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# Alveolar accumulation of fibronectin and hyaluronan precedes bleomycin-induced pulmonary fibrosis in the rat

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*Alveolar accumulation of fibronectin and hyaluronan precedes bleomycin-induced pulmonary fibrosis in the rat. J. Hernnäs, O. Nettelbladt, L. Bjermer, B. Särnstrand, A. Malmström, R. Hällgren.*

**ABSTRACT: The development of bleomycin-induced pulmonary fibrosis in rats was studied over a period of 30 days after an intratracheal instillation of bleomycin. Fibronectin was visualized in histological sections and quantified in bronchoalveolar lavage fluid (BALF) and related to simultaneous measurements of hyaluronan, collagen and albumin in BALF and/or lung tissue extracts.**

**An increase in BALF fibronectin levels was noted after 3 days and the peak value a sixty fold increase was noted at day 7. Thereafter, the fibronectin levels declined and reached control values on day 21. A pronounced, patchily distributed staining for fibronectin appeared in the injured alveolar tissue parallel to the increased lavage fluid fibronectin levels on days 3–7. A fainter, streakily distributed fibronectin staining remained within the alveolar walls in areas with proliferating fibroblasts on days 14–30.**

**Albumin in BALF increased to a peak level, 20 times control values, after 3 days and then rapidly declined. Thus, the ratio of fibronectin to albumin increased to a peak value of 43 times control values on day 7, indicating that plasma leakage cannot be the only source of the observed increase in lavage fibronectin.**

**Lung tissue hydroxyproline increased between days 7 and 30, whereas extractable hyaluronan in lung tissue and bronchoalveolar lavage fluid peaked on days 3–7 and then gradually declined towards normal values on days 21–30.**

**These data demonstrate that fibronectin accumulates in the alveolar tissue during the early inflammatory phase of the bleomycin-induced lung injury, parallelling hyaluronan accumulation and preceding the development of pulmonary fibrosis.**

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Pulmonary fibrosis is the ultimate outcome of many interstitial lung diseases (ILD) and is characterized by a massive production of fibrous connective tissue around the alveoli [1]. The mechanisms responsible, including the interaction between connective tissue components and inflammatory/immune processes, are not fully understood. Bleomycin-induced pulmonary injury/fibrosis is an established and extensively studied animal model, characterized by an initial alveolitis phase, with the common features of acute lung injury, followed by increased deposition of collagen and the gradual development of pulmonary fibrosis [2]. In this model, hyaluronan (hyaluronic acid, HA), an important connective tissue constituent in *e.g.* remodelling tissues [3], inflammatory repair [4] and wound healing [5], is transiently accumulated in the alveolar tissue during the inflammatory phase [6–8]. In a recent immunohistochemical study of the same model, increased staining of fibronectin in alveolar exudates and the interstitium was also reported [9].

Fibronectin (FN), a large glycoprotein, capable of interacting with a number of matrix molecules, is present in most tissues and body fluids [10] and it has been proposed that it is chemotactic [11] and a necessary growth factor [12] for fibroblasts in the injured lung tissue. In the normal lung, FN is localized in the interstitium and on the surface of collagen fibres [13]. In fibrotic lung disorders increased amounts of FN are found in newly synthesized connective tissue [14], as well as in bronchoalveolar lavage fluid (BALF) [15–18]. Correlations have been found between high lavage levels of FN and signs of inflammation in BALF as well as a later outcome of the disease process [18], but not to the present degree of "end-stage" fibrosis as reflected by lung function tests [15–16]. In view of these clinical findings and the biological properties of FN, it may be suggested that increased amounts of FN in BALF reflect disease activity in ILD and, hence, possibly also identify patients more inclined to deteriorate.

Similarly, other clinical studies of various ILD have suggested that increased amounts of HA in BALF are related to early inflammatory stages and not to established fibrosis [19–23].

The present study set out to evaluate the presence and levels of FN in BALF and lung tissue sections during the development of the bleomycin-induced lung injury and to investigate the significance of increased BALF FN levels as related to histological findings, the increase in HA and the development of fibrosis.

## Materials and methods

### *Animals*

Adult male Sprague-Dawley inbred rats (ALAB, Sollentuna, Sweden), weighing 190–205 g at the time of tracheostomy, were used in this study. All rats were kept in separate cages and food and water provided *ad libitum*. Animals were sacrificed by aortic exsanguination under chloralhydrate anaesthesia.

### *Induction of Bleomycin-induced lung injury*

Tracheostomies were performed on all animals to facilitate the intratracheal injection of 1.5 mg bleomycin sulphate (Lundbeck, Copenhagen, Denmark) in 0.3 ml sterile saline under chloralhydrate anaesthesia. All rats received 10 mg of sodium cefuroxime (Glaxo, Greenford, UK), intraperitoneally, immediately before and 1 day after tracheostomy, in order to minimize the risk of bacterial infections. Sham-treated control animals received 0.3 ml of sterile saline, intratracheally, in the same manner as the bleomycin-treated rats.

