ymphocyte Populations in Lung Tissue, Bronchoalveolar Lavage Fluid, and Peripheral Blood in Rats at Various Times during the Development of Silicosis[1-3]

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SUMMARY Inflammatory cells and lymphocyte populations were examined in the bronchoalveolar lavage (BAL) fluid, lung tissues, and peripheral blood from rats at various times after the intratracheal instillation of silica. In lavage fluid, there was a rapid initial increase in the percentage and number of polymorphonuclear leukocytes (PMN), which slowly decreased during the course of the experiment. In addition, compared to controls, there was an increased number and percentage of lymphocytes throughout the 75 days of the experiment. The lymphocytic populations, which were determined by an indirect immunofluorescence method with monoclonal antibodies to lymphocyte surface markers, showed a predominance of the T-helper phenotype from Day 14 through the end of the experiment (Day 75). The number of PMNs obtained from collagenase digest of the lung was increased over control levels up to Day 7 after silica administration and remained at a relatively constant level until Day 14, after which time they decreased slightly in number. The total number of lymphocytes peaked on Day 14, with cells of the T-helper phenotype predominating after this time. In the peripheral blood, T-helper cells from silicotic rats were significantly increased over control rats on Days 7 and 14 but returned to normal control values after this time. The lymphocyte subsets in the BAL, but not in the peripheral blood, more closely reflect the lymphocyte patterns in the lung. The results of these experiments suggest that T-helper cells may play an important role in the inflammatory-fibrotic events in the lungs of rats with silicosis.

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Introduction

Silicosis is a chronic progressive granulomatotic-fibrotic lung disease caused by the inhalation of various forms of crystalline silica. In the naturally occurring disease in humans and in the experimentally induced disease in animals, the initial histopathology of the silicotic nodule is characterized by aggregates of macrophages (1). This is followed by the infiltration of lymphocytes and a few neutrophils, along with alveolar type II cell hyperplasia. Later, focal fibrosis with fibroblasts surrounding the zone of lymphocytes and macrophages appears. This may enlarge progressively.

For a brief period of time after induction of silicosis, the bronchoalveolar lavage (BAL) fluid shows an inflammatory cell accumulation (2). Later, there is an increase in the number of macrophages and lymphocytes, with the occurrence of occasional neutrophils. In early studies in which the interaction between silica and alveolar macrophages was examined *in vitro*, injury occurred to alveolar macrophages after the phagocytosis of quartz particles (3). However, when alveolar macrophages from humans and animals were exposed to silica *in vivo*, injury was not apparent (2, 4, 5). The quartz particles were found to be coated with alveolar lining material which was thought to render them less toxic (6). Nevertheless, the ingestion of silica particles by the macrophages leads to the secretion of various factors, such as derivatives of arachidonic acid, monokines, and growth factors (7-10). It is thought that one of the results of the secretion of these factors was an amplification of the initial response, i.e., lymphocyte recruitment, and secretion of chemotactic and activating factors for macrophages.

In animal models of bleomycin-induced fibrosis, lymphocyte infiltration into the lung has been studied. There is a transient increase in the helper to suppressor ratio (11). Nevertheless, there was a prevalence of suppressor cells during the course of the disease, which correlated with both the decrease in collagen production and disease regression.

The purpose of this study was to examine the inflammatory cells in the lungs during the development of silicosis in the rat. We compared the lymphocyte populations present in BAL fluid to those present in lung tissue at various times during the development of the silicotic process. Our findings showed a predominance of helper T-cells relative to the suppressor T-cells in both the lavage and lung tissue during the development of silicosis. In addition, these changes in the BAL closely reflect changes that occur in the lung, whereas the peripheral blood does not.

Methods

Animals

Adult, male, pathogen-free Sprague-Dawley rats weighing 200 to 225 g were purchased from Bantin and Kingman (Fremont, CA). These animals were maintained in a horizontal laminar flow apparatus until the injection of silica, then housed in a conventional animal care facility. Before killing, all animals were anesthetized with pentobarbital (2.5 mg/kg) and anticoagulated with 1,000 U of heparin. Before processing, the lungs of control and silicotic animals were examined macroscopically to exclude pulmonary disease caused by infection.

