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Growth factor activity in the lung during compensatory growth after pneumonectomy: evidence of a role for IGF-1

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ABSTRACT: Unilateral pneumonectomy in rats causes compensatory growth of the remaining lung. During this growth, there are large increases in the cell numbers and in the rates of collagen and non-collagen protein production. We examined possible mechanisms by which these changes might occur.

Assessment of the effect of bronchoalveolar lavage (BAL) fluid on fibroblasts *in vitro* demonstrated the presence of stimulatory activity for fibroblast replication in control animals. This activity was greatly increased two and six days postpneumonectomy (115±26% and 75±18% above control values, respectively), but had returned to normal by 14 days. Preliminary characterization suggests that the activity is heat labile and consists of at least two moieties with apparent molecular weights of 5–15 kD and 70–220 kD. The activity was partially blocked by antibodies to insulin-like growth factor-1 (IGF-1), and levels of IGF-1 were increased by about 100% ($p<0.001$) two days after pneumonectomy compared with control values.

Examination of BAL cells demonstrated an early influx of leucocytes into the remaining lung of pneumonectomized rats. At two days, about 25% of the lavageable cells were neutrophils, but macrophages were the predominant cell type at all times. The extravascular albumin space of the lung increased by about 65% ($p<0.01$), six days after pneumonectomy. The influx of circulatory proteins and cells are potential sources of the increased mitogenic activity observed in the lung.

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The process of compensatory lung growth after partial pneumonectomy was first described more than a century ago [1]. The rapid growth includes large increases in cell number and total proteins, including those of the extracellular matrix, of which collagen is the major component [2–4]. The mechanisms which control this growth are still poorly understood. Several factors have been implicated, including physical forces imposed by removal of lung tissue, changes in oxygen and carbon dioxide tension, altered blood flow and hormonal effects [5–7]. Mitogenic agents in serum have also been suggested to play a role in this process. Enhanced levels of growth factors have been described in the blood [7–9] but it is not known whether these compounds enter the circulation after production at a site remote from the lungs, or are produced in the lung and subsequently enter the circulation.

In this study, we have investigated whether mitogenic agents are present in the lungs of normal animals, and in the right lung of animals that have undergone left pneumonectomy, by examining the effects of bronchoalveolar lavage fluid on fibroblasts in culture. In conjunction with this we have investigated potential sources of such agents by examining the cellular and protein influx into the right lung after pneumonectomy.

Methods

Animals

Experiments were performed on male Lewis rats, an inbred strain originally derived from Wistar rats [10], with weights of 192±2 (mean±SEM) g at the time of operation. One group of rats was anaesthetized with Hypnorm (fentanyl citrate 0.315 $mg \cdot ml^{-1}$; fluanisone 10 $mg \cdot ml^{-1}$) at a dose of 1 $ml \cdot kg^{-1}$ body weight, administered by intramuscular injection. The rats were intubated with a 16 gauge catheter and ventilated by a small animal respirator (Harvard Apparatus Ltd, Edenbridge, Kent, UK) at a rate of 54 $breaths \cdot min^{-1}$ and a stroke volume of 2.5 ml. The left side of the chest was opened at the fifth intercostal space, the

lung exposed, tied at the hilum and excised. The chest was sutured in layers, negative intrathoracic pressure re-established, and the animals allowed to recover.

Another group of rats was operated on in a similar way except that the left lung was not tied or removed (sham-operated controls). A third group of rats was used as unoperated normal controls. Animals were studied in groups of at least five, at various times between 2–28 days after operation.

Bronchoalveolar lavage (BAL) of rats and processing of the fluid obtained

Rats were deeply anaesthetized with intramuscular Hypnorm (1.5 ml·kg^{-1}) and exsanguinated by opening the peritoneal cavity and cutting the aorta. A ventral midline incision was made from the neck to the diaphragm, the left main bronchus ligated and a cannula passed through the trachea into the right bronchus. Normal saline at room temperature was instilled slowly into the right lung, *via* the cannula, in 4 ml aliquots over a period of 15 s, left in the lung for 30 s, withdrawn over 15 s and collected in a 50 ml polypropylene centrifuge tube which was kept on ice. A total of 40 ml of saline was used and in all cases >95% was recovered.

The BAL fluids were centrifuged at 4°C for 10 min at 300×g. Supernatants were retained for further analysis. The cell pellets were resuspended in 10 ml of Dulbecco's modified minimum essential medium (DMEM) and recentrifuged. The supernatant was discarded and the cell pellet resuspended in 1 ml of DMEM. An aliquot of 20 μl was taken for a total cell count in an improved Neubauer counting chamber, after mixing with 20 μl of a solution of 1% crystal violet in 1% acetic acid.