### *Experimental design*

The amounts of FN, HA and cells in BALF were studied 1, 3, 5, 7, 10 14, 21 and 30 days after bleomycin instillation. Three rats were studied at each time point. The control group consisted of untreated rats (n=3) and rats investigated seven days after an intratracheal injection of saline (sham-treated, n=2).

In a parallel study-group, the amounts of HA and hydroxyproline in lung tissue extracts from the right lung were studied 1, 3, 7, 14 and 30 days after bleomycin instillation (n=3 at each time point). Controls were either untreated (n=3) or investigated seven days after an intratracheal injection of saline (sham-treated, n=2). Tissue sections from two randomly chosen animals from each time point were investigated for the localization of fibronectin.

### *Bronchoalveolar lavage*

Lavage was performed as described previously [8]. Immediately after death, the lungs were lavaged by intratracheal infusion of 5 aliquots of 5 ml phosphate-buffered saline (PBS), under gravity, at a constant hydrostatic pressure of 25 cm. After 3 min the fluid was recovered by gravity. The recovery was 21.3±0.5 ml (85±2%). The lavage fluid was centrifuged at 400 × g for 10 min. The supernatant was kept frozen at -20°C until analysed.

### *Lung tissue preparations*

All preparations were made immediately after death. The lungs were removed en bloc and dissected free. The right lung was ligated at the hilus and weighed immediately (wet weight, w.w.) at room temperature, and then after freeze-drying (dry weight, d.w.). The dried lung was pulverized in a mortar. The homogenized lung samples were kept dry and frozen at -20°C until analysed.

### *Localization of fibronectin in lung tissue sections*

Longitudinal acetone-fixed cryostate sections, 6 μm thick, including apex, hilar tissue and the base of the left lung, were incubated in 0.6% $H_2O_2$ in methanol for 20 min. The slides were incubated for 20 min with swine serum diluted 1:10 in PBS and then incubated with a rabbit anti-rat fibronectin monoclonal antibody (immunoglobulin G (IgG), Dako A/S, Copenhagen, Denmark, final dilution 1:500) for 30 min. Swine anti-rabbit IgG (Dako A/S, Copenhagen, Denmark, final dilution 1:30) was used as secondary antibody, followed by preformed complexes of peroxidase and monoclonal rabbit anti-peroxidase antibodies (Dako A/S, Copenhagen, Denmark, final dilution 1:100). The peroxidase reaction was developed with 3-amino-9-ethyl-carbazole (Sigma Chemical Co., St. Louis, MO, USA) and the sections were counterstained with Mayer's haematoxylin for 5 min and then mounted with coverslip in gelatin-glycerin.

### *Extraction of lung tissue hyaluronan (hyaluronic acid)*

HA was extracted as described previously [7]. Briefly, 20 mg of the pulverized dried lung was extracted with 2 ml 0.5 M NaCl for 16 h with constant shaking at 40°C. The samples were then centrifuged for 15 min at 2,000 × g. The supernatants were recovered and the HA concentration analysed.

### *Analytical methods*

Rat serum albumin, hydroxyproline and 3-amino-9-ethyl-carbazole were obtained from Sigma Chemical Co., St Louis, MO, USA. Rabbit anti-rat albumin immunoglobulin fraction was bought from Cappel, Veedijk, Belgium. Rat fibronectin and goat anti-rat fibronectin antiserum were purchased from Calbiochem, La Jolla, CA, USA. Horseradish peroxidase-conjugated rabbit antiserum towards goat immunoglobulins, swine serum, a rabbit anti-rat fibronectin monoclonal antibody (IgG), swine anti-rabbit IgG, monoclonal rabbit antiperoxidase

antibodies and orthophenylenediamine were products of Dako A/S, Copenhagen, Denmark. HA-50 test, a radioassay kit for determination of hyaluronan was purchased from Pharmacia Diagnostics, Uppsala, Sweden.

*Albumin in BALF*

Rat serum albumin was radiolabelled with $^{125}I$ (Amersham, UK), using the chloramine T-method of GREENWOOD *et al.* [24], and analysed with a competition solid phase radio-immunoassay (SPRIA), principally according to DI MARIO *et al.* [25], using rabbit anti-rat albumin IgG (Cappell, Veedijk, Belgium, 5 $\mu g \cdot ml^{-1}$) for coating of tubes.

*Fibronectin in BALF*

Fibronectin was measured using enzyme-linked immunosorbent assay (ELISA), according to ENGVALL [26], with goat anti-rat fibronectin antiserum (Dako A/S, Copenhagen, Denmark, final dilution 1: 10000) as primary antibody, peroxidase-conjugated rabbit anti-goat IgG antiserum (Dako A/S, Copenhagen, Denmark, final dilution 1:500) as secondary antibody and orthophenylenediamine (Dako A/S, Copenhagen, Denmark) for detection.

Quantitative calculations of standard curves and sample concentrations for both immunochemical methods were performed using a computer programme, ELISA Soft (Perkin-Elmer, Göteborg, Sweden).

