ROLE OF MAST CELLS IN THE PATHOGENESIS OF SILICOSIS

HEIDRUN BEHRENDT • R. Ziesche • P. Stutz* • Helga Idel • K. H. Friedrichs • W. Hilshcher • H. Magnussen*

Medical Institute of Environmental Hygiene at the University, Auf'm Hennekamp 50, D-4000 Düseldorf, FRG and *Centre of Pneumology and Thoracic Surgery at the LVA-Hospital, D-2070 Großhansdorf, FRG

INTRODUCTION

Mast cells are bone marrow derived mononuclear cells containing vasoactive amines, proteoglycans and proteases within their specific granules. Under pathological conditions, mast cells play a central role in allergic inflammatory processes as well as in parasitic diseases, both in human and in animal individuals. Increased numbers of mast cells also occur under non-allergic conditions,[6] i.e. in inflammatory diseases of the intestine, rheumatoid synovitis, and fibrotic disorders of the skin, the lung and the nervous system. With respect to the lung, elevated levels of mast cells can be obtained by bronchalveolar lavage in asthmatic patients[12,17] and in patients with various fibrotic lung disorders,[5,13] including silicosis.[6]

The role of mast cells in silicosis is not well understood. We therefore investigated the effects of silica dust DQ 12 on rat mast cells *in vivo* and *in vitro*. In addition, we analyzed mast cell topography in transbronchial lung biopsies from three patients with anthracosilicosis.

MATERIAL AND METHODS

Silica Dust

Quartz dust DQ 12, particle size < 5 µm, were used in all animal experiments. For the *in vitro*-studies a stem solution (100 µg/ml) in buffer (Hanks balanced salt solution) have been prepared briefly before use, as described elsewhere.[20] For intratracheal instillation quartz dust DQ 12 has been suspended in sterile 0.9% saline. The inhalation experiments have been performed using native quartz dust.

Animals

Female Lewis rats, 8 weeks old, SPF-state, were used throughout all experiments, if not otherwise stated.

In-vitro Experiments

Rat peritoneal cells containing about 16 percent of mast cells[20] were harvested according to the method of Uvnäs and Thon.[18] Triplicate samples of 10^6 cells per ml buffer were then incubated (37°C, 10 to 120 minutes) with 3—100 µg/ml quartz DQ 12. The reaction was terminated by the addition of icecold buffer. For light microscopical investigations cytocentrifuge preparations have been performed. The cells were fixed in formaline vapour and subsequently stained with the combined alcianblue-safranine sequence.

For electron microscopy cells were fixed with glutaraldehyde, postfixed with osmium tetroxide, dehydrated and embedded in Araldite. Ultrathin sections were investigated in a Philips 400T electron microscope.

Cell viability was determined by the use of the Eosin Y dye exclusion test.

Short-term Inhalation Experiments

Short-term inhalation experiments were performed in a chamber containing a rotating wheel, as described elsewhere.[2,4] Groups of 5–6 rats were exposed to 10 mg quartz DQ 12 per cbm air, 6 hours a day, for up to 28 days. After termination of the experiment bronchoalveolar lavage (BAL) according to the method of Brain and Frank[8] was undertaken. The BAL cells were either spinned down in a cytocentrifuge followed by fixation and staining as indicated above, or processed for electron microscopical analysis.

A second group of animals were fixed by instillation of 2% buffered glutaraldehyde (10 cm H_2O, 20 minutes). After removal of total lungs, the right middle lobe was cut into small pieces. After postfixation, dehydration and embedding ultrathin sections were analyzed in the electron microscope.

For the determination of lung histamine content, from a third group of animals the left lung was removed; its wet weight was determined. It was then cut into small pieces, mechanically homogenized and resuspended in 2% perchloric acid. After sonification and centrifugation the supernatant was assayed for histamine, using the fluorimetric auto-analizer technique. Histamine content was expressed as microgram per gram wet weight of the lung.

Intratracheal Instillation Experiments

Groups of 5 female Wistar rats, SPF state, were used in these experiments. The animals received a single dose of 40 mg of quartz DQ 12 in 0.5 ml saline by instillation. Control rats were instilled with saline only. After 8 weeks the lungs were fixed and prepared for light microscopical investigations. Mast cell analysis was performed under the light microscope. 8 µm sections were stained with alcianblue-safranine O and the number of mast cells was determined.

Statistics

For statistical analysis Student's t-test has been performed.

Patients

Transbronchial lung biopsies from three male patients with

anthracosilicosis were obtained by standardized procedures. All samples were then fixed and processed for electron microscopical investigations.

