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Atypical Quartz Dust-Induced Pneumoconiosis in SPF Rats

Aspects of the Role of the Lymphatic System in the Pathogenesis of Silicosis

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Summary. Experimental exposure to quartz dust for 100 days (=700 h) induces in SPF rats histologic changes of the lungs, which have such striking similarities with human cases of pulmonary alveolar proteinosis that they may represent an animal model of human disease. Conventional stock rats, the standard model of experimental silicosis research, react upon the same dosage of quartz dust by the formation or an increase in size of perivascular lymphatic sheaths, in which epitheloid cell granulomas can arise. In SPF rats such granulomas can only be developed in the sparse pre-existent lymphatic tissue, mostly in hilar lymph nodes. The reaction of SPF rats opens an interesting aspect on the significance of the phenomenon of lymphatic "drainage," as comparable reactions are only known in conventional rats following extreme dosage and in human pathology as so-called acute silicoproteinosis.

Key words: Silicosis – SPF rats – Conventional rats – Lymphatic system – Pulmonary alveolar proteinosis.

Zusammenfassung. Unter experimenteller Quarzstaub-Exposition zeigen SPF-Ratten nach 100 Tagen Versuchsdauer histologische Lungenveränderungen, die bis zum Modellcharakter reichende Gemeinsamkeiten haben mit dem Bild der Alveolarproteinose der menschlichen Pathologie.

Konventionell aufgezogene Ratten, das Standard-Modell der experimentellen Silikose-Forschung, reagieren bei gleicher Dosierung mit einer Vermehrung perivasculärer Lymphgewebs-Manschetten im Lungenparenchym, in denen es zur Ausbildung von Epitheloidzell-Granulomen kommt. In SPF-Ratten treten solche Granulome nur auf im spärlichen präexistenten lymphatischen Gewebe, meist also nur in hilären Lymphknoten. Die Reaktion der SPF-Ratten eröffnet einen interessanten Aspekt auf die Bedeutung des Phänomens der lymphatischen „Lungenreinigung". Denn vergleichbare Reaktionen kennt man bei konventionellen Ratten nur unter extremer Dosierung sowie in der menschlichen Pathologie als sogenannte akute Siliko-Proteinose.

Introduction

The elimination of inhaled particulate matter, when deposited in the pulmonary alveoli, becomes difficult, because ciliated epithelial flow is absent beyond the respiratory bronchioles. Thus, the lymphatic route of elimination acquires great importance. In earlier experiments using conventional rats a strong proliferation of lymphatic tissue was observed in the lungs when the animals were exposed to quartz dust inhalation (von Seebach and Schoeler, 1970).

Since the role of the immune system in the development of silicosis is subject to controversy, repeated experiments have been performed under the same conditions with specified pathogen-free (SPF) rats. SPF animals are reared under specified conditions; they possess only a rather sparse lymphatic tissue. Therefore, SPF rats seemed to provide appropriate conditions for testing: a) whether or not quartz dust represents an adequate stimulus for the proliferation of lymphatic tissue and b) whether the sparse development of the lymphatic tissue, which characterizes the initial situation in SPF rats, bears consequences in the course of the evolution of quartz dust-induced pneumoconiosis.

Material and Methods

From a total of 100 male SPF rats (Chbb-Thom), 4 months old at the beginning of the experiments, and of a body weight of about 320 g, 4 were sacrificed immediately. The remaining animals were housed under standard cage conditions without maintaining their initially germ-controlled environment. Dust experiments were conducted in a generator as constructed by Polley and Friedberg (1965). Sixty-four animals were exposed to Dörentrup quartz (particle size <3 µm, airborne dust with an average concentration of about 25 mg per m^3) for 7 h a day and 5 days weekly. The remaining 32 SPF rats were kept dust-free as controls. The animals were sacrificed by ether anesthesia in groups of 4 dust-exposed and 2 dust-free SPF rats at intervals of 2 days till the 10th day, and of 5 days till the 30th day; thereafter, the intervals were 10 days until the experiments were finished on the 100th day. The lungs were removed from the thorax, fixation in formaldehyde was followed by paraffin embedding of the upper right lobe. Serial sections were made and stained with H & E, PAS, Giemsa, Gömöri.