Aliquots of 100 μl of the cell suspension were used for cytocentrifuge preparations. These were centrifuged for 10 min at 450 rpm (Cytospin 2, Shandon Southern Products Ltd, Runcorn, Cheshire, UK). The slides with the adhering cells were allowed to dry in air and then stained with May Grünwald Giemsa stain.

The cell free BAL supernatants were exhaustively dialysed against distilled water using a dialysis membrane with a nominal molecular weight cut-off of 3.5 kD. The dialysates were lyophilized, redissolved in 2 ml of DMEM and frozen at -40°C before analysis.

Fibroblast cultures

A cloned rat embryonic fibroblast cell line was used for these experiments (Rat 2, obtained from the American Type Culture Collection, Rockville, Maryland, USA). Cells were cultured in 10 cm diameter Petri dishes (Nunc Cat. No. 1-50350; Nunc, Kamstrup, Denmark) with DMEM supplemented with 5% newborn calf serum (NCS). They were incubated in a humidified atmosphere of 10% CO_2 in air at 37°C.

Fibroblast replication assay

The effect of BAL fluid on fibroblast replication was assayed as described previously [11]. Preliminary experiments were performed to determine the optimum assay conditions described below. The BAL fluid concentrates, were thawed, appropriate aliquots were centrifuged at 9,000×g for 2 min (Beckman microfuge B; Beckman - RIIC Ltd, High Wycombe, Bucks, UK) and serial twofold dilutions in DMEM were made. Assays were carried out in flat-bottomed 96 well tissue culture plates (Nunc Cat. No. 1-67008 96F; Nunc, Kamstrup, Denmark). The outermost wells, rows A and H and columns 1 and 12, were not used for assay and were filled with 100 μl of DMEM containing 2% NCS. This left the central 10×6 matrix of wells for the assay. The wells in columns 2 and 11 were filled with 50 μl of DMEM for use as medium controls. Aliquots of 50 μl of diluted sample were added to each of the six wells in columns 4 to 10.

Plates of confluent Rat 2 cells were then trypsinized, transferred to a sterile polypropylene 50 ml centrifuge tube and centrifuged at 300×g for 5 min. The cells were resuspended in DMEM containing 4% NCS at a concentration of 8×10^4 cells·ml^{-1}. Aliquots (50 μl) of this cell suspension were added to each of the wells in the inner 10×6 matrix of the assay plate, to give 4,000 cells·well^{-1} in 100 μl of DMEM with a final concentration of 2% NCS. Cells were incubated for 48 h, after which the cell layer was washed with phosphate buffered saline and fixed by adding 100 μl of 10% formol saline.

Staining and spectrophotometric assessment of cell numbers were carried out exactly as described previously [11]. Briefly, after fixing, the formol saline was removed and the cells stained by addition of 100 μl of methylene blue (1% in 0.01 M borate buffer, pH 8.5). Thirty minutes later the excess dye was removed by flicking and rinsing the stained monolayer four times in 0.01 M borate buffer (pH 8.5). The dye was eluted from the cells with 100 μl of a 1:1 solution of ethanol and 0.1 M HCl and the absorbance measured at 650 nm using a microplate spectrophotometer. Values for cells incubated in the presence of BAL fluid concentrates were compared with those for cells alone. This method depends on the binding of methylene blue dye to negatively charged moieties within cells. Although these moieties are predominantly in polynucleotides within the nucleus, some cytoplasmic molecules also bind the dye. Therefore, an increase in dye uptake could be effected by hypertrophy as well as hyperplasia of cells. For this reason, changes in fibroblast replication observed using this method were confirmed by direct counting of cell numbers.

Partial characterization of BAL fluid growth factor activity

Concentrated BAL fluid (200 μl) obtained from rats two days after pneumonectomy was fractionated by gel

filtration (Sephacryl S-200; Pharmacia LKB Biotechnology, Milton Keynes, Bucks, UK) with column dimensions of 90 cm length and 2 cm diameter. Samples were eluted with 0.02 M ammonium bicarbonate, pH 7.4, 4°C, at a flow rate of 0.5 ml·min^{-1}. Fractions were collected in 2.5 ml aliquots, freeze-dried to remove the ammonium bicarbonate and resuspended in DMEM prior to assay for fibroblast replicating activity.

The effect of heating BAL fluid, at 100°C for various times up to 60 min, on its fibroblast replicating activity was also investigated.

