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Persistent Inflammation and Impaired Chemotaxis of Alveolar Macrophages on Cessation of Dust Exposure

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Rats were exposed by inhalation to coal mine dust, titanium dioxide, or quartz. The magnitude of the consequent inflammatory response was assessed by counting numbers and types of leukocytes in the bronchoalveolar lavage fluid. The magnitude of the inflammatory response reflected the toxicity of the dusts, with quartz eliciting the greatest recruitment of inflammatory leukocytes, coal mine dust less than quartz, and titanium dioxide eliciting no inflammation. To assess the persistence of the inflammation, groups of rats were maintained in room air for 30 or 60 days after cessation of dust exposure and then numbers of leukocytes were assessed. Bronchoalveolar leukocytes in rats exposed to coal mine dust were reduced after exposure, but in the quartz-exposed rats the numbers increased with time after exposure. The chemotactic responses of bronchoalveolar leukocytes from rats inhaling coal mine dust and quartz were reduced and remained so after a 30-day recovery period. Their reduced ability to chemotact did not fully prevent macrophages from leaving the bronchoalveolar region of dust-exposed rats. However, it is likely that the delayed removal of inflammatory leukocytes with the potential to injure the lung tissue may contribute to septal damage and so contribute to the pathogenesis of pneumoconiosis.

Introduction

In recent years there has been considerable interest in the immunological/inflammatory role of leukocytes after dust deposition. Of particular importance is the function of the alveolar macrophage in clearing the lung of inhaled particles. An area where both particle clearance and the immunoinflammatory roles of bronchoalveolar leukocytes overlap is chemotaxis. Particles depositing in the alveolar region are phagocytized by alveolar macrophages and then transported intracellularly to the mucocilliary escalator (*1*). There is also firm evidence that particles are cleared from the alveolar space by transport to the lung lymph nodes (*2*). It is in such a situation that interactions between dust-laden macrophages and lymphocytes are likely to occur. Such interactions could have important consequences for the disease process, for example, through the generation of cytokines and growth factors.

A key feature of pneumoconioses is the presence of inflammatory leukocytes in the bronchoalveolar region (*3*). We have previously shown that inflammatory leukocytes have the potential to injure cells (*4*) and connective tissue molecules (*5*) of the alveolar septum. The chemotactic activity of macrophages is thus likely to be of key importance to lung defense because it may influence their ability to move out of the alveolar region and thus be of importance in limiting the extent of leukocyte-mediated tissue damage after dust exposure. We therefore assessed the persistence of the inflammatory response and the chemotactic activity of bronchoalveolar leukocytes from lungs of rats exposed to dust by inhalation.

Materials and Methods

Male SPF rats of the HAN strain were exposed to airborne mineral dusts for 8 hr/day, 5 days/week in 1-m^3 inhalation chambers as previously described (*6*).

Dusts. Coal mine dusts were sampled from the air of British Collieries using dry fabric filters and then generated as a cloud using a Timbrell dust generator. Details of coal mine dust mineralogy have been published elsewhere (*6*). The dust cloud was passed through a cyclone to produce a respirable fraction, which was then dispersed into the chamber at an airborne mass concentration of 50 mg/m^3 in experiment 1 and 10 mg/m^3 in experiment 2, as previously described (*7*). Titanium dioxide (TiO_2; rutile) was obtained from Tioxide Ltd. (Stockton on Tees, England). Quartz was the DQ12 standard preparation.

Cell Preparation. Rats were removed from the chambers at various time points after the start of dust exposure. "Recovery" animals were also removed from the chambers at selected time points and were then maintained in room air for a further 60 days (experiment 1) or 30 days (experiment 2). The animals were killed, and bronchoalveolar leukocytes (BAL) were obtained by

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lavage as previously described (5). Total cells and differential counts were performed on the BAL before use in the chemotaxis assay.

Chemotaxis Measurement. Chemotaxis was assessed by measuring the directed migration of BAL through micropore filters in Boyden chambers as previously described (8). Zymosan-activated serum was used as the chemoattractant, and the modified checkerboard technique was used to check that the migration was true chemotaxis and not chemokinesis. The chemotactic activity of the bronchoalveolar leukocytes was measured at days 8, 15, and 32 in experiment 1 and after 30 days of recovery in rats exposed for 3, 7, 15, and 30 days in experiment 2. As a measure of chemotaxis, we counted the number of migrated cells per high power field in five fields per filter and two filters per condition.

Statistical Analysis. Differences between means were tested using Student's *t*-test.