Induction of Silicosis

A tracheostomy was performed on all laboratory animals, and a single intratracheal injection of 10 mg silica (< 5 μm, Min-U-Sil; Pennsylvania Glass Sand Corp., Pittsburgh, PA) in 0.3 ml of sterile saline was given. Groups of four animals were killed at 3, 7, 14, 30, and 75 days after silica instillation.

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Control groups consisted of three animals each from four groups killed 3, 7, 14, and 30 days after intratracheal saline administration.

Cell Isolation

Bronchoalveolar cells. After the animals were exsanguinated, the lungs were collapsed by cutting the diaphragm, and the trachea was cannulated. The thoracic cavity was opened, and the left and right ventricles were incised. Next, an 18-gauge polyethylene catheter connected to a syringe filled with Hanks' balanced salt solution (without Ca^{2+} or Mg^{2+}), (HBSS) (GIBCO Laboratories, Grand Island, NY) was inserted through the right ventricle into the pulmonary artery. This was followed by perfusion of the lung circulation with HBSS until the parenchyma appeared white. Next, the cannulated trachea, heart, and lung were removed, and the lung was lavaged five times with 10 ml each time of 1% lidocaine in cold HBSS. In most experiments, we recovered approximately 80% of the instilled HBSS. The retrieved cells were washed twice with 0.1 M phosphate-buffered saline (PBS), pH 7.0. An aliquot was removed for counting and for the determination of viability. The viability of this preparation was between 80 and 90%. Also, a cytocentrifuge preparation was made and stained with Giemsa for differential counting. In order to obtain an enriched mononuclear cell preparation and to remove dead cells and debris, the remainder of the cells were overlayered on lymphocyte separation medium (LSM) (Litton Bionetics, Charleston, SC). After centrifugation at 200 × *g* for 30 min, the mononuclear cells at the interface were removed and washed three times in PBS.

Lung cells. After lavage, the lungs were inflated with 10 ml of 1 mg/ml collagenase, type I, (Sigma, St. Louis, MO) in Dulbecco's phosphate-buffered saline (DBS) (GIBCO) and incubated at 37° C for 20 min in a shaking water bath. After dissecting off the hila and major bronchi, the lungs were minced, vortexed for 1 min in 2 ml of fetal calf serum (FCS) (Hyclone Laboratories, Logan, UT), and then filtered through meshes to obtain a single cell suspension. The first passage was through a two-layer gauze filter (Parke-Davis, Detroit, MI), next a four-layer gauze filter, and finally a 20-μm mesh nylon filter (Tekto, Elmsford, NY). An aliquot of these cells was used to determine the total cells recovered, their viability and differential by Giemsa and a modified Papanicolaou for alveolar type II cells (12). The viability of these cells was greater than 80%. The remainder of the cells was separated on LSM as described above.

Peripheral blood. Blood was obtained via the aorta, diluted with an equal volume of saline, then separated on LSM to obtain the mononuclear fraction.

Identification of Lymphocyte Populations

Both B- and T-lymphocytes and T-lymphocyte subpopulations were identified by immunofluorescence using specific antibodies to cell surface antigens, and the percentage of positive cells was determined by flow cytometry with gating for lymphocytes by forward and right angle scatter (Ortho Cytofluorograph). For the detection of B-lymphocytes or surface immunoglobulin-bearing (SIg) cells, a fluorescein-conjugated $F(ab')_2$ preparation of goat IgG anti-rat IgG H and L chains (Cappel Laboratories, Downingtown, PA) was used. Total T-lymphocytes were identified by using indirect immunofluorescence and a W3/13 monoclonal antibody (Sera Labs, Westbury, NY), while cells expressing the T-helper/inducer and T-suppressor/cytotoxic phenotype were determined with W3/25 and OX-8 monoclonal antibodies (Sera Labs), respectively. To 1×10^6 of each cell preparation, 100 μl of an optimal saturating dilution of the appropriate monoclonal antibody was added and incubated at 4° C for 45 min. The optimal dilution of each antibody was determined previously on rat spleen cells. All cell suspensions were in PBS containing 0.1% sodium azide and 1% FCS. After incubation, the cells were washed with cold PBS and centrifuged at 200 × *g* for 10 min. To the pelleted cells, a fluoresceinated $F(ab')_2$ goat anti-mouse IgG (Cooper Biomedical Laboratories, Malvern, PA) was added, incubation was allowed to proceed for an additional 45 min, and the cells were washed as previously. The cells were fixed with 2% formaldehyde in PBS, and the percentage of positive cells determined by flow cytometry. Controls for T-cells consisted of the same cell populations stained with the secondary antibody only, while the control for B-cell enumeration was fluoresceinated anti-fibrinogen (Cappel Labs) of the same isotype as the B-cell marker (IgG_1). The percentage of cells positive for each marker was determined by subtracting the percentage of positive cells in the control cells from the experimental cells. In order to obtain enough cells from BAL for analysis, the cells were pooled from the non-silica-treated control groups and in the 3-day post-silica instillation group. For the other groups, a sufficient number of cells was recovered from individual animals in order to perform the lymphocyte analysis. Each group consisted of at least four animals.