Results

1. Lymphatic Tissue Reaction in SPF Rats

The nonexposed SPF-control rats show on an average small hilar and peribronchial lymph nodes. Their spread reaches down to the segmental bronchioles. In almost all of these rats peribronchial or perivascular lymphatic tissue is totally absent in the periphery of the lung (Fig. 1 a).

When exposed to quartz dust inhalation, no definite increase in size of the *hilar lymph nodes* is ascertained in the SPF rats during the 100 days' course of the experiment, because they are of very different individual size. Due to the dust inhalation, epitheloid cell granulomas are developed in hilar lymph nodes of one of the animals sacrificed on the 40th day, and in all animals after the 60th day of our experiments. These dust granulomas show little tendency toward confluence. In the *periphery of the lung* sparse lymphocytic aggre-

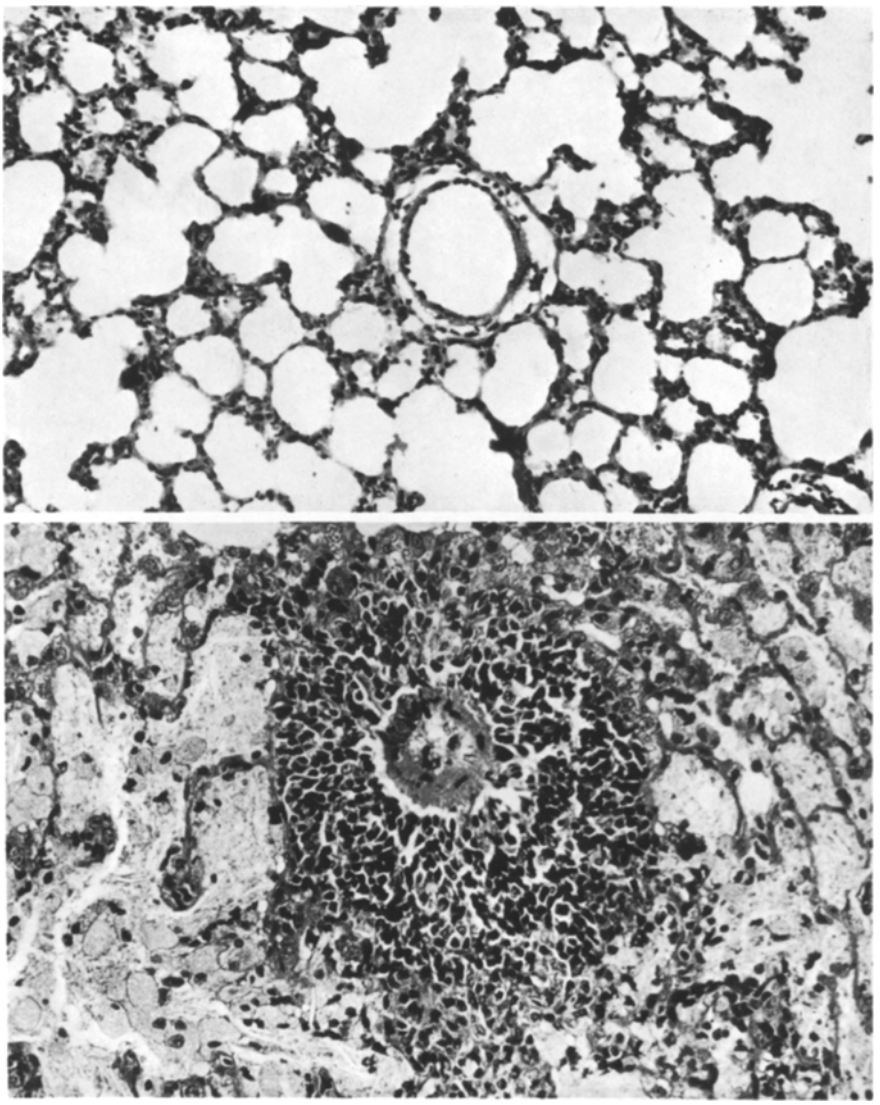

