Antibody producing cells in the spleens of mice treated with pathogenic mineral dust

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ABSTRACT Experiments were carried out to assess the effect of intraperitoneal injection of the mineral dusts, titanium dioxide, quartz, or asbestos, on splenic lymphocyte antibody forming cells in immunised mice. Titanium dioxide and quartz caused similar, about one third, reductions in plaque forming cells; asbestos caused substantial reduction to about a quarter of the number found in control spleens. The inhibition of antibody forming cells in the spleen found with chrysotile was dose dependent and both chrysotile and crocidolite asbestos were similar in activity. Systemic immunomodulation after local deposition of mineral dust may be important to the development of disease.

Asbestos and quartz (silica) are two important mineral dusts, exposure to which is associated with the development of pulmonary disease in man.¹ In the case of quartz a nodular fibrosis (silicosis) develops, whereas with asbestos, diffuse interstitial fibrosis, tumours, and various forms of pleural disease arise. Leukocytes are considered to play an important part in these pathological processes^{2,3} and the immune system is modulated in man⁴⁻⁶ and animals^{7,8} exposed to pathogenic dusts. We have ourselves studied the role of dust elicited leukocytes in inflammatory and immune responses after experimental treatment with quartz and asbestos dusts. These studies have shown inflammatory cell recruitment to sites of dust deposition,^{9,10} production of inflammatory mediators,¹¹⁻¹³ and immunomodulatory factors¹⁴ by these cells; we have also reported modulation of splenic lymphocyte T cell responses and circulating levels of specific Ig.¹⁵ This latter parameter is an indirect measure of B cell function and in the present paper we set out to directly measure plaque forming cells (PFC) in the spleens of mice treated with the pathogenic mineral dusts, quartz and asbestos. A control dust titanium dioxide that is low in activity in biological systems^{9,10} was also used.

Materials and methods

ANIMALS

Syngeneic, CBA, and C57B16 mice were used throughout the study.

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DUSTS

The following dusts were used: titanium dioxide (TiO₂, rutile form; Tioxide Limited, Stockton on Tees), UICC chrysotile asbestos standard sample 'A', and DQ₁₂ quartz.

TREATMENT

Groups of three to eight mice were injected intraperitoneally with 0.5 ml of sterile phosphate buffered saline containing 2.5 ml of dusts; occasionally lower doses of dust were used. Control mice were injected with 0.5 ml of sterile saline alone.

ASSESSMENT OF SPLENIC PFCs

Plaques formed by antibody producing cells in spleens were determined according to the method of Mishell and Dutton.¹⁶ Groups of mice were immunised by intraperitoneal injection with 0.2 ml of 10% sheep red blood cells (SRBCs). Spleens were removed from immunised mice four days later and 0.1 ml (10⁵ cells) of splenic cell suspension were mixed with 0.1 ml guinea pig serum (preabsorbed with SRBCs) as a source of complement. This was added to the following mixture and kept at 45° C: 0.5 ml 10× concentrated Hanks balanced salt solution, 0.05 ml FCS, 0.4 ml 0.5% agarose, and 0.05 ml 20-30% SRBCs. The mixture was poured on to slides, allowed to gel, and then incubated at 37° C for two hours to allow plaques to form which were counted visually. Mice were treated with 2.5 mg of mineral dust three days before immunisation.

Table 1 Inflammatory responses in the peritoneal cavity four days after injection of saline or dust (2.5 mg). Data given as $\bar{x}$ (SEM) of total numbers of leukocytes ($\times 10^6$) and $\bar{x}$ (SEM) of percentages. (Three mice in each group)

Dust	Percentage			Total No
	Macrophage	Neutrophil	Lymphocyte	
Saline	93.6 (3.0)	0.5 (0.3)	5.5 (0.6)	4.1 (1.0)
TiO ₂	97.0 (2.6)	1.7 (2.1)	1.3 (0.6)	7.0 (2.5)
Quartz	70.3 (10.9)	23.5 (9.4)	5.3 (0.5)	11.4 (2.0)
Chrysotile	63.0 (8.5)	34.5 (9.2)	2.5 (0.7)	14.2 (1.2)
Crocidolite	64.3 (9.0)	33.3 (10.8)	2.1 (2.0)	27.9 (3.3)

Significant increase ($p < 0.1-0.001$) in total numbers of cells and percentage of neutrophils, with quartz, chrysotile, and crocidolite.

