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Hyaluronic acid in bronchoalveolar lavage fluid in patients with sarcoidosis: relationship to lavage mast cells

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ABSTRACT Hyaluronate (hyaluronic acid), a potential marker for activated pulmonary fibroblasts, appears in increased concentrations in bronchoalveolar lavage fluid from patients with sarcoidosis. The mechanisms underlying fibroblast proliferation are largely unknown but activated alveolar T lymphocytes and macrophages probably play a part; the mast cell is also important for fibroblast proliferation. This study was designed to determine whether there is any association between pulmonary mast cells in lavage fluid, which are known to be increased in patients with sarcoidosis, and signs of pulmonary fibroblast activation. A strong correlation was found between lavage fluid hyaluronate and recovered mast cells ($r = 0{\cdot}72$, $p < 0{\cdot}001$). Moreover, mast cell and hyaluronate estimations correlated inversely with lung volume and transfer factor for carbon monoxide, and both indices increased with advancing radiological sarcoid stage. Macrophage and granulocyte counts were normal in lavage fluid from patients with sarcoidosis and were not related to lavage fluid hyaluronate or laboratory signs of the disease in the lungs. Lymphocytes were recovered in increased numbers ($p < 0{\cdot}001$) and were related to the lavage fluid mast cells and hyaluronate. It is concluded that in sarcoidosis release of hyaluronate into the airways is related to the degree of lung disease and to the local inflammatory reaction in the lung as defined by increased numbers of mast cells and lymphocytes in lavage fluid. The findings may reflect a link between the immune system, activation of mast cells, and a pulmonary fibroblast proliferation.

Hyaluronate (hyaluronic acid), a glycosaminoglycan, is present as part of the connective tissue in lung parenchyma.[1] Its production from fibroblasts is stimulated by various inflammatory stimuli.[2–5] Our previous finding that the appearance of increased amounts of hyaluronate in the alveolar space was related to reduced lung volume in sarcoidosis suggested that increased synthesis of hyaluronate in the lung may reflect activated interstitial fibroblasts or an expanded fibroblast mass associated with interstitial fibrosis.[6] The mechanisms underlying the accumulation and expansion of fibroblasts in sarcoidosis in the lungs are largely unknown but could be a consequence of alveolitis induced by activated alveolar T lymphocytes and macrophages.[7] The mast cell is also important for fibroblast stimulation[8] and plays a part in wound healing.[9] Nevertheless, its possible pathophysiological role in the activation of lung fibroblasts and development of lung fibrosis has not received much attention. Increased numbers of mast cells have been reported in lung tissue both in fibrotic lung disorders in man, including sarcoidosis,[10 11] and in experimentally induced lung fibrosis in rats.[12 13] Recently we reported increased numbers of mast cells in bronchoalveolar lavage fluid from patients with sarcoidosis.[14] Against this background we analysed the possible association between mast cells and increased synthesis of hyaluronate in patients with pulmonary

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sarcoidosis. In the present study we report the hyaluronate concentrations in bronchoalveolar lavage fluids from 69 patients with sarcoidosis in relation to the number of mast cells and other inflammatory cells recovered by lavage. The data obtained were correlated with the results of various pulmonary function tests and radiological criteria.

Methods

Sixty nine patients (42 women, 27 men) with sarcoidosis verified by biopsy were included in the study; their mean age was 45 (range 21–72) years. None of the patients was being treated with glucocorticoids or had been in the past. Patients with respiratory allergy or asthma were excluded from the study. Six patients were smokers. The patients had at the time of investigation a known mean duration of disease of six (range ⩽1–72) months. Ten apparently healthy volunteers (three women, seven men) underwent bronchoalveolar lavage to provide control values for lavage fluid. Sixty nine age and sex matched healthy controls served as a reference group for serum measurements of hyaluronic acid.

Vital capacity and forced expiratory volume in one second (FEV_1) were measured by standard spirometry and transfer factor for carbon monoxide (TLCO) by the single breath carbon monoxide technique. Values were expressed as a percentage of the normal predicted value. The following chest radiographic criteria were used: stage 0—no abnormal findings; stage I—bilateral hilar lymphadenopathy; stage II—bilateral hilar lymphadenopathy with parenchymal infiltrates; stage III—parenchymal infiltrates without hilar lymphadenopathy.