Fig. 1. a Blood vessel in the periphery of the lung of a dust-free control SPF-rat. Perivascular lymphatic tissue is not found in nearly all of these controls which were kept under conventional conditions for 100 days. H & E × 160. **b** Perivascular lymphatic parenchyma in the periphery of the lung of an SPF rat exposed to quartz dust for 100 days. Granulomas were never found. H & E × 160

gates appear in the perivascular spaces after a short time of quartz dust exposure; they become denser and apparently lead to the accumulation of lymphatic tissue around some blood vessels after the 40th day of quartz dust exposure (Fig. 1 b). However, granulomas are never found in the peripheral areas of the lung of SPF rats.

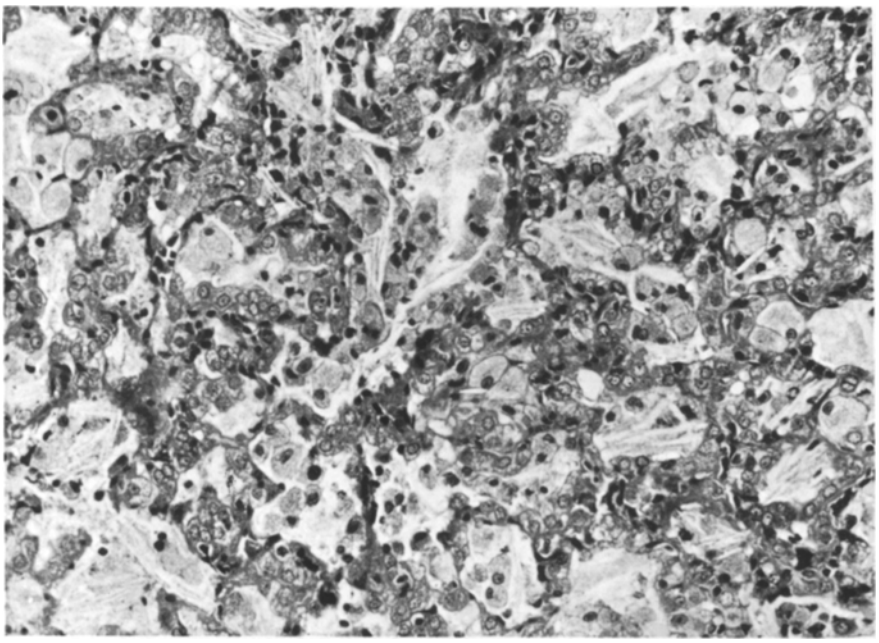

Fig. 2. Numerous large foamy macrophages or their loose floccular debris and some cholesterol crystals filling the alveolar spaces. Obviously, the densely stained cuboidal epithelial cells are granular pneumocytes. SPF rat exposed to quartz dust for 100 days. H & E × 250