RESULTS

Effect of Quartz DQ 12 on Rat Mast Cells *In Vitro*

The cytotoxic effect of quartz DQ 12 on rat peritoneal cells is time- and dose-dependent (Figure 1). At concentrations where a substantial amount of cells are still viable (12.5 µg/ml/10^6 cells) quartz DQ 12 induces a stimulatory action. Cell stimulation is proofed by the appearance of increased numbers of degranulated mast cells after incubation with low concentrations of quartz, but not at higher doses (Figure 2). During quartz-induced activation of mast cells a substantial amount of histamine is released into the incubation medium. Particle counts indicate that only 7 particles per cell (i.e. 12.5 µg/ml/10^6 cells) are responsible for cell stimulation, whereas 30 particles per cell (i.e. 50 µg/ml/10^6 cells) are already cytotoxic.[20]

Electron microscopical investigations show partially degranu-

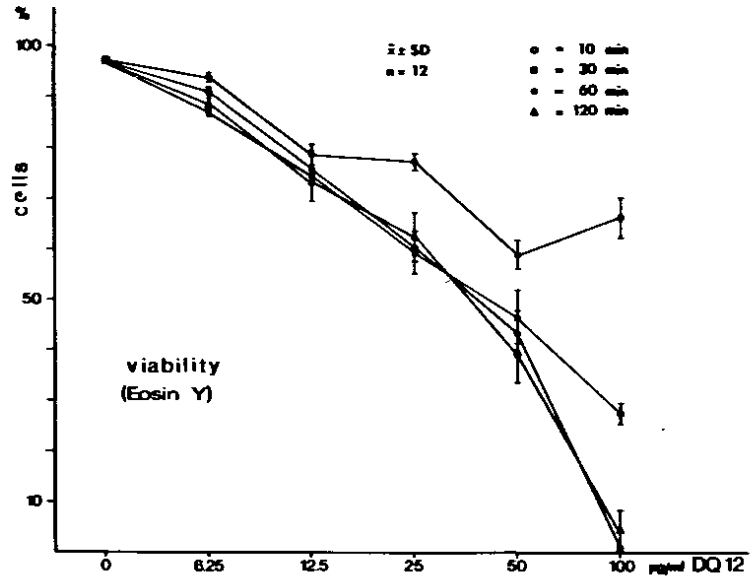

Figure 1. Dose- and time-dependent effect of quartz DQ 12 on the viability of rat peritoneal cells *in vitro*.

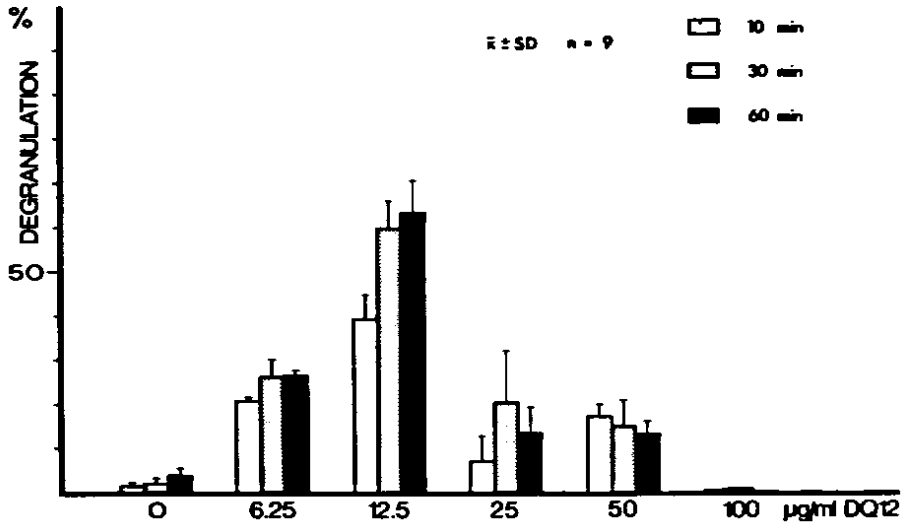

Figure 2. Dose- and time-dependent effect of quartz DQ 12 on the number of degranulated rat mast cells *in vitro*.

lated mast cells after incubation with low doses but not with high concentrations of quartz DQ 12 (Figure 3). Additionally, quartz-mediated mast cell/macrophage interactions are prominent, even under the conditions of the incubation method employed (shaking water bath). Phagocytosis of particles by mast cells could never be detected.

Effect of Short-term and Low Dose Inhalation of Quartz DQ 12 on Rat Lung Mast Cells

Early cellular events in rat lungs induced by quartz dust inhalation are characterized by an inflammatory response which has been described in detail elsewhere.[2,3,4] With respect to lung mast cells, intraepithelial mast cells occur as early as after an inhalation period of 4 days,[3] followed hy the appearance of small numbers of mast cells in the bronchial lumen at day 8 and at day 14.[4] In addition, lung histamine content is significantly reduced at day 8 and remains at lower levels during the whole inhalation period (Figure 4). No increase in the number of parenchymal mast cells could be detected.

Number and Topography of Mast Cells in Silicotic Rat Lungs after Intratracheal Instillation of Quartz Particles

Instillation of 40 mg quartz DQ 12 into the trachea of rats results in lung fibrosis after 8—12 weeks. Initiation of fibrosis is accompanied by an 68.5% increase in the number of mast cells. In addition to mast cell hyperplasia, about 50% of these cells are localized interstitially (Figure 5), indicating mast cell redistribution. Most of the mast cells display safraninophilic granules. They therefore represent the connective tissue subtype, with respect to mast cell heterogeneity. Consequently, they are situated within bundles of collagen (Figure 6). Cells who are in close contact to fibroblasts display partial degranulation. Interactions between mast cells and dust-laden macrophages are frequently seen.