To investigate whether IGF-1 or platelet-derived growth factor (PDGF) contributed to the increased fibroblast replication, induced by lavage fluid from pneumonectomized rats, neutralizing antibodies to these growth factors were employed in an attempt to abrogate the activity. Briefly, fibroblasts were seeded at a density of 4,000 cells·well^{-1} in 50 μl of DMEM containing 0.4% NCS. Twenty four hours later, 50 μl of DMEM containing BAL fluid from animals two days after pneumonectomy was added to the wells to give a final concentration of 1/16 together with either rabbit anti-human IGF-1 diluted 1:4,000 (Kabigen AB, Stockholm, Sweden) or anti-PDGF at a concentration of 50 μg immunoglobulin G (IgG)·ml^{-1} (British Biotechnology, Cowley, Oxford, UK). After a further 48 h, cell numbers were assessed as described above.

Levels of IGF-1 in BAL fluid were determined using a commercially available assay kit (Amersham International plc, Amersham, Bucks, UK) and recombinant IGF-1 as a standard (Kabigen AB, Stockholm, Sweden).

Differential cell counts

Differential cell counts of the cytocentrifuge preparations were carried out blind, *i.e.* without prior knowledge of the animals from which they were derived. Each slide was examined by light microscopy on two separate occasions, with at least 500 cells identified per slide. Cells were classified as macrophage/monocyte, neutrophil, lymphocyte and eosinophil. Cell types were either expressed as total cells in the BAL fluid or as proportions of the total number of lavageable cells.

Measurement of extravascular albumin space

The extravascular albumin space was assessed by measuring the leakage of radiolabelled albumin from the circulation into the lung parenchyma over a period of 24 h as described previously [12]. Briefly, rats were lightly anaesthetized by intramuscular injection of Hypnorm (0.1 ml) and then injected intravenously with 1 μCi of ^{125}I-labelled human serum albumin (Amersham International plc, Amersham, Bucks, UK) in 0.5 ml of normal saline. Twenty four hours later, the rats were deeply anaesthetized by intramuscular injection of Hypnorm (1.5 ml·kg^{-1}), the peritoneal cavity opened by a midline incision, the viscera laid to one side and a blood sample collected from the aorta into tubes containing ethylenediaminetetra-acetic acid (EDTA). The abdominal aorta was then cut, the thorax opened *via* a midline incision and the vena cava cannulated just above the diaphragm. The lungs were perfused, *via* the cannula, with 15 ml of phosphate buffered saline, when the lungs appeared white and the perfusate was clear. A cut was made in the ventricles to release any pressure and to prevent backflow of blood into the lungs. The right lung of each animal was removed, blotted dry, weighed and placed in a tube for determination of radioactivity. Blood samples were centrifuged at 2,000×g for 15 min and duplicate samples of 100 μl of plasma were used for radioactivity determinations. The radioactivity in the right lung was standardized to that in 1 ml of plasma and expressed with respect to lung weight.

Statistical analysis

Statistical evaluation was performed using an unpaired t-test [13]. The mean values of various parameters were said to be significantly different when the probability of the differences of that magnitude, assuming the null hypothesis to be correct, fell below 5% (*i.e.* $p<0.05$). Where mean values are calculated, standard errors of the mean (SEM) are also given.

Results

Changes in body and lung weight after left unilateral pneumonectomy

The body weights of normal control animals increased by about 60% over the 28 day period of study (table 1). Between 2–14 days after operation, sham-operated and pneumonectomized animals had lower body weights than normal controls. However, by 28 days the weight of pneumonectomized animals was not different from controls. The weight of pneumonectomized animals was lower than that of sham-operated animals at two days only. Also shown in table 1 are the changes in right lung wet weight after surgical removal of the left lung. This operation resulted in the loss of about 35% of the total lung weight. The right lung weight of pneumonectomized rats was increased by about 20% two days after operation and by 6 days had attained a weight equivalent to that of both lungs in control animals. Thereafter, the right lungs of pneumonectomized rats continued to grow at the same rate as that of the control animals. After 28 days, the right lungs of pneumonectomized rats were about 70% heavier than the right lungs of control animals but were not significantly different from the total left plus right lung weights of control animals.