*Hyaluronan (hyaluronic acid) in BALF and lung tissue*

The concentrations of HA in the lung tissue extracts and in the BALFs were determined in duplicate with a radiometric assay (HA-50 test, Pharmacia Diagnostics, Uppsala, Sweden) as described previously [7, 8].

*Collagen in lung tissue*

Since the amino acid sequences of the various collagens are made up of 10–13% hydroxyproline [27], the quantification of this amino acid is regarded as a good measurement for collagen.

Hydroxyproline was measured, according to STEGEMANN and STALDER [28] 1967, after hydrolysis of BALF or tissue samples with 6 M HCl at 100°C for 17 h and subsequent removal of the acid by freeze-drying.

*Statistical analyses*

Non-parametric Mann-Whitney U-tests, calculated with a computer programme (ASYSTANT® Macmillan Software Company, NY, USA), were used to analyse the data. A value of $p<0.05$ was considered significant.

## Results

*Histopathological features*

The same histopathological features as described previously [6] were seen. Briefly, no signs of septal oedema, inflammation or fibrosis were seen in control animals. In bleomycin-treated animals, focal signs of interstitial inflammation with oedematous septa developed progressively between days 1 and 7 and then gradually declined. Initially polymorphonuclear leucocytes (PMNs) dominated, soon followed by increasing numbers of macrophages and lymphocytes. At later stages, septal fibrosis was more advanced and bundles of proliferating fibroblasts surrounded by eosinophils appeared. These changes became apparent on days 14 and 30, when increased numbers of macrophages also remained.

*Analysis of BALF and tissue components*

Data in the text are presented as mean values (in table 1 mean±SEM). No significant differences were observed between untreated and sham-treated control rats for any given variable, therefore, the mean values for the two groups taken together are used as control values.

In control animals (untreated and sham-treated), the mean level of FN was 250 $\mu g \cdot l^{-1}$. In bleomycin-injured animals lavage FN values increased significantly and reached maximum levels on day 7

Table 1. – Fibronectin, albumin and hyaluronan in lavage fluid measured at various times after bleomycin instillation

| Day | Fibronectin $\mu g \cdot l^{-1}$ | SEM | Albumin $\mu g \cdot l^{-1}$ | SEM | Hyaluronan $\mu g \cdot l^{-1}$ | SEM | FN/Alb $\mu g \cdot mg^{-1}$ | HA/Alb $\mu g \cdot mg^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| 0 | 250 | 130 | 67 | 18 | 9.9 | 1.1 | 3.7 | 0.1 |
| 1 | 410 | 150 | 37 | 14 | 13.5 | 0.6 | 11.1 | 0.4 |
| 3 | 9800 | 1500 | 1500 | 210* | 380 | 38* | 6.7 | 0.3 |
| 5 | 14200 | 500 | 870 | 73 | 720 | 23* | 16.3 | 0.8 |
| 7 | 14800 | 600* | 350 | 44 | 420 | 44 | 42.9* | 1.2 |
| 10 | 9100 | 300 | 440 | 140 | 280 | 38 | 20.8 | 0.6 |
| 14 | 3500 | 500 | 140 | 37 | 240 | 51 | 25.9 | 1.8 |
| 21 | 130 | 100 | 10 | 2 | 10.8 | 5.1 | 10.0 | 1.2 |
| 30 | 290 | 160 | 42 | 21 | 9.3 | 5.2 | 7.1 | 0.2 |

*:$p<0.05$ compared to control (n=3 at each time point). The values at day 0 denote the mean of untreated and sham-treated rats. FN: Fibronectin; Alb: albumin; HA: hyaluronan.

(14,800 $\mu g \cdot l^{-1}$). Thereafter, lavage FN decreased slowly and was normal by day 21 (fig. 1 and table 1).

In the lung tissue of control rats, FN staining was mainly seen in the larger connective tissue structures, the adventitia of vessels, the submucosal tissue of bronchi and in the loose connective tissue surrounding larger bronchi and vessels and the pleura. Very little or no FN could be visualized in the interstitial alveolar tissue (fig. 2A). In bleomycin-injured rats, additional substantial extracellular staining for FN was seen in the interstitium on days 3–7 (fig. 2B and 2C). Furthermore, marked staining was seen in the cytoplasm of some single cells, probably macrophages, on day 3. On day 14, the degree of interstitial FN staining was reduced and became more streakily distributed. Staining was typically seen surrounding proliferating fibroblasts in scattered areas (fig. 2D).