Occurrence of Mast Cells in Transbronchial Lung Biopsies Obtained from Silicotic Patients

Numerous mast cells are present in transbronchial lung

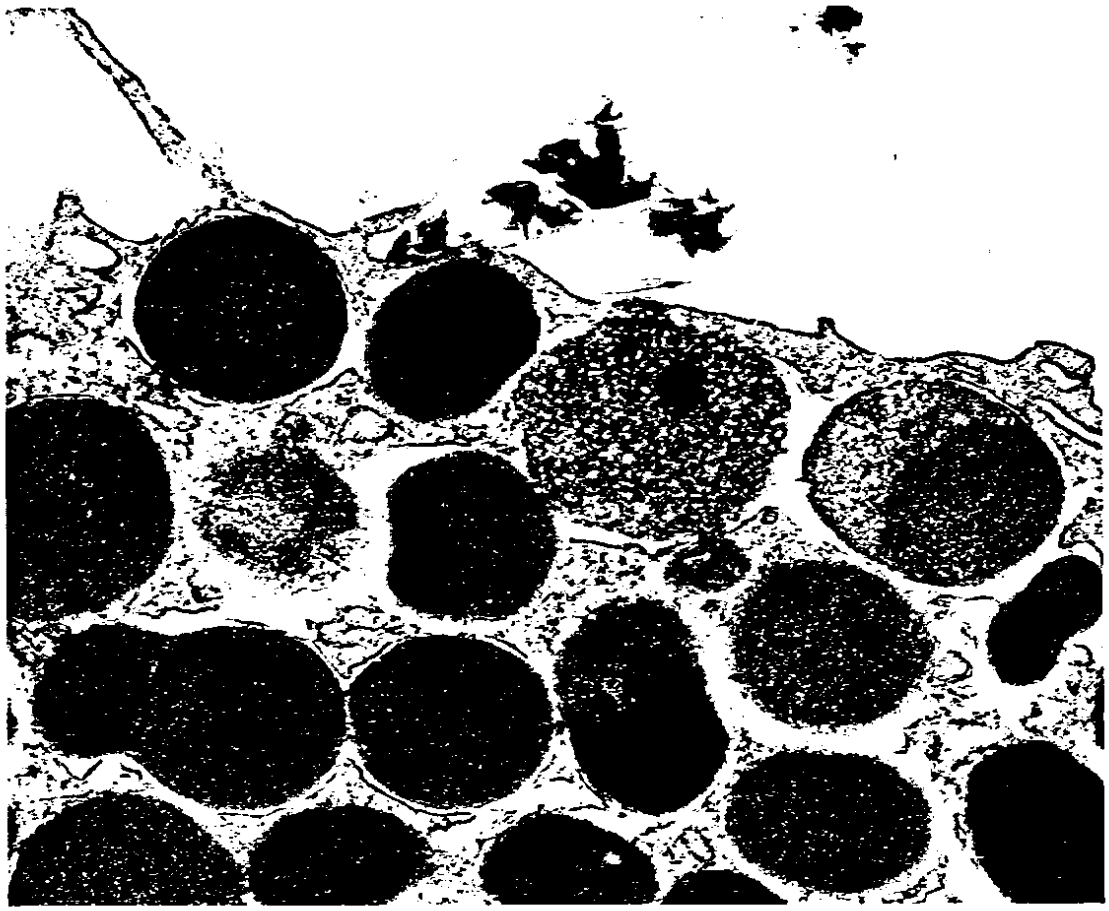

Figure 3. Electron micrograph of part of a mast cell undergoing degranulation induced by quartz DQ 12 *in vitro* (50 μg quartz DQ 12; 10 min.) Magn. x39000.

biopsies from silicotic patients (Figure 7). The cells display the species-specific whorls and scrolls within their granules. Exocytotic figures indicating mast cell degranulation are infrequently seen. If they occur they are always accompanied by fibroblast activation (Figure 8). Again, contacts between dust-laden macrophages and mast cells are visible (Figure 9). Examination of cells obtained by bronchoalveolar lavage reveal mast cells which are similar to cells seen in fibrotic lung areas and which are different from those obtained by bronchoalveolar lavage from asthmatic patients.[5]