Table 1. – Changes in body and lung weights after pneumonectomy

	Body wt	Lung wet weight g		
	g	Right	Left	Total
2 day				
Control	225±4	0.59±0.01	0.31±0.01	0.90±0.02
Sham	209±4	0.58±0.01	0.33±0.01	0.91±0.02
Pneumonectomy	189±3*	0.70±0.03*	-	0.70±0.03
6 day				
Control	231±3	0.58±0.02	0.28±0.01	0.86±0.03
Sham	216±3	0.60±0.03	0.31±0.02	0.91±0.04
Pneumonectomy	207±3*	0.88±0.05*	-	0.88±0.05
14 day				
Control	270±4	0.61±0.03	0.32±0.02	0.93±0.05
Sham	251±4	0.64±0.03	0.32±0.01	0.96±0.04
Pneumonectomy	250±4*	0.95±0.04*	-	0.95±0.04
28 day				
Control	309±10	0.73±0.08	0.40±0.04	1.13±0.11
Sham	282±9	0.73±0.03	0.37±0.03	1.10±0.06
Pneumonectomy	300±7	1.25±0.03*	-	1.25±0.03

Statistically significant difference compared with controls or sham-operated animals: *: $p<0.01$.

Effects of BAL fluid concentrates on the replication of fibroblasts in culture

Figure 1 shows the effects of BAL fluid concentrates obtained from animals six days after operation, on *in vitro* fibroblast replication. Values are expressed as percentage change from the medium control. In control groups stimulatory activity was observed, with highest values obtained at a dilution of 1/16 of the concentrated BAL fluid ($p<0.001$, for both normal control and sham-operated animals). Further dilution of the BAL concentrate diminished activity towards levels of the medium control. Stimulatory activity of BAL fluid from pneumonectomized rats was consistently higher than that observed for control or sham-operated animals and required greater dilution to negate its effect. At all time-points, the highest stimulatory activity was observed at a 1/16 dilution of the concentrated lavage fluid. Increased fibroblast replication was confirmed for samples obtained two days after pneumonectomy by direct cell counting. Stimulation of fibroblast replication above medium control was 21.0±2.6% using direct cell counts compared with 22.7±3.0% using the spectrophotometric assay, and there was no significant difference between these values.

Figure 2 shows data obtained, using BAL fluid at a 1/16 dilution, at 2, 6, 14 and 28 day time-points. Stimulatory activity was observed in the lavage of all control and sham-operated animals but there was no significant difference between these groups at any time. Therefore, values for control and sham-operated animals have been combined, for the purpose of statistical comparisons, with the activity observed for lavage fluid of pneumonectomized animals. Values were significantly raised above those of controls by 115±26% and 75±18% at 2 and 6 days, respectively. By 14 days, values were not significantly different from those of controls.

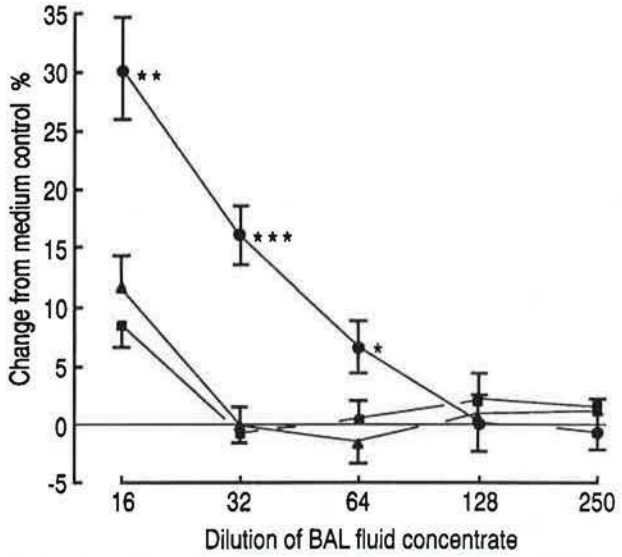

Fig. 1. – Effect of bronchoalveolar lavage fluid on fibroblast replication six days after pneumonectomy. Each point represents the mean±SEM of values for seven conrol (▲), five sham-operated (■) and six pneumonectomized (●) rats. Statistical comparisons were made at each dilution between pneumonectomized and combined sham and control values, which were not significantly different; *: $p<0.01$; **: $p<0.001$; ***: $p<0.0001$. BAL: bronchoalveolar lavage.

Studies to partially characterize the agents responsible for the increased fibroblast replication were performed on BAL fluid obtained from animals two days after pneumonectomy. Analysis of BAL fluid fractionated by gel filtration showed two major peaks of stimulatory activity for fibroblast replication ($p<0.005$ compared with medium controls in both cases). These eluted in positions which corresponded to molecular weights of between 5–15 kD, and between 70–220 kD. Heating the BAL fluid at 100°C for 15, 30 and 60 min reduced its activity by 25%, 71% and 100%, respectively.