Results

Experiment 1

The pneumoconiotic dusts (quartz and coal mine dust) elicited an inflammatory response in rat lungs after inhalation exposure at 50 mg/m³. Quartz produced the most marked response, whereas TiO_2, which is not associated with pathology in man, failed to induce inflammation except in the recruitment of polymorphonuclear neutrophils (PMN) at the latest time point. The total number of macrophages in the BAL was significantly greater ($p < 0.01$) than that in the TiO_2-exposed rats by 8 days of quartz exposure and by 16 days with the coal mine dusts (Fig. 1). On cessation of exposure to coal mine dust, macrophage numbers returned to normal, but in those animals exposed to quartz, the inflammation not only persisted but progressed markedly. The PMN response in the quartz-exposed rats reflected the macrophage response in that there was time-dependent recruitment of PMN and continuing increases in PMN numbers on cessation of dust exposure (Fig. 2). In rats exposed to coal mine dust, PMN numbers decreased during the recovery period but did not return to control levels, thus indicating persistence of the inflammatory response in these animals. Chemotaxis was assessed immediately on cessation of dusting at 8, 32, and 75 days after the start of dust exposure; at each time point there was a significant reduction in the chemotactic response of the quartz and coal mine dust-exposed leukocytes compared with controls ($p < 0.05$), but TiO_2 had no effect (Fig. 3).

Experiment 2

Having demonstrated impaired chemotactic responses of alveolar macrophages during dust exposure in experiment 1, we then went on to assess the persistence of the reduced chemotaxis. In experiment 2, inhalation of coal mine dust at 10 mg/m³ produced an inflammatory response by 15 days of exposure (Table 1). Although the increase in numbers of macrophages in the BAL was reduced when the animals were allowed to breathe room air for a further 30 days, numbers of macrophages in the BAL did not reach control levels, thus there was evidence of persistent inflammation. Chemotactic responses, measured in the recovery animals (Table 2), were impaired after 15 and 30 days of dust exposure. This result was consistent with the presence of an inflammatory response in the bronchoalveolar region. However, there was also impaired chemotaxis of BAL leukocytes after only 7 days of dust exposure, where there had been no evidence of an inflammatory response.

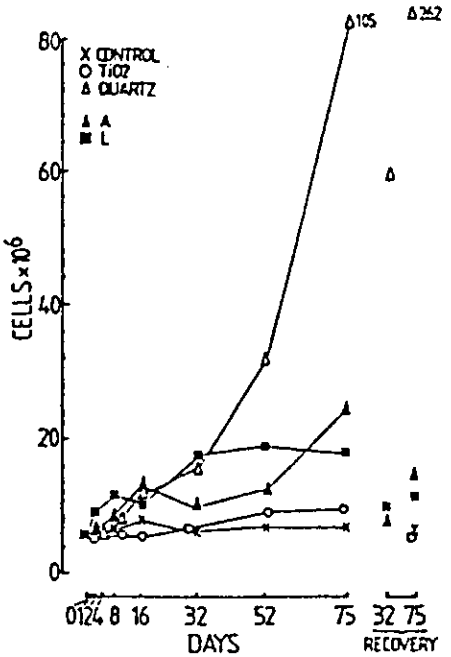

FIGURE 1. Total number of macrophages in the bronchoalveolar lavage of rats inhaling TiO_2, quartz, or coal mine dust (A,L). The last two time points represent, respectively, 32 days plus 64 days of recovery and 75 days plus 64 days of recovery. Results are the means of four rats at each time point for the dust-exposed animals and two for the controls.

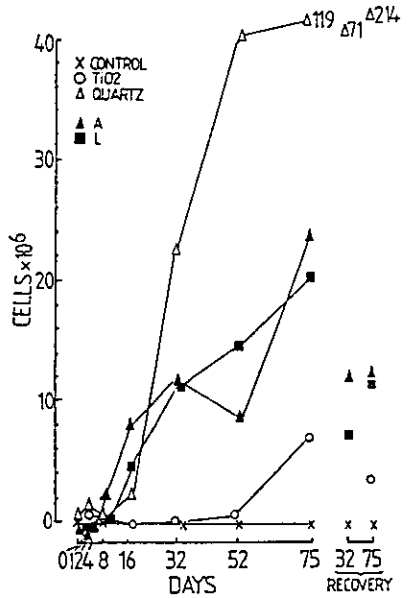

FIGURE 2. Percentage of neutrophils in the bronchoalveolar lavage with time of exposure to 50 mg/m³ of TiO_2, quartz, or coal mine dust (A,L). The last two time points are, respectively, 32 days of exposure plus 64 days of recovery and 75 days of exposure plus 64 days of recovery. Results are the means of four rats at each time point for the dust-exposed animals and two for the controls.

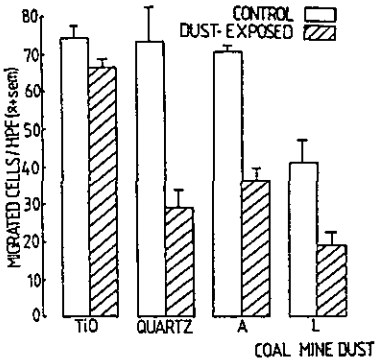

FIGURE 3. Chemotaxis of bronchoalveolar leukocytes from control rats and rats inhaling the indicated dusts at 10 mg/m^3. Results are the means (SD) of pooled data for 8, 32 and 75 days of exposure. Data were obtained from two filters, with five high-power fields per filter at each time point for each dust. Quartz and both coal mine dusts (A,L) show a significant reduction in chemotaxis ($p<0.05$).

Table 1. Total cells and differential count in the bronchoalveolar lavage fluid of untreated rats, immediately on cessation of exposure to 10 mg/m^3 coal mine dust (dust), and following a further 3 days breathing room air (recovery rats).

