0.01 diminution of initial chemotactic activity.

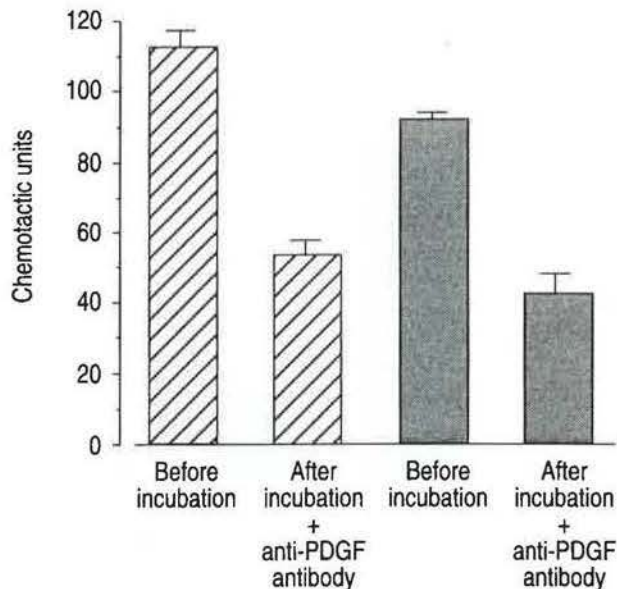


Fig. 3. - Effects of acid (▨, acetic acid pH 2.5) and neutral (■, ammonium acetate pH 7.4) extraction on the chemotactic activity for mesenchymal cells present in normal human epithelial lining fluid (ELF) before or after incubation with anti-PDGF antibody (mean±SEM). PDGF: platelet-derived growth factor.

acid or against ammonium acetate (for neutral dialysis), lyophilized, redissolved in DMEM, and then tested for chemotactic activity before or after incubation with anti-PDGF antibody. The results (fig. 3) demonstrate a significant but limited decrease of the chemotactic activity after neutral dialysis (9±2%, p<0.05). Furthermore, the reduction by incubation with anti-PDGF antibody of the chemotactic activity present in neutral-extracted samples