INFLAMMATORY RESPONSE IN THE PERITONEAL CAVITY

Inflammatory response in the mouse peritoneal cavity was assessed on cells lavaged using 3×2 ml volumes of phosphate buffered saline containing 10 units/ml of

Fig 1 Number of plaque forming cells (PFC) per 10^6 splenocytes in mice injected with 2.5 mg of indicated dusts three days previously. Results represent mean $\pm$ SEM of five experiments, four mice in each experimental group. Significantly less PFC with quartz, TiO₂, and chrysotile compared with saline ($p < 0.01-0.001$); significantly less PFC with chrysotile compared with TiO₂ ($p < 0.001$); NSD between quartz and TiO₂.

Fig 2 Dose dependent decrease in number of PFC in spleens of mice treated with chrysotile asbestos. Results represent mean $\pm$ SEM of five mice in each treatment group shown for three separate experiments.

heparin, four days after injection; a volume of > 5 ml was invariably obtained. The total number of cells obtained from individual mice was counted in a Neubauer chamber and differential counts obtained from Diff-Quik (Merz-Dade, Duding, Switzerland) stained cytocentrifuge smears.

Results

INFLAMMATORY RESPONSE TO MINERAL DUSTS

Table 1 shows the inflammatory response in the peritoneal cavity to dusts injected at the doses used for studying PFCs in spleen. These showed that TiO₂ had no inflammatory effect other than slight increase in macrophage numbers. Quartz and the two types of asbestos produced substantial amounts of inflamma-

Table 2 Effect of intraperitoneal crocidolite or chrysotile on antibody producing cells in the spleen three days after injection

Saline	Treatment*	
	Chrysotile	Crocidolite
1327 (229)†	539 (266)	589 (191)

*0.5 ml of saline or saline containing 2.5 mg of crocidolite or chrysotile asbestos.

† $\bar{x}$ (SEM) of plaque forming cells per 10^6 splenocytes. Significant ($p < 0.01$) reductions with asbestos compared with saline alone.

tion at day 4, as assessed by increased macrophages and neutrophils; crocidolite asbestos produced more inflammation than quartz or chrysotile.

PFCs IN THE SPLEENS OF RATS TREATED WITH MINERAL DUST

In fig 1 the results from five different experiments show that a single intraperitoneal injection of TiO_2 or quartz both produced about one third reductions in the numbers of splenic PFCs. By contrast, chrysotile caused a far greater reduction than both TiO_2 and quartz, down to about a quarter of the number found in control immunised spleens.

DOSE RESPONSE OF INHIBITION OF PFCs CAUSED BY CHRYSOTILE

Figure 2 shows that, in three different experiments, there was a pronounced dose response in the inhibition of splenic PFCs caused by injection of chrysotile asbestos.

COMPARISON OF CHRYSOTILE AND CROCIDOLITE ASBESTOS IN CAUSING REDUCTION IN SPLENIC PFCs

Table 2 shows the results of a single experiment where equal doses of crocidolite or chrysotile asbestos were used and shows similar reductions with both asbestos types in the number of PFCs present in the spleens of the injected mice.

Discussion

These experiments have shown that after exposure to mineral dust by single intraperitoneal injection the number of antibody producing cells in the spleens of immune mice is reduced. This reduction was caused by both TiO_2 , a non-pathogenic dust, a fibrogenic dust quartz, and asbestos which is both fibrinogenic and carcinogenic. The decrease, however, was much greater with both chrysotile and crocidolite asbestos than with quartz or TiO_2 . The ability of the dusts to cause this type of immunosuppression was not simply related to their ability to cause inflammation at the site of deposition, since quartz and chrysotile asbestos caused much more inflammation than TiO_2 , yet

chrysotile was much more immunosuppressive than quartz. Additionally, the immunosuppression caused by chrysotile and crocidolite was similar although crocidolite caused more inflammation at the time studied.

The inhibition of PFCs found with the inert dust TiO_2 is in keeping with several previous studies showing that inhalation exposure to a particulate load of relatively inert dust, such as fly-ash¹⁷ or olivine, can cause modulation of immune function. Previous work from our own laboratories suggest that intraperitoneal TiO_2 has little inflammatory effect⁹ or effect on splenic lymphocyte responses to T cell mitogens.¹⁵ The present study has shown inhibition of specific humoral immune response, as assessed by PFCs in spleens, on injection of TiO_2 . This indicates that a complex systemic immunomodulatory effect is present in the spleen of mice exposed to TiO_2 which may involve induction of suppressive T cells in the complex environment of the spleen.