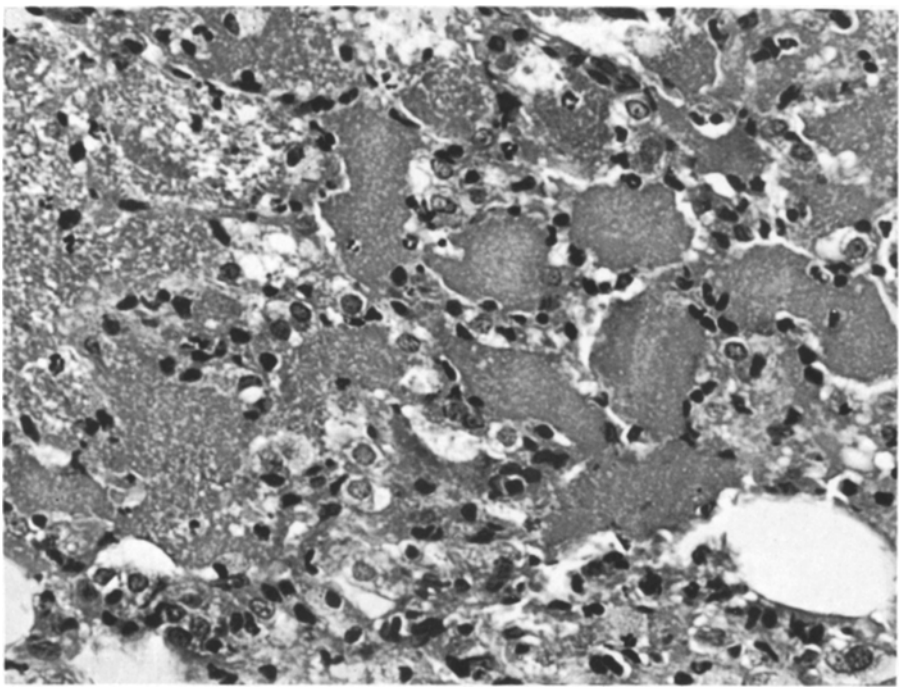

Fig. 3. Typical aspect of pulmonary alveolar proteinosis. A strongly PAS-positive amorphous as well as a more granular material filling the alveolar spaces. SPF rat exposed to quartz dust for 100 days. PAS-hematoxylin × 350

2. Reaction of the Lung Tissue

The ingestion of inhaled particles in the SPF rats is performed by macrophages, which accumulate in the alveoli surrounding the peripheral bronchioles and the blood vessels. When they increase in number, epithelial cells with a markedly basophilic cytoplasm begin to protrude into the alveolar lumina, at first preferably in the alveolar niches. At a later stage, the alveolar wall may at some places be lined by these cells only (Fig. 2). At this time the phagocytes have acquired the appearance of swollen cells with abundant foamy cytoplasm. Many of them fill the alveoli mostly in a diffuse distribution throughout the lungs (Fig. 2). As they disintegrate cholesterol crystals can be formed (cf. Fig. 2) and large areas of the lung exhibit fine granular deposits within the alveolar spaces, which condense to form a homogeneous, deeply eosinophilic, strongly PAS-positive material (Fig. 3), preferably in the alveoli around peripheral blood vessels. At the 40th day, when these intraalveolar changes begin, they are limited to focal areas of the lung parenchyma. Subsequently, these foci expand and show a pronounced tendency towards confluence, while nearly all the rest of the lung is stuffed with macrophages or their debris.

Discussion

The knowledge in experimental silicosis research is based mainly upon findings in conventional rats. Therefore, we compared the slides of this experiment with those of a former series done with 120 conventional (non-SPF) rats under the same experimental conditions (von Seebach and Schoeler, 1970). This comparison discloses interesting histologic differences:

1. Lymphatic Tissue Reaction in Conventional Rats

Nonexposed conventional rats show larger hilar and peribronchial lymph nodes than SPF animals, lymphatic sheaths around and along bronchial branches and, very characteristically, lymphoid tissue around normal peripheral pulmonary blood vessels (Fig. 4a), apparently depending upon specific living conditions. There are, however, great individual differences between single animals.

Quartz dust exposure induces a marked proliferation of hilar and peribronchial lymphatic tissue with some interesting histologic phenomena (von Seebach and Schoeler, 1970) and formation of epitheloid or spindle cell granulomas. But by comparison, we feel now as a consequence of the study with SPF animals, that, from a theoretic point of view, the more important result is the conspicuous proliferation of perivascular lymphatic tissue. And all granulomas found in the pulmonary periphery do arise in these perivascular lymphatic sheaths (Fig. 4b)—quite in contrast to the findings obtained in SPR rats.