Before bronchoscopy patients and control subjects were given atropine or scopolamine, usually combined with morphine or pethidine chloride, subcutaneously. The upper respiratory tract was anaesthetised with lignocaine hydrochloride. A fibreoptic bronchoscope (Olympus BF IT or BF 4B2, Tokyo, Japan) was wedged in the anterior segmental bronchus of the lingula and 240 ml sterile Krebs Ringer phosphate buffer (pH 7·3) at 37°C was infused in boluses of 60 ml. The fluid was aspirated immediately after each instillation. The volume of instilled fluid recovered was 47% (SD 13%) in patients and 45% (8%) in control subjects. The total number of cells in the lavage fluid was counted in a Bürker chamber. The lavage fluid was kept on ice and filtered through a nylon filter (pore diameter 100 μm, Syntab Product AB, Malmö, Sweden). The cells were then collected by centrifugation at 400 *g* for 15 minutes. The supernatant was kept frozen at −70°C before analysis. The cells were gently resuspended in balanced salt solution to a concentration of 10^6 cells/ml, excluding epithelial cells. Preparations for cytological studies were made in a cytocentrifuge (Cytospin Shandon, Southern Prod Ltd, Runcorn) with 5×10^4 non-epithelial cells per slide. The cytocentrifuge preparations were stained with May-Grünwald-Giemsa before differential counting. Mast cells were stained with acid toluidine blue and counterstained with Mayers acid haematoxylin.[15] Numbers of lymphocytes, polymorphonucleocytes, and monocytes were expressed both as percentages of 200 cells (except epithelial cells) counted and as actual lavage fluid concentrations. The relative counts of these cells were normally distributed. Mast cells were counted as the number present in 10 visual fields with × 16 magnification and expressed as percentages of all non-epithelial cells. The mast cell counts were log normally distributed.

Hyaluronate concentrations were analysed in duplicate in serum and lavage fluid by a radioassay as previously described.[16] All samples were analysed in sequence. Albumin was measured by fluorescence nephelometry (Multistat III, Instrumental Laboratory, Lexington, Montana) at the Department of Clinical Chemistry, University Hospital of Uppsala. Lavage fluid hyaluronate concentration was divided by the lavage fluid albumin concentration to normalise hyaluronate for increased leakage over the capillary-alveolar barrier.

The study was approved by the local ethical committee and performed according to the Declaration of Helsinki with free and informed consent of all volunteers and patients.

For the statistical analyses we used Wilcoxon's rank sum test and Spearman's rank correlation test. Hyaluronate concentrations and cell counts in lavage fluid were logarithmically transformed for all calculations because of their skewed distribution. The means of the log transformed values are presented as the antilog of the means and the ±1 SD values (SD range).

Results

LAVAGE FLUID CONTENT OF HYALURONATE IN RELATION TO RECOVERED MAST CELLS AND OTHER INFLAMMATORY CELLS

The mean lavage fluid concentration of hyaluronate in patients with sarcoidosis was 31 (SD range 12–81) μg/l. Of the 10 healthy control subjects, six had hyaluronate concentrations above the detection limit (⩽5 μg/l). The mean hyaluronate concentration was ⩽7 and the SD range ⩽5–11 μg/l ($p < 0{\cdot}001$ in the comparison with patients' values). The mean serum hyaluronate concentration was 47 (SD 25) μg/l in the patients; this did not differ from the mean value (49 (33) μg/l) found in a healthy reference population. Lavage fluid

hyaluronate was not influenced by age, sex, or duration of disease.