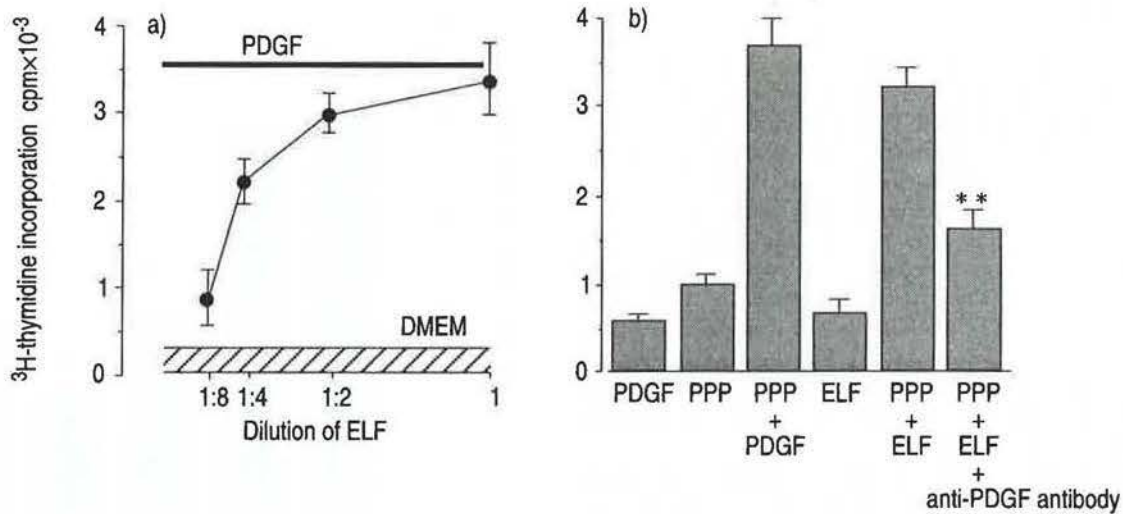


Fig. 4. - Presence of a "competence" growth activity for fibroblasts in normal human epithelial lining fluid (ELF). Acid-extracted samples of ELF obtained by BAL were tested for the presence of a growth activity for fibroblasts using ³H-thymidine incorporation. a) Dilution curve of ELF growth activity for fibroblasts in the presence of optimal concentration of platelet poor plasma (PPP). The shaded area corresponds to the growth activity obtained in the presence of DMEM alone. This growth activity is compared to the growth activity obtained in the presence of platelet purified human PDGF (10 ng·ml⁻¹). Shown is one representative example (mean±SEM for one subject) obtained from five different subjects tested. b) Characterization of the "competence" growth activity present in ELF. ELF samples from 6 subjects (mean±SEM) were tested in a "complementation" test using fibroblasts as target cells. PDGF, PPP, and ELF samples were added in different combinations to fibroblasts in culture and their replication was measured by ³H-thymidine incorporation. In some experiments ELF samples were incubated with anti-PDGF antibody before testing. **: p<0.01 compared to PPP+ELF alone. For further abbreviations see legend to figure 1.

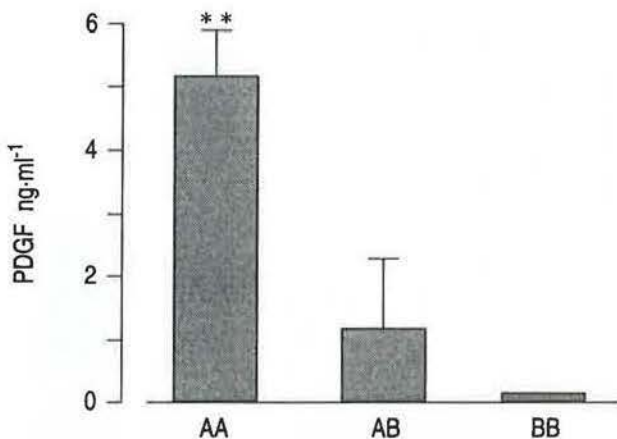


Fig. 5. - Characterization of platelet-derived growth factor (PDGF) dimers present in normal human lung epithelial lining fluid (ELF) (mean±SEM). ELF samples obtained by BAL of normal subjects (n=13) were dialysed against acetic acid, lyophilized, and tested for the presence of PDGF AA, AB and BB dimers using specific ELISA tests. **: p<0.01 AA vs AB.

suggests that acid extraction plays a limited role in ELF PDGF biological activity (acid extraction 53±4 chemotactic units versus neutral extraction: 42±5 chemotactic units, p>0.05).

Fibroblast growth activity in ELF

The induction of fibroblast replication, a biological activity of PDGF, was detected in ELF by the addition to Balb/c3T3 fibroblasts of serial dilutions of ELF samples in the presence of a constant amount of PPP. Addition

of ELF resulted in a dose-dependent increase of fibroblast replication (fig. 4a). Since fibroblasts require two signals to divide [3, 4], the first acting in the early stage of G1 cell cycle and given by a "competence" factor (such as PDGF), and the second signal acting later in G1 and given by a "progression" factor (such as insulin-like growth factor-1 (IGF-1)), to further characterize PDGF growth activity in ELF, ELF samples were tested in a "complementation" test (fig. 4b), demonstrating that a "competence" growth activity was present in ELF, since a significant fibroblast proliferation was observed in the presence of a "progression" factor (PPP). Furthermore, this "competence" activity was partially suppressed by the incubation of ELF samples with anti-PDGF antibody (1.6±0.2 10³ cpm vs 3.2±0.2 10³ cpm, p<0.01).

PDGF dimer presence

Finally, to determine which PDGF dimers are present in ELF, aliquots of ELF were evaluated by three ELISAs, each specific for one PDGF dimer (fig. 5). Interestingly, whilst no significant amount of PDGF BB dimer was detectable in any sample, PDGF AB dimers were present at a mean concentration of 1.2±0.4 ng·ml⁻¹ ELF and PDGF AA dimers at a mean concentration of 5.2±0.7 ng·ml⁻¹ (p<0.01, AA vs AB dimers). For all subjects tested, the amount of PDGF AA dimers present in ELF was higher than the amount of PDGF AB dimers, with a mean ratio AA/AB dimers of 4.3±0.5. Furthermore, acid or neutral extraction did not result in a significant change of PDGF dimer levels in ELF (data not shown).