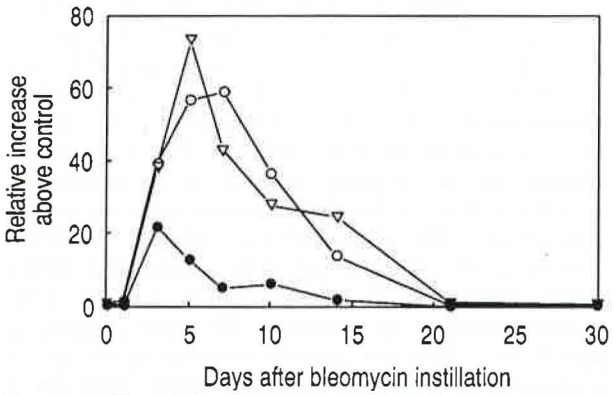


Fig. 1. – The relative mean concentrations (increase above control) of fibronectin (O), hyaluronan (∇) and albumin (●) in bronchoalveolar lavage fluid from rats investigated at different times (n=3 at each day) during a 30 day period after bleomycin administration. Control denotes untreated and sham-treated rats.

To investigate the possibility that the increase in FN is due to a plasma leakage, the presence of albumin in BALF was measured. Albumin reached a maximum on day 3, earlier than FN (fig. 1 and table 1). The ratio of FN to albumin varied from 3.7 μg FN·$mg^{-1}$ albumin in controls to a maximum of 42.9 $\mu g \cdot mg^{1}$ day 7 (table 1). This also means that the relative increase in albumin is smaller than that of FN, 20 timés control values as compared to a sixty fold increase in FN. Albumin then declined faster than FN, and control values were reached already on day 14.

Hyaluronan (hyaluronic acid) in BALF increased in the same order of magnitude as fibronectin and reached its maximum on day 5, somewhat earlier than the peak in FN. The values then declined, initially at a somewhat faster rate, reaching a plateau between days 10 and 14, but regained control values on day 21 (fig. 1). The ratio of HA to albumin varied from 0.1μg HA·$mg^{-1}$ albumin in controls, to a maximum of 2 $\mu g \cdot mg^{-1}$ on day 14 (table 1), whereas control values were reattained on days 21–30.

The total extractable amount of HA from lung tissue in control animals (untreated and sham-treated) was 95 $\mu g \cdot g^{-1}$ dry weight. In bleomycin-treated animals the HA-concentrations increased to reach a maximum on day 3 (201 $\mu g \cdot g^{-1}$ d.w., fig. 3). This is earlier than the increase in BALF, which occurred on day 5 (fig. 1). Thereafter, the values declined to 125 $\mu g \cdot g^{-1}$ d.w. on day 30.

Hydroxyproline in lung tissue increased from control values 9.7 $\mu g \cdot mg^{-1}$ d.w., to a maximum of 16.8 $\mu g \cdot mg^{-1}$ d.w. at day 30. However, hydroxyproline initially decreased between control level and day 3, 6.9 $\mu g \cdot mg^{-1}$ d.w. At day 7, control values were reattained (fig. 3).

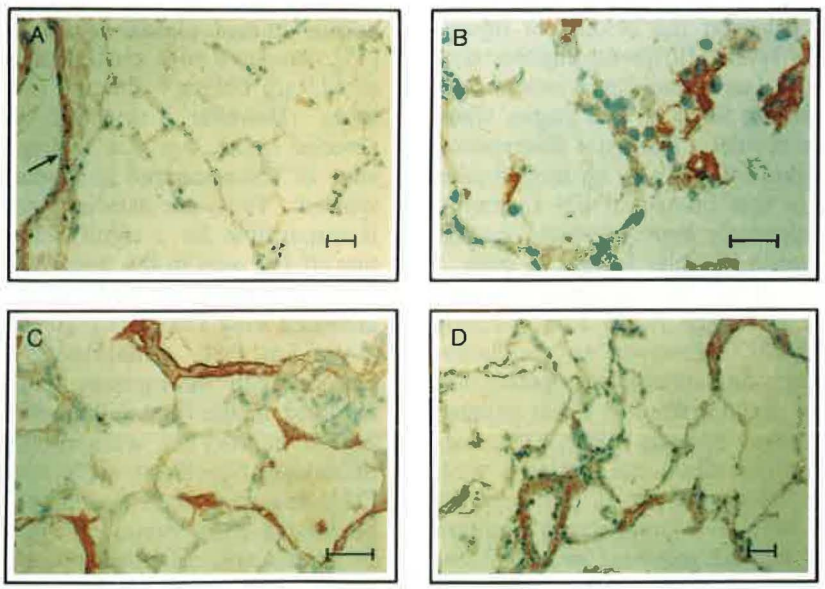


Fig. 2. – Immunoperoxidase staining of fibronectin on cryostat sections of rat lungs. The sections were counterstained with Mayer's haematoxylin. (Bars =2.4 mm). A) An untreated rat. Postive staining only in loose connective tissue structures surrounding a larger bronchus (arrow) and vessels but no visible staining in the alveolar walls. B) Three days after an intratracheal injection of 1.5 mg bleomycin. Fibronectin staining is seen in a patchy "cell close" pattern. C) Seven days after bleomycin instillation. Positive fibronectin staining is seen diffusely distributed in the inflamed alveolar septa. D) Fourteen days after bleomycin instillation. Fibronectin staining has faded to become more streakily distributed in the fibrotic alveolar wall, typically surrounding proliferating fibroblasts.