The relative numbers of mast cells recovered in lavage fluid were ⩽0·02% of all non-epithelial cells from control subjects and 0·42% (SD range 0·08–2·1%). The absolute mast cell concentration in lavage fluid from the patients was 28 × 10^4 (SD range 5–165) cell/l and was significantly correlated with hyaluronate concentrations in lavage fluid ($r = 0·72$, $p < 0·001$; fig 1). The relative mast cell counts were also related to the hyaluronate concentrations in lavage fluid ($r = 0·68$, $p < 0·001$). The patients with sarcoidosis had significantly higher concentrations of lymphocytes in lavage fluid than the control subjects (table 1) and a relative increase in lymphocyte and a relative decrease in macrophage numbers. There was a significant relationship between mast cell counts and both absolute and relative lymphocyte counts ($r = 0·47$ and 0·39 respectively, $p < 0·001$). Lavage fluid hyaluronate correlated significantly with the relative ($r = 0·48$, $p < 0·001$) and absolute ($r = 0·39$, $p < 0·001$) number of lymphocytes. Lavage hyaluronate was also related to the relative (but not the absolute) number of granulocytes ($r = 0·29$, $p < 0·01$) and macrophages ($r = -0·58$, $p < 0·001$) recovered.

The lavage fluid albumin concentration was 131 (SD 122) mg/l in the patients and 43 (23) mg/l in the control subjects. When lavage fluid hyaluronate concentration was normalised for albumin the relation between hyaluronate and mast cells remained ($r = 0·47$, $p < 0·001$), whereas the relationship between hyaluronate and lymphocytes became non-significant ($r = 0·14$, $p > 0·05$).

LAVAGE FLUID HYALURONATE, MAST CELLS, AND OTHER INFLAMMATORY CELLS IN RELATION TO PULMONARY FUNCTION (table 2)

In the patients with sarcoidosis mean (SD) values for vital capacity were 92% (15%) (range 53–132%) of the predicted value, FEV_1 94% (18%) predicted (range 54–136%), and TLCO 82% (14%) (range 45–116%). There were significant inverse correlations between lavage fluid hyaluronate concentrations and results of the three lung function tests (table 2). The significance of the relationships remained after normalisation of hyaluronate concentrations for albumin. Mast cells in lavage fluid were also inversely related to lung function. Polymorphonuclear cell counts tended to correlate inversely with vital capacity and TLCO ($p < 0·05$); the numbers of lymphocytes and macrophages recovered had no relation to lung function.

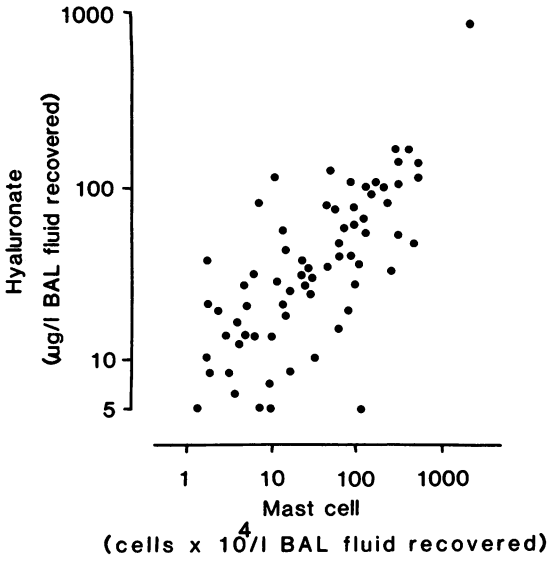

Fig 1 *Relation of hyaluronate concentrations and mast cell counts in bronchoalveolar lavage (BAL) fluid in patients with sarcoidosis.*

LAVAGE FLUID HYALURONATE AND MAST CELLS IN RELATION TO PULMONARY RADIOLOGICAL CRITERIA

The hyaluronate concentrations increased significantly and the mast cell counts tended to increase with radiographic stages (fig 2). High lymphocyte counts were found in lavage fluid from patients with radiological stage I disease. The polymorphonuclear cell and monocyte counts did not vary with radiographic stage.