Statistics

Statistical analyses were carried out by a one-way analysis of variance.

Results

Lung Injury

The histologic evidence of lung injury was similar to what has been reported previously after intratracheal administration of silica (13). The two major lesions present in the lungs of silica-treated rats were silica-containing granulomas or silicotic nodules and variable lipoproteinosis. These granulomas, some discrete and some coalescing, were scattered throughout the lung lobes, with some localization around airways. Within the granulomas, macrophages were seen surrounded by lymphocytes. In addition, there were scattered neutrophils and eosinophils in the early disease; however, the eosinophils were not present in the advanced silicotic lung. Furthermore, there was focal interstitial fibrosis with alveolar type II hyperplasia occurring first on Day 14.

BAL Cells

There was a rapid increase in the number of inflammatory cells in the BAL fluid after instillation of silica (table 1). During the first 2 wk, the change in percentage of cells was attributable primarily to an increase in neutrophil percentage. The number of neutrophils was also relatively high in those control animals killed 3 days after saline instillation; however, they never exceeded 6% of the total cell count (data not shown). The number of BAL lymphocytes increased with disease progression, with an increase in both the

TABLE 1

CELL TYPES RECOVERED FROM BAL FLUID

Cell Type	Controls†‡	Days After Silica Administration* 3‡	7	14	30	75
Macrophages, %	93 ± 2.0¶	69 ± 3.0	60 ± 3.0	50 ± 4.0	53 ± 3.0	54 ± 3.0
Lymphocytes, %	2 ± 0.5	4 ± 1.0	15 ± 2.0	29 ± 3.0	35 ± 4.0	35 ± 3.0
Neutrophils, %	4 ± 0.5	22 ± 3.0	20 ± 2.0	16 ± 2.0	11 ± 1.0	9 ± 1.0
Eosinophils, %	1 ± 0.5	4 ± 1.0	4 ± 1.0	4 ± 1.0	1 ± 0.5	1 ± 0.5
Total	8 ± 1.0§	12 ± 2.0	20 ± 3.0	22 ± 2.0	20 ± 2.0	19 ± 2.0

* All the results for animals receiving silica are statistically significant ($p < 0.05$) from control results except for the percentage of eosinophils at Days 30 and 75 after silica administration ($p > 0.05$)

† The control group consisted of three animals each from four groups killed on Days 3, 7, 14, and 30 after intratracheal saline administration

‡ In order to obtain enough cells for analysis, the differential counts for the control group and the group of animals receiving silica and killed 3 days later represent the number of each cell type/animal from four experiments with the cells pooled from three animals/experiment. For the control group, the four experiments are from animals killed on Days 3, 7, 14, or 30 after saline administration.

¶ Mean percentage of cells/animal ± SEM

§ Total number of cells × 10^6/animal ± SEM

TABLE 2

TOTAL LYMPHOCYTE POPULATIONS IN BAL FLUID ($\times 10^6$ CELLS)