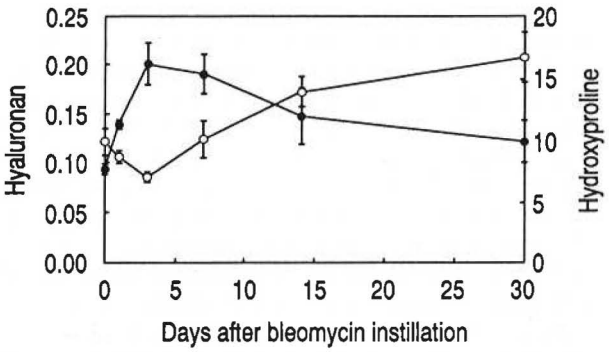


Fig. 3. – Concentrations (mg·g$^{-1}$ dry weight, mean±SEM) of hydroxyproline (○) and hyaluronan (●) in lung tissue extracts from the right lung of rats investigated at different times (n=3 at each day) during a 30 day period after bleomycin administration. The values at day 0 denote untreated and sham-treated rats.

Due to the insensitivity of the method used in this study, no hydroxyproline could be detected in BALF.

## Discussion

The results in the present study demonstrate that FN accumulates in the alveolar tissue during the development of bleomycin-induced pulmonary fibrosis as reflected by BALF levels and immunohistochemistry. The accumulation profile in BALF, as well as the histological localization of FN, parallels the appearance of HA in the same model, as described in this and previous studies [6–8], and precedes collagen accumulation. In the normal lung tissue FN could only be located to larger connective tissue structures surrounding bronchi and vessels and only trace amounts were found in BALF. In contrast, a pronounced increased staining is seen in the alveolar interstitium during the alveolitis phase of the bleomycin injury, paralleling peak BALF levels. However, positive septal FN staining, although less prominent, was still seen in the fibrous remodelling tissue at later stages, when lavage FN levels were normal. The latter discrepancy suggests that BALF either reflects only an intraalveolar accumulation of FN or that interstitial FN appearing at later stages, in a supposedly more organized matrix, is not accessible to bronchoalveolar lavage, or both.

Clinical studies have shown that increased amounts of fibronectin appear in lavage fluids from patients with various ILD [15–18]. However, no correlation between BALF FN levels and simultaneous pulmonary function tests could be demonstrated in either patients with idiopathic pulmonary fibrosis (IPF), [15], sarcoidosis [15, 16], or mesothelioma patients with hemithorax irradiation. Bitterman *et al.* [18] reported that the presence of both the "competence" growth factor fibronectin and the "progression" growth factor alveolar macrophage-derived growth factor (AMDGF) correlated with functional deterioration in IPF, sarcoidosis and "other ILD" after a mean observation time of 2.6 yrs. In contrast, O'Connor *et al.* [16] found no correlation between BALF FN levels and the progression of disease in sarcoidosis. The results in this study clearly demonstrate that increased BALF levels of FN are seen only during the inflammatory phase of the bleomycin-induced alveolar injury and not in the late fibrotic phase. Thus, data indicate that increased levels of FN in BALF reflect remodelling of the connective tissue and (inflammatory) disease activity in ILD, rather than the present degree of pulmonary fibrosis.

The alveolar accumulation of fibronectin, as observed in this study, may result from either a plasma leakage and/or a local production in the tissue. In a recent immunohistochemical and ultrastructural study of the bleomycin model, Lazenby *et al.* [9] state that FN appearing early probably originates from plasma leakage, whereas local production is a more likely source at day 10 and later stages. The concept of a local production is further supported by increased FN-messenger ribonucleic acid (mRNA) expression in fibroblasts observed in this model [29, 30] and the observation that, in human pulmonary fibrosis, increased amounts of cellular but not plasma fibronectin are seen in the connective tissue matrix [14]. Although not fully conclusive, our data also indicate that the increased amounts of FN observed at days 5 and later in this model cannot originate merely from plasma leakage for several reasons. In lavage fluid the peak values of albumin, supposed to reflect plasma leakage to the alveolar tissue, and FN differ in time, resulting in increased FN/albumin ratios on days 5–14. Also, the relative increase in lavage FN is three times higher than the relative increase in albumin, and on day 7 the FN/albumin ratio has increased to 42.9 μg FN·mg$^{-1}$ albumin compared to 3.7 μg FN·mg$^{-1}$ albumin in controls. Finally, although neither rat fibronectin nor albumin serum values were recorded here, the ratio of fibronectin to albumin in normal human plasma is 5–15 μg FN·mg$^{-1}$ albumin [15] compared to a maximum rat BALF mean value of 42.9 μg FN·mg$^{-1}$ albumin, recorded on day 7 in this study. However, a selective "trapping" of FN in the alveolar tissue, *e.g.* due to impaired lymphatic clearance of FN compared to albumin, must also be considered. Thus, our data indicate that local production is responsible for a significant part of the accumulation of FN seen in the injured lung.