2. Reaction of the Lung Parenchyma in Conventional Rats

The wide involvement and disseminated intraalveolar distribution of phagocytes observed in quartz-exposed SPF rats contrasts remarkably with their more circumscribed accumulation around intrapulmonary peripheral blood vessels in

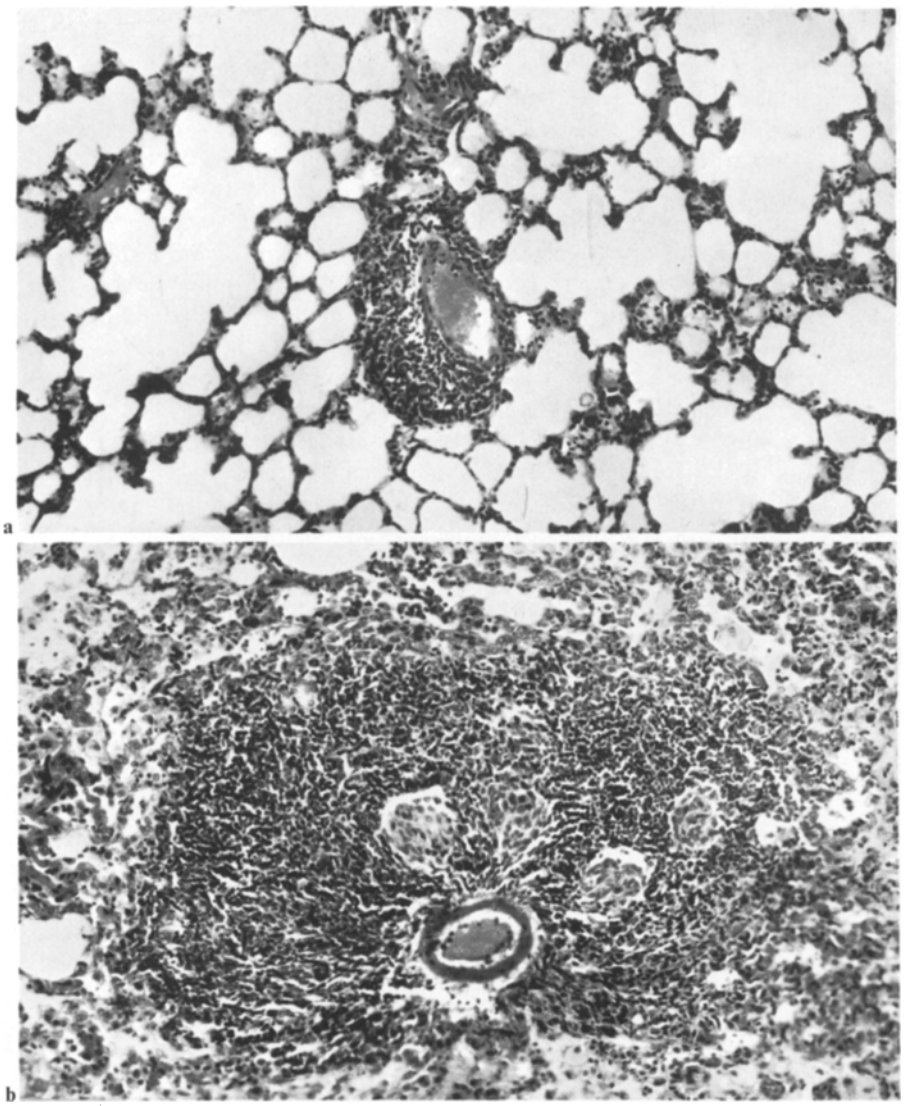

Fig. 4. a Sheath of lymphoid tissue around a blood vessel as found in the periphery of the lung of all non-SPF controls. H&E × 140. **b** Broad perivascular lymphatic sheath with epitheloid cell granulomas. Non-SPF rat exposed to quartz dust for 68 days. H & E × 140

the exposed non-SPF rats. Mainly the so-called alveolar niches usually contain masses of trapped macrophages or their debris, then showing also a cuboid basophilic pneumocytic lining. The proteinaceous intraalveolar deposits seen in exposed SPF rats, however, were never observed.