Cell Phenotype	Controls†‡	Days After Silica Administration* 3‡	7	14	30	75
Lymphocytes	0.16 ± 0.01§	0.48 ± 0.03	3.00 ± 0.06	6.38 ± 0.15	7.00 ± 0.24	6.65 ± 0.24
Total T (W3/13)	0.08 ± 0.01	0.28 ± 0.03	1.85 ± 0.07	3.60 ± 0.24	5.94 ± 0.23	4.68 ± 0.34
T-helpers (W3/25)	0.05 ± 0.01	0.16 ± 0.02	0.90 ± 0.09	2.45 ± 0.07	4.45 ± 0.23	3.16 ± 0.23
T-suppressors (OX-8)	0.04 ± 0.01	0.13 ± 0.02	1.00 ± 0.10	1.63 ± 0.09	1.53 ± 0.14	1.86 ± 0.12
B-cells (SIg)	0.02 ± 0.01	0.07 ± 0.01	0.62 ± 0.04	1.20 ± 0.18	0.75 ± 0.11	0.84 ± 0.12

* All the values for the silica-treated rats are statistically different from the control values ($p < 0.05$)

† The control group consisted of three animals each from four groups killed on Days 3, 7, 14, and 30 after intratracheal saline administration

‡ In order to obtain enough cells for analysis, the differential counts for the control group and the group of animals receiving silica and killed 3 days later represent the number of each cell type/animal from four experiments with the cells pooled from three animals/experiment. For the control group the four experiments are from animals killed on Days 3, 7, 14, or 30 after saline administration

§ Mean number of positive cells/animal ± SEM

total number of T- and B-cells (table 2). There was a nearly equal ratio of T-helper to T-suppressor cells through Day 7 after silica administration, with a slight inversion of the ratio on Day 7 (figure 1a). However, on Days 30 and 75 there was a significant increase in this ratio ($p < 0.05$).

Lung Tissue Cells

There was an increase in the total number of inflammatory cells obtained from the dissociated lung tissue through the first 14 days after silica injection, although the increase in the total number of cells was proportionally less dramatic than that observed for the BAL cells (table 3). On Day 75, a slight reduction in the total number of cells from Day 30 was observed, although the number of cells recovered at this time was still higher than the number of cells recovered from the control rats. The increased number of inflammatory cells infiltrating the lung tissue during the course of the disease was attributed to a generalized increase in all types, including both lymphocytes and macrophages. The examination of neutrophil presence within the lungs showed a peak at Day 7, with a return to control levels by Day 75 after silica administration. A similar pattern was noted for the percentage of eosinophils infiltrating the lungs. The number of lymphocytes obtained from the digested lung tissue showed an increase through 14 days after silica administration, after which time there was a slight decrease in the total number recovered (table 3). However, animals killed at 75 days had levels of lymphocytes nearly three times that of the control animals. The numbers of both B- and T-lymphocytes showed a slight increase through Day 14 followed by a slight decrease through Day 75. The T-helper to T-suppressor cell ratio on Days 3 and 7 after silica administration was similar to the ratio obtained for the control animals, with an inversion of the ratio at 3 days followed by a further drop in the ratio at 7 days. Finally, there was a change to a higher ratio on Days 15, 30, and 75 ($p < 0.05$, figure 1b).

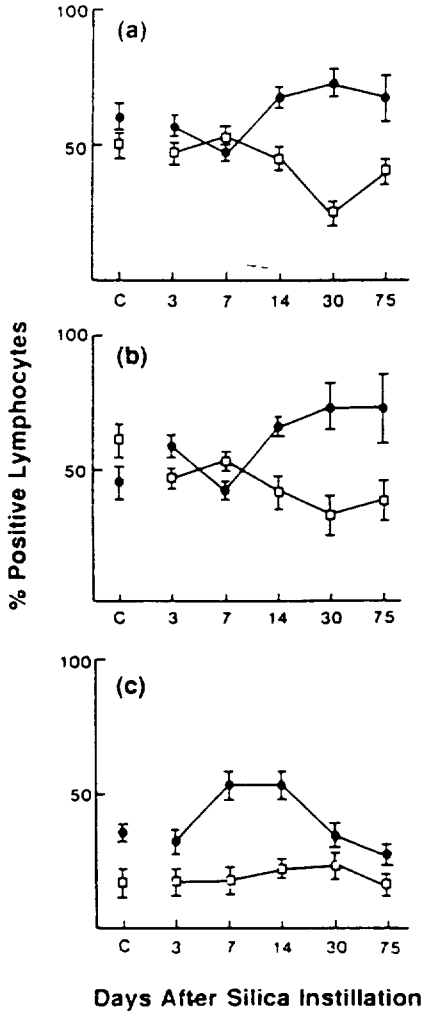

Fig. 1. Percentage of T-lymphocytes bearing the T-helper (*circles*) or T cytotoxic-suppressor (*squares*) phenotype obtained from BAL fluid (a), collagenase-digested lung tissue (b), and the peripheral blood (c) from rats at various times after the administration of silica. The C in each panel refers to the control, non-silica-treated rats.