An accumulating body of evidence indicates that activated lung fibroblasts are of prime importance in a yet not fully understood, complex network of cytokine-cell interactions, regulating the processes involved in the remodelling of the connective tissues in the healthy as well as the injured lung [31]. Stimulated fibroblasts are a source of FN in the lung [32], and may also, including when stimulated with bleomycin [33], produce high amounts of HA [34]. Furthermore, although the results of *in vitro* experiments with a single cytokine do not necessarily explain their role in a complex *in vivo* system, recent studies have shown that *e.g.* both transforming growth factor-β (TGF-β) and interleukin-1 stimulate the production of FN [35–37], and HA [38, 39], as well as collagen [40], in cultured lung fibroblasts. However,

macrophages [11] and epithelial cells [41], are other possible cellular sources of FN in the alveolar compartment(s), whereas HA may originate from lung endothelial cells [42]. The appearance of FN in some scattered macrophage-like cells on day 3, seen in this study, also suggests that these cells may either be a local source of FN, or, less likely, phagocytosing FN.

As shown in this study, the accumulation profile of HA parallels that of FN in lavage fluid and, furthermore, when previous observations on HA accumulation are taken into account [6], these two matrix components appear in the same compartments in normal as well as in bleomycin-injured lung tissue. *In vitro* experiments have shown that FN is an important chemoattractant [11, 15, 43] and growth factor [12] for fibroblasts and also augments phagocytosis by human alveolar macrophages [44], whereas HA can regulate a variety of cellular functions, *e.g.* phagocytosis [45, 46], and agglutinate alveolar macrophages [47]. Furthermore, FN binds HA and other glycosaminoglycans [48–50] and several studies indicate that interactions between FN and HA may determine parts of their function *in vivo, e.g.* cell adhesion [51], cell detachment [52], and have been suggested to play an important role in building up the initial clot during wound healing [53]. Thus, although both FN and HA may have various separate functions in the extracellular matrix and in controlling cellular activity, HA-FN interactions may be important for fibroblast detachment, and hence migration, as well as early matrix formation during tissue repair. Interactions between HA and FN are also in agreement with the simultaneous and colocated appearance of both of these macromolecules in the alveolar tissue seen in this study.

In summary, the results of this study confirm previous clinical and experimental studies indicating that the appearance of increased amounts of FN in BALF reflects an early inflammatory remodelling phase in the process of pulmonary fibrosis rather than the degree of established end-stage disease. Our data also show that increased amounts of FN and HA appear parallel in the alveolar compartment and BALF, preceding collagen deposition, indicating that FN-HA interactions may be important for the further development of pulmonary fibrosis. Furthermore, the accumulation of these two extracellular matrix substances may also, in part, be regulated by common mechanisms.

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## References

1. Clark JG. – The molecular pathology of pulmonary fibrosis. *In*: Connective Tissue Disease. J. Uitto, A.J. Pereida. eds, Marcel Dekker Inc., New York 1987; pp: 321–343.
2. Thrall RS, McCormick JR, Jack RM, McReynolds RA, Ward PA. – Bleomycin-induced pulmonary fibrosis in the rat. Inhibition with indomethacin. *Am J Pathol*, 1979; 85: 117–130.
3. Toole BP. – Glycosaminoglycans in morphogenesis. *In*: Cellular Biology of Extracellular Matrix. E.D. Hay ed., Plenum Publ. Corp., New York 1981; pp. 251–294.
4. Whiteside TL, Buckingham RB. – Interactions between cells of the immune system and hyaluronate synthesis by human dermal fibroblasts. *In*: The Biology of Hyaluronan. D. Evered, J. Whelan eds, Ciba Foundation Symp. 143, Wiley, Chichester, UK, 1989; p. 170–186.
5. Irvin TT. – The healing wound. *In*: Wound Healing, Principles and Practice. T.T. Irvin ed., Year Book Medical Publishers Inc, New York, 1981; pp. 1–33.
6. Nettelbladt O, Bergh J, Schenholm M, Tengblad A, Hällgren R. – Accumulation of hyaluronic acid in the alveolar interstitial tissue in bleomycin-induced alveolitis. *Am Rev Respir Dis*, 1989; 139: 759–762.
7. Nettelbladt O, Tengblad A, Hällgren R. – Accumulation of hyaluronan (hyaluronic acid) in lung tissue during experimental alveolitis parallels development of interstitial edema. *Am J Physiol (Lung Cell Mol 1)*, 1989; 267: L379–L384.
8. Nettelbladt O, Hällgren R. – Hyaluronan (hyaluronic acid) in bronchoalveolar lavage fluid during the development of bleomycin-induced alveolitis in the rat. *Am Rev Respir Dis*, 1989; 140: 1028–1032.
9. Lazenby AJ, Crouch EC, McDonald JA, Kuhn C III – Remodeling of the lung in bleomycin-induced pulmonary fibrosis in the rat. An immunohistochemical study of laminin, type IV collagen and fibronectin. *Am Rev Respir Dis*, 1990; 142: 206–214.
10. Ruoslahti E, Engvall E, Hayman EG. – Fibronectin: current concepts of its structure and function. *Coll Rel Res*, 1981; 1: 95–128.
11. Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG. – Production of fibronectin by the human alveolar macrophage: Mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung disease. *Proc Nat Acad Sci USA*, 1981; 78: 7147–7151.
12. Bitterman P, Rennard S, Adelberg S, Crystal R. – Role of fibronectin as a growth factor for fibroblasts. *J Cell Biol*, 1983; 97: 1925–1932.
13. Gil J, Martinez-Hernandez A. – The connective tissue of the rat lung: electron immunohistochemical studies. *J. Histochem Cytochem*, 1984; 32: 230–238.
14. Kuhn C III, Boldt J, King TE Jr, Crouch E, Vartio T, McDonald JA. – An immunohistochemical study of architectural remodeling and connective tissue synthesis in pulmonary fibrosis. *Am Rev Respir Dis*, 1989; 140: 1693–1703.
15. Rennard SI, Crystal RG. – Fibronectin in human bronchopulmonary lavage fluid. Elevation in patients with interstitial lung disease. *J Clin Invest*, 1981; 69: 113–122.
16. O'Connor C, Odlum C, Van Breda, A, Power, C, Fitzgerald MX. – Collagenase and fibronectin in bronchoalveolar lavage fluid in patients with sarcoidosis. *Thorax*, 1988; 43: 393–400.
17. Maasilta P, Salonen EM, Vaheri A, Kivisaari L, Holsti LR, Mattson K. – Procollagen-III in serum, plasminogen activation and fibronectin in bronchoalveolar lavage fluid during and following irradiation of human lung. *Int. J Radiat Oncol Biol Phys*, 1991; 20: 973–980.
18. Bitterman P, Rennard S, Keogh B, Adelberg S, Crystal RG. – Chronic alveolar macrophage release of fibronectin and alveolar macrophage derived growth factor correlates with functional deterioration in fibrotic lung disease. (Abstract). *Clin Res*, 1983; 31.
19. Hällgren R, Eklund A, Engström-Laurent A, Schmekel B. – Hyaluronate in bronchoalveolar lavage fluid,