Independent of whether SPF or non-SPF rats are concerned, the rapid incorporation of dust particles by macrophages in the pulmonary alveoli is a

process of phagocytosis, including lysosomal attachment of phagosomes, enzyme discharge, and formation of secondary lysosomes (Allison et al., 1966). Innocuous dust particles leave these cell organelles intact, so that the vitality of the macrophages is not seriously impaired. In contrast, on account of the catalytic activities of their crystal surface (Robock, 1968), the quartz dust particles lead to changes of the permeability of the lysosomal membrane system, so that lysosomal enzymes kill the cell (Allison et al., 1966; Nash et al., 1966; Nadler and Goldfischer, 1970).

The lung tissue around the blood vessels represents "pulmonary dust sumps," where dust cells mainly accumulate (Macklin, 1955). After repeated phagocytosis and killing of macrophages, the quartz dust particles can easily reach the interstitium of the lung to come into contact with histiocytes which have, at least in conventional animals, a close relation to the lymphatic tissue, where the dust is deposited (cf. Macklin, 1955) and induces the formation of epitheloid cell granulomas, the precursors of silicotic nodules (Belt and King, 1945).

In the SPF rats as well as in the non-SPF rats, the macrophages are at first stored in the lung tissue around in the blood vessels. The SPF rats, however, show morphologic evidence of eliminatory insufficiency. Thus, at a later stage, almost the entire lung tissue is filled with macrophages or their debris. The same condition can be attained in conventional rats by an exceptionally high experimental quartz dust concentration requiring more macrophages for their engulfment (Gross and de Treville, 1968). Both conditions lead to pulmonary alveolar proteinosis (Heppleston, 1967; Gross and de Treville, 1968), as in human cases of so-called acute silicoproteinosis (Hoffmann et al., 1973).

From electron-microscopic investigations evidence is reported that in the formation of the proteinaceous intraalveolar material in addition to phagocytic remnants another component derived from the granular pneumocytes is necessary (Divertie et al., 1966; Kuhn et al., 1966; Corrin and King, 1969, 1970; Heppleston et al., 1970; Heppleston and Young, 1972).

The granular pneumocytes represent a population of secretory alveolar epithelial cells, that possess osmiophilic laminated bodies as typical cell organelles (Klaus et al., 1962; Bensch et al., 1964; Faulkner, 1969; Askin and Kuhn, 1971). As the source of surface-active phospholipids, these corpuscles are physiologically secreted to constitute the pulmonary surfactant, which has protective functions for the lung parenchyma (Macklin, 1954; Avery and Said, 1965; Leeson and Leeson, 1966; Kikkawa et al., 1970).

Granular pneumocytes—our cuboidal alveolar epithelial cells seem to be such pneumocytes (cf. Heppleston et al., 1970)—can be stimulated by different irritants both to proliferate (Evans et al., 1973) and to increase secretion of their lamellar bodies (Valdivia and Sonnad, 1966; Yuen and Sherwin, 1971). As they constitute the site of phospholipid synthesis in the lung, increased amounts of these lipids, as demonstrated in conventional animals in earlier silicotic stages (Fallon, 1937; Marks and Marasas, 1960), can only originate from the metabolism of these cells (Buckingham et al., 1966). This reaction can possibly be stimulated by the presence of macrophages. In SPF rats, the huge masses of macrophages may induce a vicious increase of the intraalveolar phospholipid content. The macrophages, which phagocytize not only the quartz

dust particles, but also the unphysiologically increased phospholipid material, acquire a foamy appearance (Heppleston et al., 1970). At a later stage, obviously in places of highest concentration around the blood vessels, their loose floccular cell debris condenses to form a homogeneous intraalveolar material, typical of pulmonary alveolar proteinosis (Rosen et al., 1958). Thus, the pneumoconiotic lesion in SPF rats seemingly represents a more severe reaction with considerable damage of the granular pneumocytes and macrophages. In human pathology as well as in conventional stock rats this pattern is only known from extreme dosage.

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