Discussion

This study demonstrated that ELF obtained by BAL from normal subjects contains significant amounts of PDGF AA and AB dimers, and this cytokine is partly responsible for the chemotactic and "competence" growth activity for mesenchymal cells present in normal ELF. This observation suggests that PDGF, normally present in the lung, is involved in the local processes of tissue maintenance and repair.

PDGF is a 30 kDa glycoprotein composed of two chains (A and B) bound by disulphide bridges and encoded by genes located, respectively, on chromosome 7 and 22 [5-7]. Both PDGF chains share 60% homology, and three different dimers are possible: AA, AB and BB. Initially purified from platelets (where it is stored in α granules), PDGF is a strong chemotactic factor and a "competence" growth factor for mesenchymal cells, and has been shown to be involved in the processes of tissue repair and fibrosis. Subsequently, PDGF was shown to be released by several cell types (blood monocytes, alveolar macrophages, endothelial cells, vascular SMC), and transformed cells [8-12]. PDGF exerts its activities by binding with specific receptors on target cells [14-18]. Two types of PDGF receptor subunits have been identified: the α subunit that binds PDGF A and B chains, and the β subunit that binds only PDGF B chain. The effects of the different forms of PDGF on each type of target cell are modulated by the differential and specific expression of both types of PDGF receptor subunits by each type of cells [19, 20].

Following the observation of the release of PDGF by activated blood monocytes, alveolar macrophages obtained from normal individuals were shown to spontaneously release biologically active PDGF [9, 10]. More recently, about 20% of lung interstitial macrophages were characterized by immunohistochemistry to be positively stained by an anti-PDGF antibody, suggesting the normal presence of PDGF in the lung and its relationship with macrophages [30].

In this study, we used 50 ml aliquots and the urea method to evaluate PDGF concentration in the lung. As suggested by MARCY *et al.* [31], this method tends to underestimate the concentrations of proteins in the *in situ* ELF and, thus, PDGF concentrations might be slightly higher than suggested by our work.

It is not yet possible to ascertain the origin of ELF PDGF, since, if human platelet PDGF composition is well-characterized [32], little is known about the nature of PDGF released by normal cells. Platelet PDGF is mainly composed of AB dimers and, to a lesser extent, of BB dimers. Thus, our observation suggests that platelets might be only a limited source of ELF PDGF, and it concurs with the fact that platelets are normally absent from the alveolar space, and none or very few platelets are recovered by BAL procedure due to local injury [24]. With regard to the different types of PDGF producing cells present in the normal lung, limited data are available about the composition of the PDGF they release, and their individual contribution is to be further evaluated. In contrast, tumour cells mainly release PDGF AA dimers

[33, 34], and cells transfected with both A and B chain complementary deoxyribonucleic acid (cDNA) express both genes, produce AA, AB and BB dimers [35], but release almost exclusively PDGF AB dimers. In this respect, PDGF BB dimers are currently thought not to be secreted by several cell types that produce them, but to stay membrane bound. Due to the fact that ELF contains both PDGF AA and AB dimers, PDGF can act on mesenchymal cells expressing PDGF receptor α and β subunits. However, the predominance of AA dimers suggests that ELF PDGF might be more active on mesenchymal cells expressing PDGF receptor α subunit. A better understanding of ELF PDGF *in vivo* biological activity requires the specific observation of PDGF receptor subunits expression by lung mesenchymal cells. Despite this limitation, it can be postulated that ELF PDGF plays a role in the mesenchymal cell turnover that maintains normal lung architecture and controls the repair processes. Interestingly, PDGF B chain is the product of the *c-sis* proto-oncogene and PDGF BB homodimer is capable of inducing cell transformation [36]. In this respect, the absence of significant amounts of BB homodimer in ELF PDGF suggests a protection against a chronic exposure of lung cells to a transforming activity.

Finally, PDGF is only one of the mediators present in ELF capable of playing a role in normal mesenchymal cell biology, in addition to fibronectin (a chemotactic and attachment mediator for mesenchymal cells [28, 37]), and TGF- β (a powerful chemotactic factor for fibroblasts with inhibitory effects on fibroblast replication [29, 38]). Furthermore, other biologically active relevant compounds are present in ELF, such as prostaglandin E₂ (PGE₂) which inhibits fibroblast replication [39]. Thus, the final result of the local presence of these different mediators depends upon the delicate balance of their biological activities and of target cell specific receptivity [23].

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