a new marker in sarcoidosis reflecting pulmonary disease. *Br Med J*, 1985; 290: 1778–1781.
20. Hällgren R, Samuelsson T, Laurent TC, Modig J. – Accumulation of hyaluronan (hyaluronic acid) in the lung in adult respiratory distress syndrome. *Am Rev Respir Dis*, 1989; 139: 682–687.
21. Bjermer L, Engström-Laurent A, Lundgren R, Rosenhall L, Hällgren R, – Hyaluronic acid and procollagen III peptide in bronchoalveolar lavage fluid as indicators of lung disease activity in farmers lung. *Br Med J*, 1987; 295: 801–806.
22. Bjermer L, Engström-Laurent A, Thunell M, Hällgren R. – Hyaluronic acid in bronchoalveolar lavage fluid in patients with sarcoidosis. The relationship to lavage mast cells. *Thorax*, 1987; 42: 933–938.
23. Bjermer L, Lundgren R, Hällgren R. – Hyaluronan and type III procollagen peptide concentrations in bronchoalveolar lavage fluid in idiopathic pulmonary fibrosis. *Thorax*, 1989; 44: 126–131.
24. Greenwood FC, Hunter WM, Glover JS. – The preparation of $^{125}$I-labelled human growth hormone of high specific activity. *Biochem J*, 1963; 89: 114–123.
25. Di Mario U, Pietravalle P, Napoli A, Morano S, Mancuso M, Gambardella S, Anreani D. – A sensitive routine assay for urinary albumin based on the competitive binding to anti-albumin antibodies in solid phase. *Horm Metab Res*, 1986; 18 (10): 689–692.
26. Engvall E. – Enzyme immunoassay ELISA and EMIT. *Methods Enzymol* 1980; 70: 419–439.
27. Miller EJ. – Chemistry of the collagens and their distribution. *In*: Extracellular Matrix Biochemistry. K.A Piez A.H. Reddy eds, Elsevier Publishing Co. Inc., NY, 1984; pp. 41–81.
28. Stegemann H, Stalder K. – Determination of hydroxyproline. *Clin Chim Acta*, 1967; 18: 267–273.
29. Kelley J, Chrin L, Shull S, Rowe, DW, Cutroneo KR. – Bleomycin selectively elevates mRNA levels for procollagen and fibronectin following acute lung injury. *Biochem Biophys Res Commun*, 1985; 131: 836–843.
30. Raghow R, Lurie S, Seyer JM, Kang AH. – Profiles of steady-state levels of messenger RNAs coding for type I procollagen, elastin and fibronectin in hamster lungs undergoing bleomycin-induced interstitial pulmonary fibrosis. *J Clin Invest*, 1985; 76: 1733–1739.
31. Kelley J. – Cytokines of the lung. *Am Rev Respir Dis*, 1990; 141: 765–788.
32. Mosher DF, Furcht LT. – Fibronectin: review of its structure and possible function. *J Invest Dermatol*, 1981; 77: 75–180.
33. Otsuka K, Murota SI, Mori Y. – Stimulatory effect of bleomycin on the hyaluronic acid synthetase in cultured fibroblasts. *Biochem Pharmacol*, 1978; 27: 1551–1554.
34. Fraser JRE. – Hyaluronan: sources, turnover and metabolism. *In*: Clinical impact of bone and connetive tissue markers (Pharmacia Diagnostics Clinical Symposia). E. Lindh, J.I. Thorell eds, Academic Press, Harcourt Brace Jovanowich Publishers, London San Diego, 1989; pp. 31–49.
35. Varga J, Rosenbloom J, Jimenez SA. – Transforming growth factor-β (TGF)-β) causes a persistent increase in steady-state amounts of type I and type III collagen and fibronectin mRNAs in normal dermal fibroblasts. *Biochem J*, 1987; 247: 597–604.