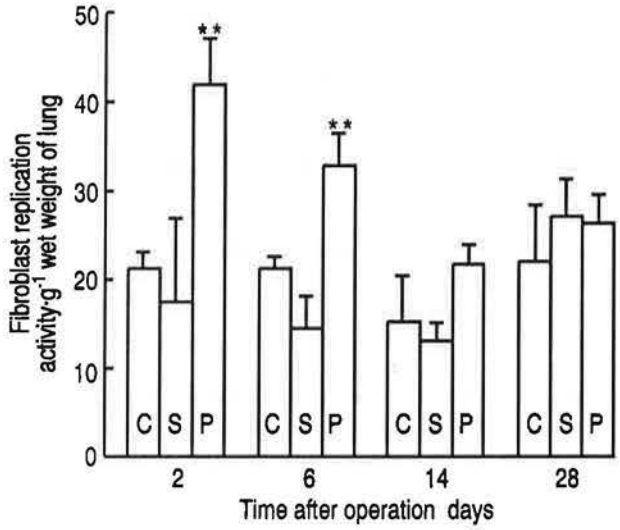

Fig. 2. – Activity of bronchoalveolar lavage fluid from control, sham-operated and pneumonectomized rats on fibroblast replication during compensatory lung growth. The effect of BAL fluid on fibroblast replication *in vitro* is shown as the percentage stimulation on fibroblast replication compared with medium controls (as shown in figure 1) divided by the lung weight. Values represent the mean±SEM for groups of at least five animals. Statistical comparisons were made at each time, between the pneumonectomy (P) group and combined sham (S) and control (C) groups, which were not significantly different; **: p<0.005. BAL: bronchoalveolar lavage.

Figure 3 shows the effects of neutralizing antibodies to IGF-1 (fig. 3A) and PDGF (fig. 3B) on the fibroblast stimulating activity in the BAL fluids from rats two days after pneumonectomy. The BAL fluid stimulated fibroblast replication by 33.6±2.4% compared with fibroblasts exposed to media alone (fig. 3A). The presence of an antibody to IGF-1 reduced this stimulation by about one-third. Antibodies to PDGF had no effect on BAL fluid-induced fibroblast replication (fig. 3B). Anti-bodies alone did not significantly affect fibroblast replication. Both antibodies were capable of blocking the effects of their respective growth factors. Addition of a stimulatory dose of IGF-1 to BAL fluid did not further enhance fibroblast replication, compared with BAL fluid alone and addition of antibody reduced the stimulation to that of BAL fluid plus antibody (fig. 3A). Addition of a stimulatory dose of PDGF to BAL fluid further enhanced fibroblast replication to 59.1±3.5% (fig. 3B). This additional replication was completely blocked by addition of antibodies to PDGF.

Estimates of the levels of IGF-1 in the BAL fluid of control, sham-operated and pneumonectomized rats at times between 2–28 days are shown in table 2. Two days after pneumonectomy, levels of IGF-1 were double those of control animals, expressed either as total amounts or per gram wet lung weight. At the same time, levels for two out of five sham-operated animals were also raised compared with control animals; the values for this group were significantly different from both control and pneumonectomy groups ($p<0.05$). Six days after pneumonectomy, total levels of IGF-1 were still 60% above those of controls but when expressed per gram wet lung weight the values were within the normal range. There were no differences between control or sham-operated animals at this time. By 14 days, values had returned to normal, although at 28 days there was an apparent decrease in IGF-1 levels of the pneumonectomy group when expressed per gram wet lung weight.