	Total BAL leukocytes, $\times 10^6$ [a]		%PMN in BAL[a]	
	Control	Dust	Control	Dust
3 days	4.8 (0.5)	4.5 (1.0)	0 (0)	0 (0)
3 days + recovery	7.8 (0.1)	7.3 (0.7)	1.3 (0.9)	0.5 (0.4)
7 days	5.4 (0.7)	7.8 (1.2)	0 (0)	13.7 (5.9)
7 days + recovery	6.9 (0.2)	7.9 (0.6)	0 (0)	1.3 (0.7)
15 days	4.3 (0.4)	12.2 (1.0)	0 (0)	29.0 (5.0)
15 days + recovery	6.9 (1.6)	14.9 (2.1)	0 (0)	17.3 (1.7)
30 days	5.3 (0.1)	29.5 (4.5)	0.7 (0.6)	41 (5.8)
30 days + recovery	4.6 (0.8)	14.4 (1.7)	1.3 (2.7)	27.3 (2.7)

Abbreviations: BAL, bronchoalveolar lavage; PMN, polymorphonuclear neutrophils.

[a]Values are means; SEM in parentheses.

Table 2. Chemotaxis of bronchoalveolar leukocytes from rats inhaling coal mine dust and then breathing room air for an additional 30 days.[a]

	Control		Coal mine dust	
Time	Mean	SEM	Mean	SEM
3 days + recovery	37.0	3.3	43.2	4.7
7 days + recovery	66.9	7.8	40.6	6.3
15 days + recovery	21.7	3.2	5.4	1.7
30 days + recovery	26.5	3.9	3.7	0.9

[a]Results are the counts of five fields per filter and two filters for each rat, with three rats per group at each time point.

Discussion

In this study, we have confirmed previous work demonstrating the recruitment of inflammatory leukocytes to the bronchoalveolar region in response to the inhalation of mineral dust in man (*5*) and in experimental animals (*9*). The magnitude of the inflammatory response was related to the pathogenic potential of the dusts, with quartz proving to cause more inflammation than coal mine dust. The failure of titanium dioxide to elicit an inflammatory response in the present study reflects the innocuous effects of this mineral, which is widely used in industrial processes but is not associated with pathology in man (*10*). We have also demonstrated reduced chemotactic responses of bronchoalveolar leukocytes obtained from rats inhaling pneumoconiotic dusts but not TiO_2. These results are consistent with previous reports of impaired chemotaxis after exposure to quartz (*11*) and chrysotile asbestos (*12*).

The mechanisms governing the reduction in chemotaxis are as yet unclear, but we have previously reported that the chemotaxis deficit was largely due to impaired alveolar macrophage function and was not due to the presence of PMN in the BAL (*8*). Further evidence that PMN do not contribute substantially to the impaired chemotaxis of inflammatory bronchoalveolar leukocytes was obtained in the present study where reduced chemotaxis was observed in macrophages from rats allowed to recover in room air for 30 days after dust exposure, by which time there were no PMN remaining in the BAL. Interestingly, there was also decreased chemotaxis of alveolar macrophages from rats exposed to coal mine dust for 7 days and then allowed to recover for a further 30 days. In these animals there was no evidence of PMN or macrophage recruitment to the bronchoalveolar region at any time. This suggests that the changes that occur to alter macrophage chemotactic responses after mineral dust exposure are subtle and can occur in the absence of overt inflammation. The failure of TiO_2 to elicit any such changes in macrophage chemotaxis suggests that the effect may be due to a direct interaction between toxic dust particles and the macrophages. However, we have shown that *in vitro* exposure to dust does not alter chemotaxis (*8*), and so a direct effect of the dust *in vivo* is unlikely.

One of the interesting findings of the present study was that, although quartz caused more inflammation than coal mine dust, there was no significant difference in the extent of the chemotaxis deficit between the quartz and coal mine dust-elicited leukocytes. Taken together with the reduced chemotaxis of leukocytes before the onset of bronchoalveolar inflammation in rats exposed to coal mine dust, this suggests that a factor similar to migration inhibition factor may be released as part of the early response to any toxic mineral dust.

In this study, bronchoalveolar macrophage numbers decreased on cessation of exposure to coal mine dust and by 60 days had returned to normal control levels. Impaired chemotaxis was therefore not sufficient to fully abrogate macrophage clearance. However, the delay in clearance may be of importance in the development of pneumoconiosis. Activated alveolar macrophages release proteases that can damage the alveolar septum (*5*). In addition, we have demonstrated recently that inflammatory macrophages from dust-exposed rats secrete increased amounts of interleukin 1 (*13*) and tumor necrosis factor (Brown et al., manuscript in preparation). These cytokines can generate chemotaxins and cause increased recruitment of inflammatory leukocytes to the alveolar region. They can also stimulate proliferative responses in mesenchymal cells. The delayed removal of inflammatory macrophages may thus contribute to the persistence of inflammation in the bronchoalveolar region of the lung and in the long term may play a role in the pathogenesis of pneumoconiosis.

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