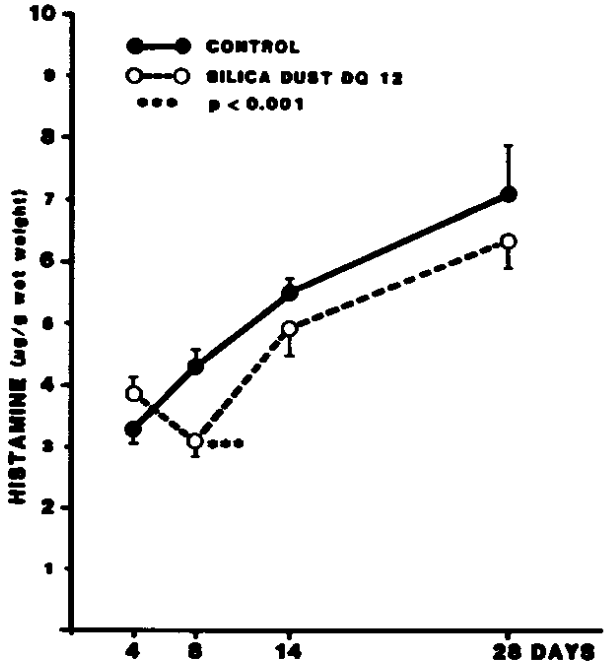

Figure 4. Effect of quartz dust inhalation (10 mg/cbm, 6 hrs per day) on total lung histamine content. Values are expressed as µg histamine per g wet weight of the lung.

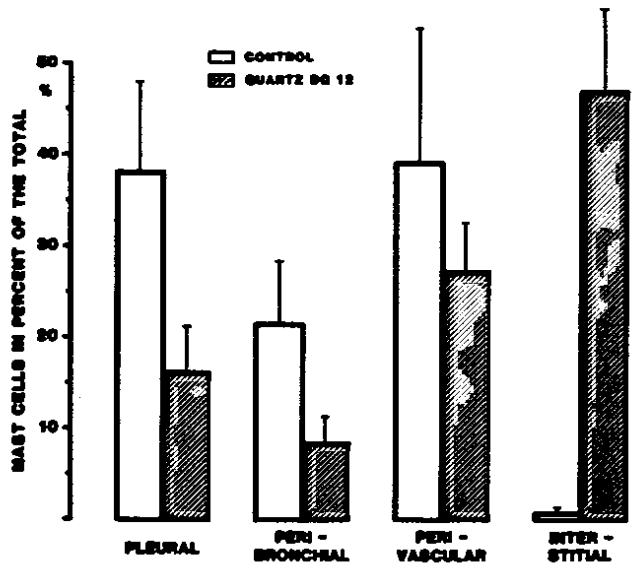

Figure 5. Distribution of mast cells in the rat lung 8 weeks after a single intratracheal dose (40 mg) of quartz DQ 12.

DISCUSSION AND CONCLUSIONS

This paper describes the occurrence, topography and functional behaviour of lung mast cells in response to cytotoxic quartz particles. The results obtained show that mast cells participate in the generation of silicotic pulmonary fibrosis. As is evident from our *in vitro* and *in vivo* experiments in the rat, mast cells are clearly involved in both, the initial inflammatory process and in fibrogenesis. Since continuous inflammation is an important link between inhaled quartz particles and the development of fibrosis in the lung,[7] mast cells are supposed to be one important element influencing and modulating this process. It is well known that mast cells release a variety of chemically active mediators of inflammation including diverse chemotactic factors upon appropriate stimuli.[14] Macrophages have been reported to release products with histamine liberating activity[16] as is true for oxygen radicals.[11] Histamine itself is able to potentiate the phagocytic activity of alveolar macrophages.[10] For this functional interaction between mast cells and macrophages in the lung the morphological equivalent is demonstrated in this paper. The phenomenon is not only relevant in the pathogenesis of experimental silicosis but is also present in transbronchial lung biopsies from silicotic patients. Therefore, mast cell—macrophage cooperation seems to be a general mechanism involved in the pathogenesis of silicosis.

The role of mast cells within fibrotic lung areas is not well understood. Mast cells can activate fibroblasts to divide,[15] and mast cell granules have been shown to affect fibroblast functions.[1] Fibroblasts in reverse are needed for the differentiation, development and granule synthesis of connective tissue mast cells.[9] Additionally, mast cells are able to influence some extracellular components of the connective tissue itself.[1] Furthermore, mast cell hyperplasia has been reported to occur in lung parenchyma of chronically hypoxic rats.[19] Therefore, mast cells can influence a variety of parameters which are necessary for and involved in fibrogenesis induced by toxic silica particles.

REFERENCES

1. Atkins, F.M., Friedman, M.M., Subba Rao P.V., Metcalfe, D.D.: Interactions between mast cells, fibroblasts and connective tissue components. *Int. Archs. Allergy Appl. Immun.* 77:96-102 (1985).
2. Behrendt, H., Friedrichs, K.H.: Effect of low dose and short time inhalation of silica dust on the morphology of rat lungs. *Aerosols: Formation and Reactivity*, 2nd Int. Aerosol Conf. Berlin, pp. 279-281, Pergamon Press (1986).
3. Behrendt, H., Friedrichs, K.H.: Influence of quartz dust DQ 12 inhalation on early cellular reaction in the rat lung. II. Electron microscopical analysis of the lung parenchyma. *Silicosis Report North-Rhine Westfalia* Vol. 16:231-245 (1987).
4. Behrendt, H., Friedrichs, K.H., Gentz, R., Kainka-Stänicke, E.: Influence of quartz dust DQ 12 inhalation on early cellular reaction in the rat lung. I. Electron microscopical analysis of the bronchoalveolar lavage cells. *Silicosis Report North-Rhine Westfalia* Vol. 16:217-230(1987).