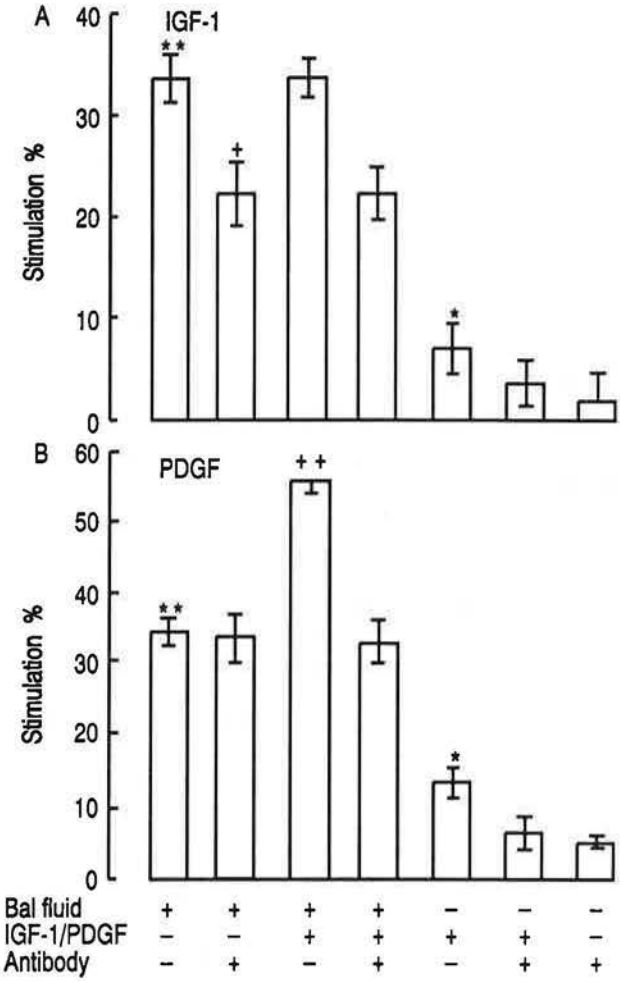

Fig. 3. – Effect of antibodies to IGF-1 and PDGF on bronchoalveolar lavage fluid-induced fibroblast replication after pneumonectomy. Each bar represents the percentage stimulation in fibroblast replication compared with replication of fibroblasts exposed to media alone. A) shows results of addition of BAL fluid, IGF-1 and antiserum to IGF-1, either alone or in the combinations indicated. B) shows results of addition of BAL fluid, PDGF and antibodies to PDGF, either alone or in the combinations indicated. Significant differences from cells exposed to media alone: * p<0.01; **: p<0.001; or from BAL fluid alone: +: p<0.05; ++: p<0.02. BAL: bronchoalveolar lavage; IGF-1: insulin-like growth factor-1; PDGF: platelet-derived growth factor.

Changes in bronchoalveolar lavage (BAL) cell populations

The total number of cells lavaged from the right lungs of control, sham-operated and pneumonectomized animals are shown in table 3, expressed per gram wet weight of lung. Values for control and sham-operated rats were similar for all groups. Two days after

pneumonectomy, the mean value for total cells lavaged was more than double that of controls but this was not statistically significant due to the large variation observed between animals. Six days after pneumonectomy, between 3–55% more cells were recovered compared with control groups.

Table 2. – Changes in the levels of IGF-1 in bronchoalveolar lavage fluid during compensatory lung growth

	Total lavaged IGF-1	
	ng	·g^{-1} wet lung weight
2 day		
Control	25.9±1.5	43.9±2.5
Sham	37.7±4.4*	65.0±7.6*
Pneumonectomy	61.5±4.4*+	87.9±6.3*+
6 day		
Control	26.2±1.2	45.2±2.1
Sham	28.1±0.9	46.7±1.5
Pneumonectomy	42.1±1.0*+	47.9±1.1
14 day		
Control	29.5±1.0	48.4±1.6
Sham	29.6±0.8	46.3±1.3
Pneumonectomy	41.3±6.2	43.5±6.5
28 day		
Control	31.5±1.5	43.1±2.0
Sham	26.7±2.9	36.5±4.0
Pneumonectomy	34.7±2.8	27.7±2.2*

Statistically significant difference compared with controls: *: p<0.05; **: p<0.001; or sham-operated animals: +: p<0.05; ++: p<0.001. IGF-1: insulin-like growth factor-1.

Also shown in table 3 are the numbers of lavageable macrophages, neutrophils, lymphocytes and eosinophils as well as their proportions relative to the total number of cells lavaged. In all cases the macrophage was the predominant cell type, and represented >90% of the total cells in all control and sham-operated groups. Six days after pneumonectomy, the number of macrophages recovered from the lavage fluid increased by between 25–40% compared with control values. However, the proportion of macrophages decreased to about 90% because of increased numbers of neutrophils and lymphocytes in the BAL fluid. Two days after pneumonectomy, there was about a 35 fold increase in the number of neutrophils lavaged from the right lung, when compared with sham-operated controls. The values at 6 and 14 days postpneumonectomy were about three to five times those of control groups.

Lymphocyte numbers increased more slowly, with peak values obtained six days after pneumonectomy and were about three to seven times those of control groups. Eosinophils were not detected in the lavage of most animals of the control groups but were clearly present in the lavage of pneumonectomized animals, with peak values obtained six days after operation.