Discussion

The increased amounts of hyaluronate in bronchoalveolar lavage fluid in patients with

Table 1 *Absolute and relative numbers of lymphocytes, macrophages, and leucocytes in lavage fluid in patients with sarcoidosis and control subjects (mean values with standard deviations or SD range in parentheses)*

		Lymphocytes		*Macrophages*		*PMNs*	
	n	(× 10^7/l)	(%)	(× 10^7/l)	(%)	(× 10^7/l)	(%)
Sarcoidosis	69	2·4(0·9–6·9)***	40 (21)***	3·3(1·4–7·6)	54 (21)***	0·12(0·02–0·84)	4·2 (7)
Control	10	0·4(0·2–1·0)	10 (8·3)	4·6(2·9–7·1)	88 (9)	0·08(0·02–0·23)	2·0 (1·9)

***$p < 0·001$ compared with the control subjects (Wilcoxon's test).
PMNs—polymorphonuclear cells.

Table 2 *Correlation coefficients between lung function and hyaluronate and mast cells in lavage fluid in patients with sarcoidosis*

	Vital capacity (% pred)	*FEV_1 (% pred)*	*TLCO (% pred)*
Hyaluronate (μg/l)	−0·47***	−0·38***	−0·44***
Hyaluronate (μg/mg albumin)	−0·42***	−0·37***	−0·46**
Mast cells (%)	−0·40***	−0·29**	−0·42***
Mast cells (No/l)	−0·38***	−0·27**	−0·41***

**p < 0·01, ***p < 0·001, as tested by Spearman's rank correlation test.
TLCO—transfer factor for carbon monoxide.

sarcoidosis suggest an enhanced synthesis of this glycosaminoglycan in the lung parenchyma. Permeability of the capillary membrane is increased in sarcoidosis and passive leakage may allow a smaller fraction of plasma components to appear in the alveolar space. The circulating concentrations of hyaluronate in our patients were similar to the concentrations in the bronchoalveolar lavage fluid, so an increase in passive permeability over the capillary-alveolar barrier has presumably had only a minor influence on the amount found in the lavage fluid. The serum albumin:lavage fluid albumin ratio is calculated to be around 300 in our patients with sarcoidosis, whereas their serum hyaluronate:lavage fluid hyaluronate ratio was on average only 1·5. When we "normalised" lavage hyaluronate, using correction factors based on recovered albumin, the findings were essentially the same for measured and for normalised hyaluronate values in the correlative analyses.

The mechanisms leading to fibroblast accumulation and activation in the sarcoid lung are not fully recognised, nor has the source of the increased hyaluronate synthesis been identified. We may reasonably assume, however, that the enhanced synthesis of hyaluronate reflects activated fibroblasts or an increased fibroblast mass in the lung, since hyaluronate is a connective tissue element normally present in lung parenchyma.[1] Hyaluronate is released into the culture medium of growing fibroblasts and when fibroblast synthesis is stimulated by growth factors from various inflammatory cells.[2-5] Indirect support for the hypothesis that activated lung fibroblasts synthesise the major portion of hyaluronate in inter-