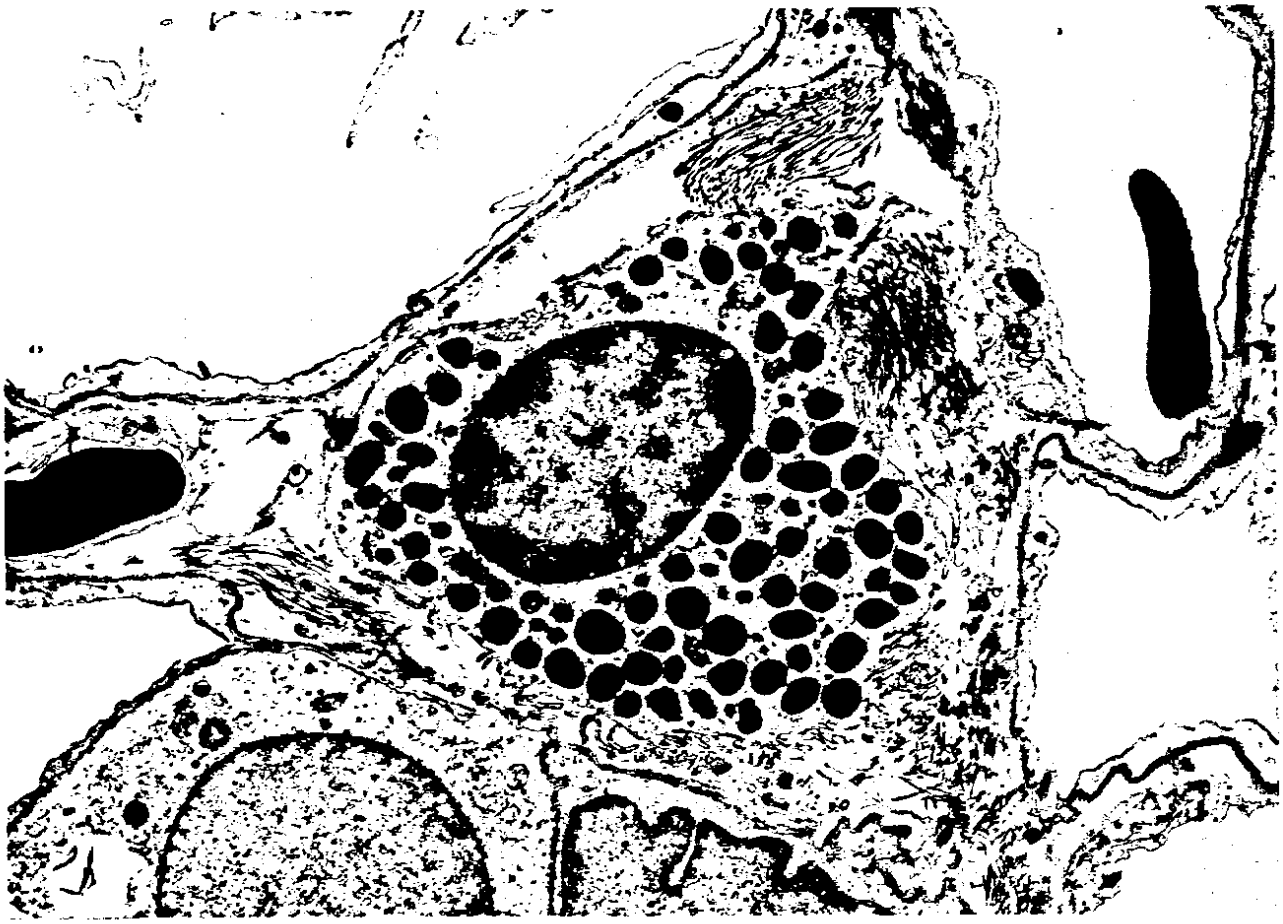

Figure 6. Rat lung mast cell surrounded by bundles of collagen fibres 8 weeks after intratracheal application of 40 mg quartz DQ 12. Electron micrograph. Magn. x9000.