36. Krane SM, Dayer JM, Simon LS, Byrne MS. – Mononuclear cell-conditioned medium containing mononuclear cell factor (MCF), homologous with interleukin-1 stimulates collagen and fibronectin synthesis by adherent rheumatoid synovial cells: effect of prostaglandin $E_2$ and indomethacin. *Coll Rel Res*, 1985; 5: 99–117.
37. Roberts CJ, Birmenmeier TM, McQuillan JJ, Akiyama SK, Yamada SS, Chen WT, Yamada KM, McDonald JA. – Transforming growth factor beta stimulates the expression of fibronectin and both subunit of the human fibronectin receptor by cultured human lung fibroblasts. *J Biol Chem*, 1988; 263: 4586–4592.
38. Hamerman D, Wood DD. – Interleukin-1 enchances synovial cell hyaluronate synthesis. *Proc Soc Exp Biol Med*, 1984; 177: 205–210.
39. Westergren-Thorsson G, Särnstrand B, Fransson LÅ, Malmström A. – TGF-β enhances the production of hyaluronan in human lung but not in skin fibroblasts. *Exp Cell Res*, 1990; 186: 192–195.
40. Fine A, Goldstein RH. – The effect of transforming growth factor-β on cell proliferation and collagen formation by lung fibroblasts. *J Biol Chem*, 1987; 262: 3897–3902.
41. Shoji S, Rickard KA, Ertl RF, Robbins RA, Linder J, Rennard SI. – Bronchial epithelial cells produce lung fibroblast chemotactic factor: fibronectin. *Am J Respir Cell Mol Biol*, 1989; 1 (1): 13–20.
42. Sampson P, Oarshley MS, Mandi I, Turino GM. – Glycosaminoglycans in tissue cultures of rat lung cells. *Conn Tissue Res*, 1975; 4: 441–451.
43. Postlethwaite AE, Keski-Oja J, Balian G, Kang AH. – Induction of fibroblast chemotaxis by fibronectin. *J Exp Med*, 1981; 153: 494–499.
44. Czop JK, McGovan SE, Center DM. – Opsonin-independent phagocytosis by human alveolar macrophages: augmentation by human plasma fibronectin. *Am Rev Respir Dis*, 1982; 125: 607–609.
45. Ahlgren T, Jarstrand C. – Hyaluronic acid enhances phagocytosis of human monocytes *in vitro*. *J Clin Immunol*, 1984; 4: 246–249.
46. Forrester JV, Balazs EA. – Inhibition of phagocytosis by high molecular weight hyaluronate. *Immunol*, 1980; 40: 435–446.
47. Love SH, Shannon BT, Myrvik QN. – Additional evidence for the role of hyaluronic acid in the macrophage disappearance reaction. *Immun Comm*, 1980; 9: 735–746.
48. Isemura M, Yosizawa Z, Koide T, Ono T. – Interaction of fibronectin and its proteolytic fragments with hyaluronic acid. *Biochem J*, 1982; 91: 731–734.
49. Ruoslahti E, Engvall E. – Complexing of fibronectin, glycosaminoglycans and collagen. *Biochem Biophys Acta*, 1980; 631: 350–358.
50. Yamagata M, Yamada KM, Yoneda M, Suzuki S, Kimata K. – Chondroitin sulphate proteoglycan (PG-M like proteoglycan) is involved in the binding of hyaluronic acid to fibronectin. *J Biol Chem*, 1986; 261: 13526–13535.
51. Culp LA, Rollins BJ, Buniel J, Hitri S. – Two functionally distinct pools of glycosaminoglycans in the substrate adhesion of site of murine cells. *J Cell Biol*, 1978; 79: 788–801.
52. Laterra J, Culp LA. – Differences in hyaluronate binding to plasma and cell surface fibronectins. *J Biol Chem*, 1982; 257: (2) 719–726.
53. Weigel PH, Fuller GM, LeBoef RD. – A model for the role of hyaluronic acid and fibrin in the early events during the inflammatory response and wound healing. *J Theor Biol*, 1986; 119: 219–234.