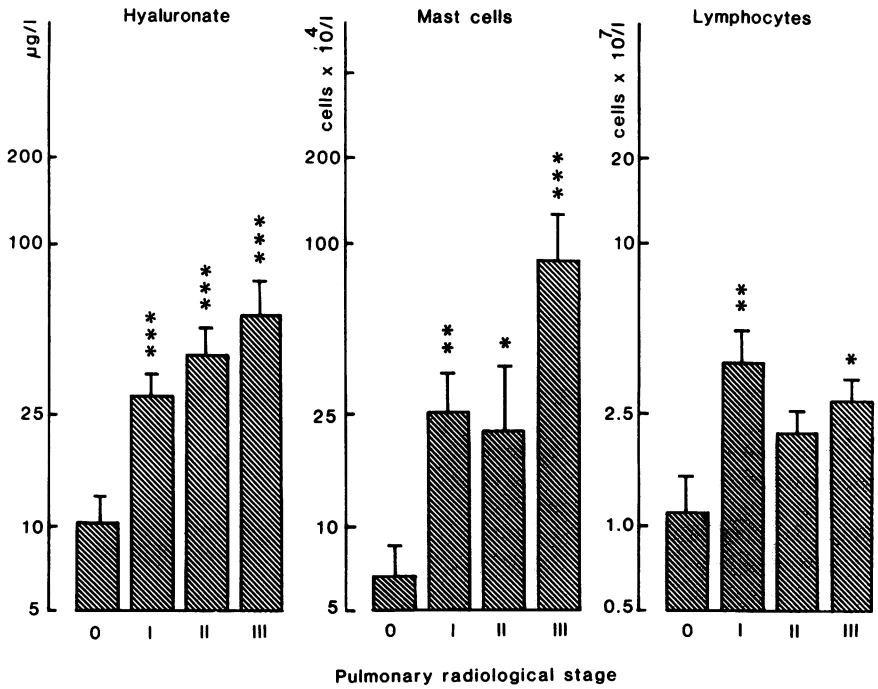

Fig 2 *Concentrations of hyaluronate, mast cells, and lymphocytes in lavage fluid from patients with sarcoidosis subgrouped with respect to pulmonary radiological criteria—stage 0 (n = 11), stage I (n = 23), stage II (n = 13), and stage III (n = 22). The bars represent geometric means with standard errors. Statistical differences between stage 0 and other stages are asterisked: *p < 0·05, **p < 0·01, ***p < 0·001 (rank sum test).*

stitial lung diseases is provided by the relationship between the increased bronchoalveolar concentrations of hyaluronate and type III procollagen peptide, a potential marker of collagen type III production (L Bjermer *et al,* to be published). The present observation that bronchoalveolar lavage hyaluronate concentration was correlated inversely with lung volume and function, and increased with advancing radiographic stage, further supports the contention that the appearance of hyaluronate in the alveoli reflects an altered connective tissue reaction.

The development of lung fibrosis in sarcoidosis coincides with the recruitment of fibroblasts and the production by them of connective tissue matrix. During recent years it has been proposed that the increases in fibroblasts in sarcoid lungs is a consequence of the intensity of the local lung T lymphocyte activation.[7] Alveolar macrophages may also play a significant part by releasing active mediators.[17,18] Until now, it has not been generally considered that mast cells may have a role in the pathogenesis of fibrosis in sarcoidosis. Pulmonary fibrosis in man, however, whether idiopathic or due to connective tissue diseases, is associated with mast cell accumulation in the alveolar epithelial cell layer.[10] Moreover, experimental studies on rats have shown that radiation[12] as well as bleomycin[13] induced pulmonary fibrosis is accompanied by a massive increase in mast cell numbers in the lung parenchyma. Our observations that lavage mastocytosis in patients with sarcoidosis is correlated with increased hyaluronate concentrations in the lavage fluid, impaired lung function, and more advanced disease as indicated by chest radiographs provide further arguments favouring a role for mast cells in lung fibrosis.

Although the correlation between pulmonary mastocytosis and various laboratory indices of lung in disease are intriguing, we have no explanation of the possible mechanisms. One mast cell activity, however, relevant to the observations in the present study is the ability of mast cell granules to interact with fibroblasts.[8] Animal experimental studies have suggested that mast cell degranulation is accompanied by an increase in the proliferative rate of adjacent fibroblasts,[19-20] a mitogenic effect partly ascribed to histamine.[21,22] Other observations suggest alternative mechanisms by which mast cells may affect fibroblast proliferation. Extracellularly, released mast cell granules are taken up by phagocytic cells[23] and fibroblasts[24] and later degraded.[23,24] In the case of fibroblasts, this process is accompanied by release of fibroblast derived proteolytic enzymes,[8] which with released heparin may affect ground substance components directly.[8] These interactions might, in certain circumstances, initiate or perpetuate lung injury and could account for the association in our patients with sarcoidosis between increased numbers of mast cells in lavage fluid, increased pulmonary production of hyaluronate, and impaired lung function.

Recently it has been reported that especially high numbers of mast cells are seen in lavage fluid in patients with extrinsic allergic alveolitis.[25] Since this finding is particularly apparent in the acute phase of the disease before there is evidence of fibrosis, the hypothesis of a link between mast cell accumulation and the presence of lung fibrosis has to be challenged. We have observed that the acute phase of farmer's lung is characterised not only by lavage fluid mastocytosis but also by very high concentrations of hyaluronate and procollagen III peptide in the lavage fluid (Bjermer *et al,* unpublished findings). After avoidance of contact with mouldy plant material the mast cell number and the hyaluronate and procollagen concentrations in lavage fluid returned towards normal. Furthermore there was a close relationship in these patients with farmer's lung between lavage fluid mast cells and lavage fluid hyaluronate and procollagen. These findings suggest that lavage fluid mastocytosis may be associated with (*a*) reversible non-fibrotic lung diseases accompanied by laboratory signs of transient fibroblast activation and (*b*) fibrotic lung diseases with laboratory signs of longstanding fibroblast activation or proliferation.