Peripheral Blood Lymphocytes

Some degree of fluctuation occurs in the peripheral blood lymphocyte populations during the course of the disease (figure 1c). The most dramatic change oc-

TABLE 3

INFLAMMATORY CELLS* RECOVERED FROM COLLAGENASE-DIGESTED LUNG TISSUE

Cell Type	Controls‡§	Days After Silica Administration† 3§	7	14	30	75
Macrophages, %	20 ± 3.0‖	25 ± 2.8	30 ± 2.9	30 ± 3.0	30 ± 3.0	30 ± 3.2
Lymphocytes, %	10 ± 2.0	15 ± 1.5	18 ± 1.6	30 ± 3.2	27 ± 2.8	25 ± 3.0
Neutrophils, %	2 ± 0.2	4 ± 1.0	13 ± 2.2	10 ± 1.4	3 ± 0.5	2 ± 0.5
Eosinophils, %	1 ± 0.2	3 ± 1.0	5 ± 1.5	5 ± 1.1	2 ± 1.0	0
Total	80 ± 2.2¶	85 ± 2.4	95 ± 1.9	110 ± 1.1	110 ± 1.2	95 ± 1.1

* The remainder of the cells represents fibroblasts, epithelial alveolar cells, mast cells, and various other nonidentified lung parenchymal cells.

† All the results are statistically significant ($p < 0.05$) from control results except for the percentage of neutrophils on Day 75 after silica administration ($p > 0.05$).

‡ The control group consisted of three animals each from four groups killed on Days 3, 7, 14, and 30 after intratracheal saline administration.

§ In order to obtain enough cells for analysis, the differential counts for the control group and the group of animals receiving silica and killed 3 days later represent the number of each cell type/animal from four experiments with the cells pooled from three animals/experiment. For the control group, the four experiments are from animals killed on Days 3, 7, 14, or 30 after saline administration.

‖ Mean percentage of cells/animal ± SEM.

¶ Total number of cells/animal × 10^4 ± SEM.

curs on Days 7 and 14 after silica administration, where the percentage of T-helper cells increases from 35 to 54% ($p < 0.05$, figure 1c). This increase in T-helper cells precedes the increase noted in the BAL and lung tissue by 1 wk. There is no statistically significant difference at any of the other time points for the total number of peripheral blood T-cells, T-suppressor cells, or B-cells between control animals and animals receiving silica ($p > 0.05$).

Discussion

The results of this study demonstrate an increase in the total number of inflammatory cells including lymphocytes and macrophages in the lungs of rats during silica-induced disease. This increase occurs both in the lung tissue and in the BAL fluid. Initially, PMNs are recruited to the lung, and later these are replaced by mononuclear cells. This sequence of events is in accordance with earlier reports although there was no enumeration of the cells (13, 14). A previous study had suggested that the BAL cells may be representative of the cell populations in the lung (15); however, our data show that there is an earlier and larger percent increase of PMN in the BAL. Nevertheless, it may provide insight into changes occurring in the lung tissue.

The concomitant increase in the number of B-lymphocytes is not surprising because it is known that silicotic lung disease is accompanied by the presence of immunoglobulin in the lavage fluid of persons exposed to silica (16) as well as by the presence of autoantibodies and immune complexes in the serum of patients with silicosis (17). However, the role of B-cell or B-cell products in the fibrotic response of the lung is unknown. An increase in the total number of T-cells in the lung tissue was observed 3 days after silica exposure and this increased progressively until 14 days, when the number of cells recovered plateaued. Furthermore, there was a dramatic increase in the number of lymphocytes obtained in the BAL. The T-helper to T-suppressor cell ratio in the lung of rats was inverted on Day 3 but then reverted back to a ratio on Day 7, nearly equivalent to that of the control rats. The ratio increased on Day 14 and was nearly 2:1 on Days 30 and 75. A similar alteration in the T-helper to T-suppressor ratio occurred in the BAL, but without the inversion of the ratio on Day 3. In the peripheral blood, an increase in the T-helper to T-suppressor cell ratio occurred 1 wk before the alterations in the BAL and lung tissue. This suggests the origin of the T-helper cells in the lung and BAL may be from the blood, perhaps in response to mediators released by the initial inflammatory cells in the lung. One could speculate that the inversion of the T-helper to T-suppressor cell ratio in the lung on Day 7 may represent a migration of sensitized lung T-helper cells into the blood or lymph nodes where systemic amplification might result in a re-entry of an expanded population of these cells back to the lung at a later time.