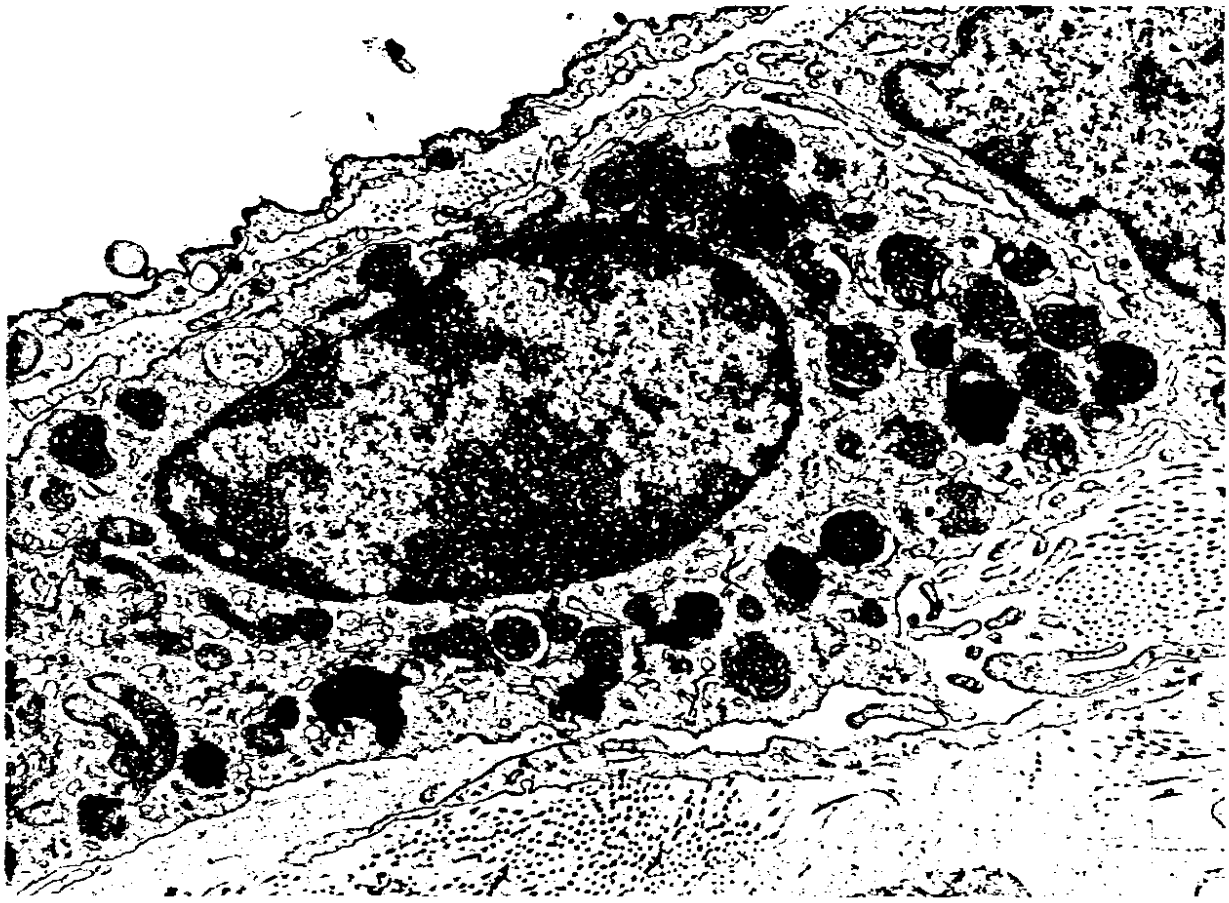

Figure 7. Human mast cell lying within the interstitium of a fibrotic lung area from a silicotic patient. Transbronchial biopsy. Electron micrograph. Magn. x17000.