Another explanation for the relationship between mast cells and hyaluronate in lavage fluid comes from possible effects of local immune activation on mast cells and fibroblasts. Although the mast cell type in the lung is largely independent of the T lymphocyte,[26] its proliferation seems to be regulated by T cells.[27] Moreover, products of activated T cells and mononuclear-lymphocyte cultures can stimulate the growth of mast cells from bone marrow precursors.[28] Stimulated T cells also elaborate factors that stimulate fibroblast proliferation and collagen synthesis.[29-31] Thus the appearance of mast cells and hyaluronate in the alveolar space may be parallel events, both being regulated by activated lymphocytes. In support of this notion we observed in this study that amounts of mast cells as well as hyaluronate recovered during lavage were related to numbers of recovered lymphocytes. Our findings may also support the model of T cell-mast cell interrelation proposed by Claman,[32] which is that activated T cells may stimulate fibroblasts either directly or indirectly, in the latter case by stimulating mast cell proliferation and thereby mast cell interaction with fibroblasts.

Thus this study has added a further layer of complexity to the pathophysiology of sarcoidosis by indirectly demonstrating a potential role for pulmonary mast cells in the enhanced synthesis of hyaluronate in the lung. We hope that current longitudinal studies

in patients with sarcoidosis will further elucidate the underlying mechanisms and also the possible prognostic use of measurements of hyaluronate and mast cells in bronchoalveolar lavage fluid.