Lymphocytes and macrophages appeared in close proximity to one another in developing silicotic nodules in both animal models and human cases. Increased proportions of lymphocytes were found in BAL specimens from animals developing silicosis (18) and from humans with silica dust exposure (3, 19). However, the lymphocyte subpopulations and their traffic during the development of the disease were not studied. The cell-mediated immune responses have been reported to be normal in silicosis (20). In our study, the absence of significant change in the lymphocyte subpopulations in peripheral blood of rats with silicosis could support these findings. In contrast, in sarcoidosis, which is also characterized by lung lymphocytosis with helper preponderance, there is a decrease in cell-mediated immunity (21).

TABLE 4

TOTAL LYMPHOCYTE POPULATIONS IN LUNG TISSUE OF RATS (× 10^4 CELLS)

	Controls†‡	Days After Silica Administration* 3‡	7	14	30	75
Lymphocytes	8.0 ± 1.0§	12.8 ± 2.0	17.1 ± 2.0	33.0 ± 3.0	29.7 ± 3.0	23.8 ± 3.0
Total T (W3/13)	4.7 ± 0.2	7.2 ± 0.3	9.9 ± 0.2	19.7 ± 1.6	18.3 ± 1.9	15.2 ± 1.7
T-helpers (W3/25)	2.2 ± 0.2	4.3 ± 0.2	4.3 ± 0.1	13.4 ± 0.9	13.6 ± 1.6	11.2 ± 1.6
T-suppressors (OX-8)	2.9 ± 0.2	3.4 ± 0.2	5.4 ± 0.1	8.1 ± 0.8	6.2 ± 1.0	5.9 ± 1.0
B-cells (SIg)	1.0 ± 0.1	2.5 ± 0.2	2.7 ± 0.5	5.6 ± 1.0	4.8 ± 1.4	3.1 ± 1.1

* All the values for the silica-treated rats are statistically different from the control values ($p < 0.05$).

† The control group consisted of three animals each from four groups killed on Days 3, 7, 14, and 30 after intratracheal saline administration.

‡ In order to obtain enough cells for analysis, the differential counts for the control group and the group of animals receiving silica and killed 3 days later represent the number of each cell type/animal from four experiments with the cells pooled from three animals/experiment.

§ Mean number of positive cells/animal ± SEM.

The role of T-helper cell influx and persis[illegible] in the lungs of silicotic animals is u[illegible]ear. In a previous investigation in which bleomycin was used to induce lung fibrosis (12), it was shown that at 14 days after bleomycin treatment, when there was a T-helper cell predominance in the lungs, the rate of collagen synthesis was significantly elevated, whereas at 30 days, when there was a predominance of suppressor cells, the rate of collagen synthesis was within normal limits. It has been suggested that the T-helper cells in the lung of bleomycin-treated animals may play a role in enhancing collagen synthesis (21). In silicosis, there is a progressive increase in lung collagen. Hence, the correlation between increased T-helper cells and collagen deposition may also be true in silicotic lung as well.

In summary, we were able to show that in the animals with silicotic lung disease the increase in the percentage of cells expressing the T-helper phenotype preceded the increase of these cells in the BAL and lung tissue digest. Although the blood T-helper and T-suppressor ratios returned to levels comparable to levels obtained in control animals, the T-helper [illegible]ance in the BAL and lung digest persists.