With quartz our previous studies have shown that intraperitoneal exposure causes inhibition of mitogenic T cell responses.¹⁵ In inhalation and intraperitoneal studies quartz has been reported to cause suppression of humoral and cellular immune responses both locally and systemically (in the spleen).⁷ Splenic PFC responses in mice inhaling quartz and the non-toxic dust olivine have also shown reductions in immune responsivity with both quartz and the non-toxic dust; however, the suppression was greatest with quartz.¹⁸

With chrysotile asbestos our previous studies have shown reduction in serum antibody response after both intraperitoneal and inhalation exposure.¹⁵ By contrast, epidemiological studies on workers exposed to asbestos have tended to show suppression of T cell immunity with a slight adjuvant effect on humoral immunity.^{5,19,20} These findings in exposed workers are in contradiction to the inhibition of antibody forming cells in the spleens of mice treated with asbestos by intraperitoneal injection. The present study, however, was carried out three days after exposure to a large dose of the mineral and experiments carried out using eight day exposures showed a substantial decrease in the inhibitory activity of the asbestos on splenic PFC number (data not included). The responses shown here may therefore represent an acute response to asbestos deposition whereas chronic exposure may well result in a different pattern of change in the systemic immune system.

The experimental model used here could be criticised on the basis that the immunogen was deposited in the peritoneal cavity where dust induced inflammation had already been caused. This, however, mimics the situation in the lung where deposited mineral dusts such as asbestos and quartz cause alveolitis with

leukocyte recruitment.¹⁰ The aim of the present study was to examine the effect of just such an inflammation on the response to any immunogen depositing in the lung. The model, therefore, is an appropriate one for studying immune responses imposed on mineral dust induced inflammation in the peritoneal cavity.

The immune response modifier which is responsible for affecting splenic lymphocyte responses may be assumed to pass from the peritoneal cavity, where the dust is deposited, to the spleen itself. Although this could be dust itself, which in the case of amosite can directly inhibit Ig production by B cells *in vitro*,²¹ there is no convincing evidence that dust is transferred to the spleen in large amounts. Evidence that the signal for immunomodulation may be produced in the spleen itself has come from studies where we injected a lysate of washed splenocytes from quartz exposed rats into naive recipients and assessed their splenic mitogen responses. This showed that splenocytes from dust exposed rats, but not control rats, released factors causing profound immunosuppression.²² Leukocyte traffic from the peritoneal cavity could be responsible and many products, now known to be released by dust exposed leukocytes, could be important in causing immunomodulation. Macrophages from rats exposed to silica⁸ and asbestos²³ are reported to produce increased amounts of Interleukin-1; this would, however, be expected to produce enhanced B cell responses⁸ rather than the inhibition evident in the present study. Other potentially important leukocyte products with ability to affect lymphocytes and known to be released by dust treated macrophages are reactive oxygen intermediates,¹¹ proteases,¹³ and prostaglandins.²⁴ A low molecular weight inhibitor of splenocyte mitogenesis released from dust exposed peritoneal macrophages¹³ may be of particular importance.

Other potentially important substances which could have systemic effects are the acute phase proteins. We have shown that these are found in greatly raised amounts in the serum of mice exposed to asbestos, compared with those exposed to TiO₂.²⁵ Furthermore, the levels produced by quartz were similar to those produced by TiO₂ (data not published) mimicking the pattern of response found here for immunosuppression in the spleen.

The results have shown no difference between chrysotile and crocidolite asbestos in causing immunosuppression, although crocidolite caused more inflammation, at the time studied, than chrysotile.

The implications of the present study are, of course, that inhaled dust causes important immunomodulation systemically which could be important for development of dust related disease or responses to other substances—for instance, infectious agents. In

particular, the immunosuppression associated with the carcinogenic asbestos fibres could be important.

The peritoneal model differs from inhalation with regard to (a) site of deposition and (b) single hit nature of the stimulus in the peritoneal model compared with the multi-hit nature of daily inhalation exposure. Further work is clearly warranted to determine the importance of the immunomodulatory effects described here and of the peritoneal cavity as a site to study the effect of dusts on the immune system.

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