Changes in extravascular albumin space

Table 4 shows the values obtained for estimates of extravascular albumin space for the right lungs of

Table 3. – Changes in lavage cell profiles during compensatory lung growth

	Number of cells lavaged (×10^{-5})·g^{-1} wet lung weight (proportion of total cells lavaged %)				
	Total	Macrophage	Neutrophil	Lymphocyte	Eosinophil
2 day					
Control	13.6±4.6	13.4±4.5 (98.9)	0.05±0.04 (0.3)	0.11±0.04 (0.8)	ND
Sham	13.1±3.1	12.3±3.1 (92.8)	0.27±0.08 (2.6)	0.50±0.24 (4.5)	ND
Pneumonectomy	29.1±5.2	19.0±3.4 (71.4)	9.23±3.09+ (26.2)	0.83±0.32 (2.3)	0.01±0.01 (0.1)
6 day					
Control	13.3±2.9	12.9±2.8 (96.9)	0.09±0.03 (0.5)	0.34±0.05 (2.7)	ND
Sham	11.5±0.8	11.3±0.8 (97.8)	0.09±0.02 (0.8)	0.14±0.04 (1.2)	ND
Pneumonectomy	17.9±1.8++	16.2±1.8* (89.9)	0.46±0.06++ (2.8)	1.16±0.16++ (6.7)	0.08±0.04 (0.5)
14 day					
Control	12.3±2.1	11.9±2.1 (96.4)	0.14±0.09 (1.2)	0.34±0.19 (2.4)	ND
Sham	11.5±0.7	11.2±0.6 (97.5)	0.11±0.05 (0.9)	0.20±0.09 (1.6)	ND
Pneumonectomy	16.1±2.0	15.0±1.9 (93.4)	0.35±0.06* (2.3)	0.61±0.10 (4.0)	0.05±0.02 (0.3)

Numbers of cells in the right lung for groups of control, sham and pneumonectomized animals ·g^{-1} wet lung weight are shown at two, six and 14 days after operation. Values for individual cell types are derived from the total and differential cell counts with the proportion of the total cells in parentheses. ND: not detectable. Values which were significantly different from those of control and sham-operated animals: *: p<0.05; +: p<0.02; and ++: p<0.01.

Table 4. – Changes in extravascular albumin space of the right lung during compensatory growth

	Plasma albumin cpm·ml^{-1}	Lung albumin cpm·g^{-1}	Extravascular albumin space ·unit^{-1} wet lung weight
2 day			
Control	19600±1450	2610±180	0.134±0.003
Sham	20100±800	2860±100	0.143±0.009
Pneumonectomy	22600±1050	4420±270*	0.189±0.010*
6 day			
Control	22700±1000	3170±210	0.140±0.006
Sham	23750±900	3840±240	0.162±0.007
Pneumonectomy	21800±1250	5000±240*	0.232±0.014*
14 day			
Control	20900±850	3150±160	0.151±0.006
Sham	22450±1550	3240±270	0.145±0.005
Pneumonectomy	20550±900	3280±120	0.160±0.003*
28 day			
Control	26750±1550	4100±390	0.153±0.006
Sham	24350±1400	3890±230	0.160±0.008
Pneumonectomy	25800±950	4150±270	0.161±0.009

Significantly different from control and sham values: *: $p<0.01$; or from sham values: *: $p<0.05$.

control, sham-operated and pneumonectomized rats, together with the radioactivity measurements in the plasma and lungs from which they were derived. The radioactivity measurements in plasma were not significantly different between groups at any time. At two and six days after pneumonectomy, radioactivity measurements in the lung increased compared with sham-operated and normal control values. The extravascular albumin space for control and sham-operated rats, was similar at all times. However, after pneumonectomy values increased to a maximum of about 65% above control values at six days and then decreased to control levels by 28 days.

Discussion

After left unilateral pneumonectomy the remaining right lung responded rapidly, so that six days after operation the wet weight of the remaining lung was equivalent to that of both lungs in control animals. Thereafter, the weight of the right lung of pneumonectomized rats increased at the same rate as seen for both lungs of control animals. Studies in our laboratory and others have shown that this increased lung weight is associated with increased numbers of cells and deposition of proteins [2–4, 14–16]. In this study, we have begun to investigate some potential mechanisms by which this rapid change in the apparent rate of lung growth might occur.

After pneumonectomy, stimulation of fibroblast replication was greatly augmented. The highest activity was observed two days after pneumonectomy, when stimulation of fibroblast replication was double that observed for control animals. Values declined thereafter and were not significantly different from control values by 14 days, suggesting that the normal lung contains mediators capable of stimulating fibroblast replication and that these mediators can be increased during times of rapid growth or possibly after injury.

Antibody blocking experiments were performed in an attempt to identify possible mediators of fibroblast growth in the BAL fluid of pneumonectomized rats. Antibodies to IGF-1 decreased the activity by one-third and radioimmunoassay data confirmed that levels of IGF-1 were increased in these fluids. This suggests a potential role for IGF-1 in the process of compensatory lung growth after pneumonectomy. No evidence was found to indicate the presence of PDGF in BAL fluid.