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References

1. Spencer H. Pathology of the lung. 3rd ed. Philadelphia: WB Saunders, 1977; 379–95.
2. Davis GS, Hemenway DR, Evans JN, Lapenas DJ, Brody AR. Alveolar macrophages stimulation and population changes in silica-exposed rats. Chest 1981; 80(S):8–10.
3. Allison AC, Harington JS, Birbeck M. An examination of the cytotoxic effects of silica on macrophages. J Exp Med 1966; 124:141–54.
4. Christman JW, Emerson RJ, Graham WGB, Davis GS. Mineral dust and cell recovery from the bronchoalveolar lavage of healthy Vermont granite workers. Am Rev Respir Dis 1985; 132:393–9.
5. Brody AR, Roe MW, Evans JN, Davis GS. Deposition and translocation of inhaled silica in rats. Quantification of particles distribution, macrophage participation, and function. Lab Invest 1982; 47:533–42.
6. Emerson RJ, Davis GS. Effect of alveolar lining material-coated silica on rat alveolar macrophages. Environ Health Perspect 1983; 51:81–4.
7. Schmidt JA, Oliver CN, Lepe-Zuniga JL, Green I, Gery I. Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin-1. A potential role for interleukin-1 in the pathogenesis of silicosis. J Clin Invest 1984; 73:1462–72.
8. Lugano EM, Dauber JH, Daniele RP. Silica stimulation of chemotactic factor release by guinea pig alveolar macrophages. J Reticuloendothel Soc 1981; 30:381–90.
9. Lugano EM, Dauber JH, Elias JA, Bashey RI, Jimenez SA, Daniele RP. The regulation of lung fibroblast proliferation by alveolar macrophages in experimental silicosis. Am Rev Respir Dis 1984; 129:767–71.
10. Martin TR, Altman LC, Albert RK, Henderson WR. Leukotriene B_4 production by the human alveolar macrophages: a potential mechanism for amplifying inflammation in the lung. Am Rev Respir Dis 1984; 129:106–11.
11. Thrall RS, Barton RW. A comparison of lymphocyte populations in lung tissue and in bronchoalveolar lavage fluid of rats at various times during the development of bleomycin-induced pulmonary fibrosis. Am Rev Respir Dis 1984; 129:279–83.
12. Kikkawa Y, Yoneda K. The type II epithelial cell of the lung. I. Method of isolation. Lab Invest 1974; 30:76–80.
13. Reiser KM, Haschek WM, Hesterberg TW, Last JA. Experimental silicosis. II. Long-term effects of intratracheally instilled quartz on collagen metabolism and morphologic characteristics of rat lungs. Am J Pathol 1983; 110:30–40.
14. Lugano EM, Dauber JH, Daniele RP. Acute experimental silicosis. Lung morphology, histology, and macrophage chemotaxin secretion. Am J Pathol 1982; 109:27–36.
15. Hunninghake GW, Kawanami O, Ferrans VJ, Young RC, Roberts WC, Crystal RG. Characterization of the inflammatory and immune effector cells in the lung parenchyma of patients with interstitial lung disease. Am Rev Respir Dis 1981; 123:407–12.
16. Calhoun WJ, Christman JW, Ershler WB, Graham WGB, Davis GS. Raised immunoglobulin concentrations in bronchoalveolar lavage fluid of healthy granite workers. Thorax 1986; 41:266–73.
17. Doll NJ, Stankus RP, Hughes J, *et al.* Immune complexes and autoantibodies in silicosis. J Allergy Clin Immunol 1981; 68:281–5.
18. Callis AH, Sohnle PG, Mandel GS, Weissner J, Mandel NS. Kinetics of inflammatory and fibrotic pulmonary changes in murine model of silicosis. J Lab Clin Med 1985; 105:547–53.
19. Schuyler M, Gauner HR, Stankus RP, Kaimal V, Hoffman E, Salvaggio J. Bronchoalveolar lavage in silicosis. Lung 1980; 157:95–102.
20. Schuyler M, Zisking M, Salvaggio J. Cell-mediated immunity in silicosis. Am Rev Respir Dis 1977; 116:147–51.
21. Phan SH, Thrall RS, Williams C. Bleomycin-induced pulmonary fibrosis. Effects of steroid on lung collagen metabolism. Am Rev Respir Dis 1981; 124:428–34.

Bronch in Heal

JEANNE MA and DENISE

Although the bestos exposure recognized sinc tury, the pathc ing to asbesto related disease on animal moc tems (6–9) have the acute and su fiber deposition cases, asbestos not detected ur posure (8). It h during this lag chemical and c ed with retentio lung, eventuall

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