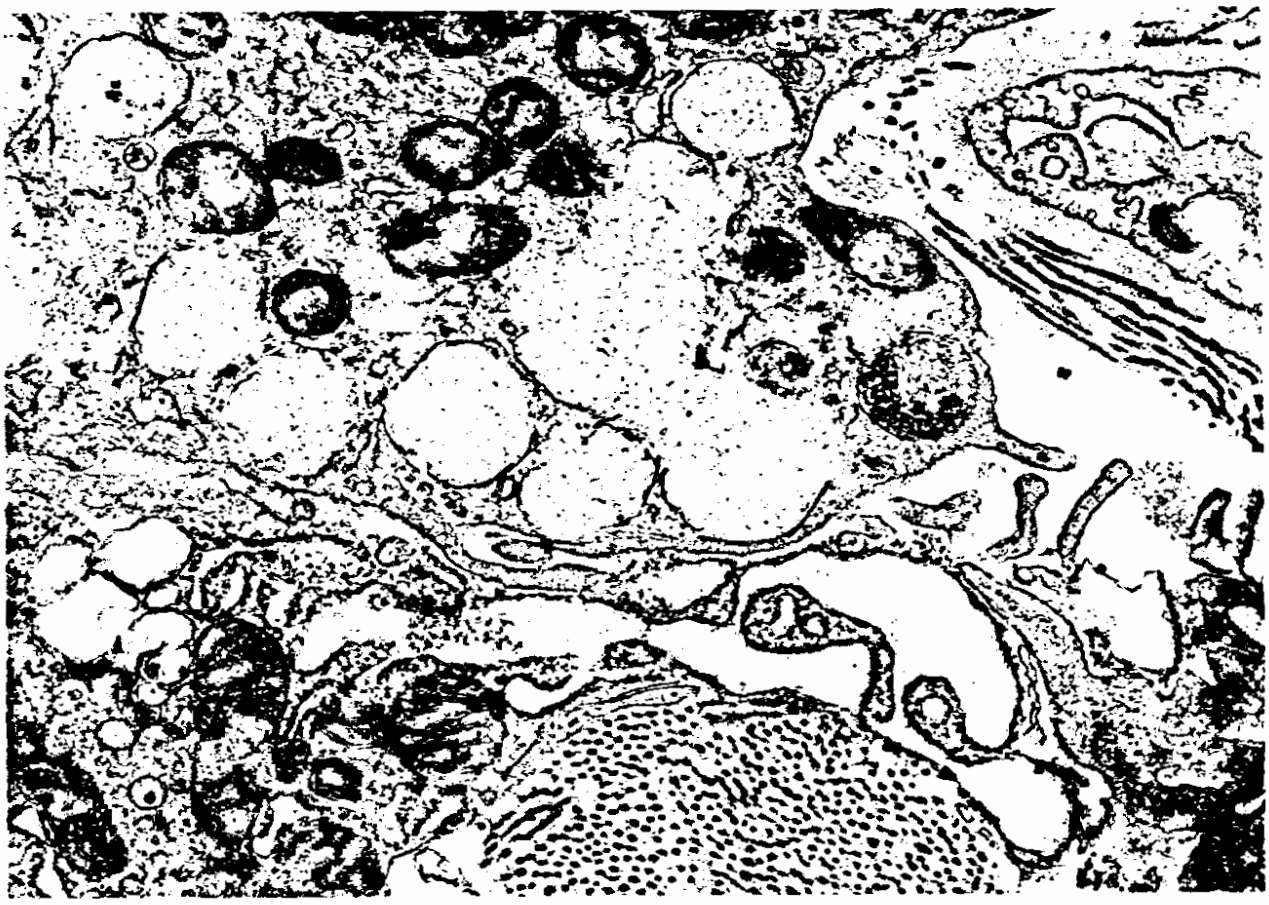

Figure 8. Part of a parenchymal mast cell undergoing degranulation. The cell is closely connected to a fibrocyte. Transbronchial lung biopsy. Electron micrograph. Magn. x28000.

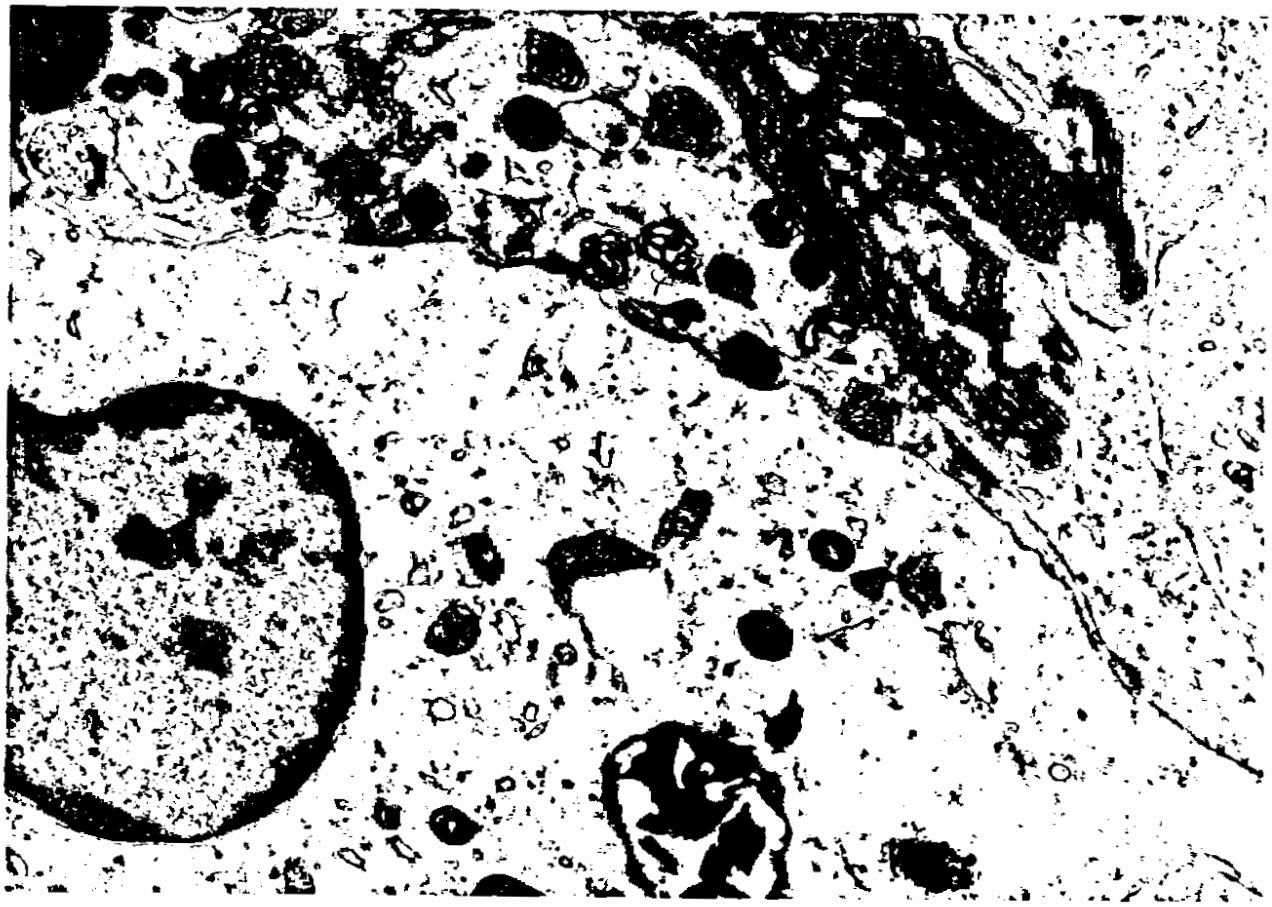

Figure 9. Interaction between a mast cell and a dust-containing macrophages in lung parenchyma of a silicotic patient. Transsbronchial biopsy. Electron micrograph. Magn. x17000.