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References

1 Wusteman FS. Glycosaminoglycans of bovine lung parenchyma and pleura. *Experientia* 1972;**28**:887–8.
2 Sisson JC, Castor CW, Klavons JA. Connective tissue octivation. XVIII—Stimulation of hyaluronic acid synthetase activity. *J Lab Clin Med* 1985;**96**:189–97.
3 Harnerman D, Wood DD. Interleukin 1 enhances synovial cell hyaluronate synthesis. *Proc Soc Exp Biol Med* 1984;**177**:205–10.
4 Yaron M, Yaron I, Wiletzki C, Zor U. Interrelationship between stimulation of prostaglandin E and hyaluronate production by poly (I) and (C) and interferon in synovial fibroblast culture. *Arth Rheum* 1978;**21**:694–8.
5 Engström-Laurent A, Feltelius N, Hällgren R, Wasteson Å. Elevated serum hyaluronate in scleroderma. An effect of growth factor induced activation of connective tissue cells. *Ann Rheum Dis* 1985;**44**:614–20.
6 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**:1778–81.
7 Crystal RG, Roberts WC, Hunninghake GW, Gadek JE, Fulmer JD, Line BR. Pulmonary sarcoidosis: a disease characterized and perpetuated by activated T lymphocytes. *Ann Intern Med* 1981;**94**:73–94.
8 Atkins FM, Friedmann MM, Subba Rao PV, Metcalfe DD. Interactions between mast cells, fibroblasts and connective tissue components. *Int Arch Allergy Appl Immun* 1985;**77**:96–102.
9 Persinger MA, Lepage P, Simard JP, Parker GH. Mast cell numbers in incisional wounds in rat skin as a function of distance, time and treatment. *Br J Dermatol* 1983;**108**:179–87.
10 Kawanami O, Ferrans VJ, Fulmer JD, Crystal RG. Ultrastructure of pulmonary mast cells in patients with fibrotic lung disorders. *Lab Invest* 1979;**40**:717–34.
11 Lykke AW, Schonell ME, Stewart BW. Atypical mast cell degranulation and focal hydropic degeneration of venular endothelium in diffuse fibrosing alveolitis. *Experentia* 1979;**35**:1492–3.
12 Watanabe S, Watanabe K, Ohishi T, Motohiko A, Kageyama K. Mast cells in the rat alveolar septa undergoing fibrosis after ionizing irradiation. Ultrastructural and histochemical studies. *Lab Invest* 1974;**31**:555–67.
13 Goto T, Befus D, Low R, Bienenstock J. Mast cell heterogeneity and hyperplasia in bleomycin-induced pulmonary fibrosis of rats. *Am Rev Respir Dis* 1984;**130**:797–802.
14 Bjermer L, Bäck O, Roos G, Thunell M. Mast cells and lysozyme positive macrophages in bronchoalveolar lavage from patients with sarcoidosis: valuable prognostic and activity marking parameters of disease? *Acta Med Scand* 1986;**220**:161–6.
15 Strobel S, Miller HRP, Ferguson A. Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. *J Clin Pathol* 1981;**34**:851–8.
16 Engström-Laurent A, Laurent UBG, Lilja K, Laurent TC. Concentration of sodium hyaluronate in serum. *Scand J Clin Lab Invest* 1985;**45**:497–504.
17 Hunninghake GW. Release of interleukin-1 by alveolar macrophages of patients with active pulmonary sarcoidosis. *Am Rev Respir Dis* 1984;**129**:569–72.
18 Bitterman PB, Rennard SI, Hunninghake GW, Crystal RG. Human alveolar macrophage growth factor for fibroblasts. Regulation and partial characterization. *Clin Invest* 1982;**70**:806–22.
19 Norrby K. On the 48/80-induced secretion of tissue mast cells and its mitogenic effect on nearby cells in the intact rat. *Virchows Arch (Cell Pathol)* 1981;**38**:57–65.
20 Norrby K. Intradermal mast cell secretion causing cutaneous mitogenisis. *Virchows Arch (Cell Pathol)* 1983;**42**:263–9.
21 Norrby K. Mast cell histamine, a local mitogen acting via H2-receptors in nearby tissue cells. *Virchows Arch (Cell Pathol)* 1980;**34**:13–20.
22 Russel JD, Russel SB, Trupin KM. The effect of histamine on the growth of cultured fibroblasts isolated from normal and keloid tissue. *J Cell Physiol* 1977;**93**:389–93.
23 Lindahl U, Pertoft H, Seljelid R. Uptake and degradation of mast cell granules by mouse peritoneal macrophages. *Biochem J* 1979;**182**:189–93.
24 Atkins FM, Metcalfe DD. Degradation of the heparin matrix of mast cell granules by cultured fibroblasts. *Immunol* 1983;**131**:1420–5.
25 Haslam PL, Dewar A, Butchers P, Primett ZS, Newman-Taylor A, Turner-Warwick M. Mast cells, atypical lymphocytes, and neutrophils in bronchoalveolar lavage in extrinsic allergic alveolitis. *Am Rev Respir Dis* 1987;**135**:35–47.
26 Kitamura Y, Shimada M, Go S, Matsuda H, Hatanaka K, Seiki M. Distribution of mast cell precursors in hematopoietic and lymphopoietic tissues of mice. *Exp Med* 1979;**150**:482–90.
27 Dy M. Increase in histamine production and mast cell proliferation during allograft rejection. *Transpl Proc* 1982;**14**:575–80.
28 Haig DM, McKee TA, Jarett EE, Woodbury R, Miller HR. Generation of mucosal mast cells is stimulated in vitro by factors derived from T cells of helminth infected rats. *Nature (Lond)* 1982;**300**:188–90.
29 Hibbs MS, Postlethwaite AE, Mainardi CL, Seyer JM, Kang AH. Alterations in collagen production in mixed mononuclear leukocyte-fibroblast cultures. *J Exp Med* 1983;**157**:47–59.
30 Wahl SM, Gately CL. Modulation of fibroblast growth by a lymphokine of human T cell continuous T cell line origin. *J Immunol* 1983;**130**:1226–30.
31 Postlethwaite AE, Smith GH, Mainardi CL, Seyer JM, Kang AH. Lymphocyte modulation of fibroblast function in vitro: stimulation and inhibition of collagen production by different effector molecules. *J Immunol* 1984;**132**:2470–7.
32 Claman HN. Mast cells, T cells and abnormal fibrosis. *Immunology Today* 1985;**6**:192–5.