IGF-1 is a heat labile, single chain polypeptide with a molecular weight of 7.65 kD. However, in the free form it has a very short half-life and is usually present in association with binding proteins. The most abundant binding protein for IGF-1 is insulin-like growth factor binding protein-3 (IGFBP-3) which forms a 150 kD complex with IGF-1. We have demonstrated that the fibroblast growth promoting activity present in BAL fluid after pneumonectomy is heat labile and when fractionated by gel filtration at neutral pH the activity eluted in two fractions with estimated molecular weights of 5–15 kD and 70–220 kD. These fractions may represent IGF-1 and IGF-1 bound to IGFBP-3, respectively. Further studies are required to confirm that these fractions are associated with IGF-1.

To our knowledge, this is the first report to demonstrate increased levels of IGF-1 in compensatory lung growth. However, it has been implicated previously in the developmental regulation of lung growth [17, 18] and in fibrotic lung disease [19]. Increased levels of IGF-1 or its messenger ribonucleic acid (mRNA) have also been implicated in compensatory growth of other tissues including kidney [20, 21], skeletal muscle [22, 23] and the pancreas [24]. Together, these studies suggest an important role for IGF-1 in the regulation of normal tissue growth, compensatory tissue growth and in repair.

It is possible that the enhanced mitogenic activity demonstrated here in BAL fluid reflects changes in the interstitium, where it could act on interstitial cells *in vivo*. Evidence to support this concept comes from autoradiographic studies of ^{3}H-thymidine uptake in mice, rats and rabbits, which suggest that interstitial cells, as well as other cell types, replicate during compensatory lung growth [14–16]. Although fibroblasts were the target cell in these studies, their is no evidence that the growth factors observed are specific for fibroblasts. Indeed, IGF-1 has been shown to be mitogenic for several lung cells, including fibroblasts and epithelial cells [25, 26].

As well as stimulating cell replication, IGF-1 has also been reported to stimulate the production of connective tissue components such as collagen, elastin and proteoglycans [27–29]. We have previously

demonstrated a threefold increase in collagen synthesis rates during compensatory lung growth after pneumonectomy [3]. This could be due, at least in part, to the increased levels of IGF-1 observed in this study.

The source of the mediator, or mediators, observed here in BAL fluid, is unknown. Data presented in this study, from measurements of the extravascular albumin space of the lung, demonstrated increased amounts of albumin in the lungs of pneumonectomized rats compared with control animals. This increased albumin content of the lung could be due to either increased vascular permeability or decreased lymphatic flow, since the method used takes no account of the changes in rates of flux in these systems [30]. However, the evidence that albumin, a serum protein of approximately 67 kD, is present in increased amounts in the lung within two days of pneumonectomy demonstrates the potential for a rapid influx of proteins into the lung from the circulation, which could be retained in this expanded pool and may affect cell replication during compensatory lung growth. It is possible, that the high molecular weight stimulatory fraction observed in these studies could represent an influx of IGF-1 bound to IGFBP-3 from the circulatory system, although this has not been confirmed.

Resident lung cells or cells entering the lung from the circulation are another possible source of mediators, which may act in a paracrine or autocrine fashion. Lung fibroblasts are capable of synthesizing and releasing IGF-1 [25, 31]. Macrophages also produce mediators which stimulate cell replication. These include interleukin-1 [32, 33], tumour necrosis factor [34] and β-interferon [35]. Furthermore, during pulmonary fibrosis, alveolar macrophages have been shown to spontaneously release PDGF [36] and an IGF-1-like molecule [19]. In this study, we have shown from morphometric studies of BAL fluid that increased numbers of macrophages are present in the lung after pneumonectomy and are, thus, a potential source of mediators. Further studies are required to identify the source of the mediator(s) observed during compensatory lung growth.

In summary, we have demonstrated the presence of growth promoting activity in BAL fluid of normal rats, which stimulates the replication of fibroblasts *in vitro*. Stimulatory activity was greatly enhanced in fluid from pneumonectomized animals during the early phase of compensatory growth. At least part of this activity can be attributed to IGF-1. The extravascular albumin space of the lung was expanded and increased numbers of leucocytic cells were observed in the lung. An influx of circulating proteins, cells or resident cells of the lung are all potential sources of the increased levels of IGF-1. Further studies are required to elucidate the precise source and nature of this IGF-1 and other as yet unidentified mediator(s).

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