5. Behrendt, H., Stiller-Winkler, R., Stutz, P., Magnussen, H.: Morphological evidence for two mast cell populations in bronchoalveolar lavage obtained from asthmatic and nonasthmatic patients. Allergy 43:72(1988).
6. Bienenstock, J., Befus, A.D., Denburg, J.A.: *Mast cell heterogeneity: Basic questions and clinical implications. Mast cell differentiation and heterogeneity*, pp. 391-402. A. D. Befus et al., Eds Raven Press, New York (1986).
7. Bowden, D.H., Adamson, Y.R.: The role of cell injury and the continuing inflammatory response in the generation of silicotic pulmonary fibrosis. *J. Pathol.* 144:149-161(1984).
8. Brain, J.D., Frank, N.R.: Recovery of free cells from rat lungs by repeated washings. *J. Appl. Physiol.* 25:63-69(1968).
9. Davidson, S., Kinarty, A., Coleman, R., Reshef, A., Ginsburg, H.: Fibroblasts are required for mast cell granule synthesis. *Mast cell differentiation and heterogeneity*, pp. 115-124. A.D. Befus et al., Eds. Raven Press, New York (1986).
10. Diaz, P., Jones, D.G., Kay, A.B.: Histamine coated particles generate superoxide (O_2-) and chemiluminescence in alveolar macrophages. *Nature (Lond.)* 279:454-456(1979).
11. Faiman, M.D., Januskiewicz, A.J.: Pulmonary oxygen in the rat and the role of histamine. *Biochem. Pharmacol.* 35:3889-3892(1986).
12. Flint, K.C.: *Bronchoalveolar mast cells and asthma*, Springer, London (1987).
13. Goto, T., Befus, A.D., Low, R., Bienenstock, J.: Mast cell heterogeneity and hyperplasia in bleomycin-induced pulmonary fibrosis of rats. *Am. Rev. Respir. Dis.* 130:797-802(1984).
14. Metcalfe, D.D., Kaliner, M., Donlon, M.: The mast cell. *CRC Crit. Rev. Immunol.* 3:23-74(1981).
15. Norrby, K., Enerb ck, L., Franzen, L.: Mast cell activation and tissue cell proliferation. *Cell Tiss. Res.* 170:289-303(1976).
16. Schulman, E.S., Liu, M.C., Proud, D., MacGlashan, D.W., Lichtenstein, L.M., Plaut, M.: Human lung macrophages induce histamine release from basophils and mast cells. *Am. Rev. Respir. Dis.* 131:230-235(1985).
17. Tomioka, M., Ida, S., Shindoh, Y, Ishihara, T., Takishima, T.: Mast cells in bronchoalveolar lumen of patients with bronchial asthma. *Am. Rev. Respir. Dis.* 129:1000-1005(1984).
18. Uvnäs, B., Thon, I.L.: Isolation of 'biologically intact' mast cells. *Expt. Cell Res.* 18:512-520(1959).
19. Williams, A., Heath, D., Kay, J.M., Smith, P.: Lung mast cells in rats exposed to acute hypoxia, and chronic hypoxia with recovery. *Thorax* 32:287(1977).
20. Ziesche, R., Wenners, J., Behrendt, H.: Morphological investigations on the initial effects of quartz DQ 12 on membranes in vitro. *North-Rhine Westfalia Silicosis Report* Vol. 15: 331-344(1985).

Proceedings of the VIIth International Pneumoconioses Conference *Part*
Transactions de la VIIe Conférence Internationale sur les Pneumoconioses *Tome*
Transaciones de la VIIa Conferencia Internacional sobre las Neumoconiosis *Parte*

II

Pittsburgh, Pennsylvania, USA—August 23–26, 1988
Pittsburgh, Pennsylvanie, Etats-Unis—23–26 aout 1988
Pittsburgh, Pennsylvania EE. UU—23–26 de agosto de 1988

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

CDC
CENTERS FOR DISEASE CONTROL

Sponsors

International Labour Office (ILO)
National Institute for Occupational Safety and Health (NIOSH)
Mine Safety and Health Administration (MSHA)
Occupational Safety and Health Administration (OSHA)
Bureau of Mines (BOM)

November 1990

DISCLAIMER

Sponsorship of this conference and these proceedings by the sponsoring organizations does not constitute endorsement of the views expressed or recommendation for the use of any commercial product, commodity, or service mentioned.

The opinions and conclusions expressed herein are those of the authors and not the sponsoring organizations.

DHHS (NIOSH) Publication No. 90-108 Part II