Inflammation in the lungs of rats after deposition of dust collected from the air of wool mills: the role of epithelial injury and complement activation

K Donaldson, Geraldine M Brown, D M Brown, Joan Slight, R T Cullen, R G Love, C A Soutar

Abstract
In a previous study assessing respiratory symptoms in individuals employed in wool textile mills in the north of England relations between symptoms of chronic bronchitis, breathlessness and wheeze, and rhinitis and current exposure to airborne mass concentration of dust were shown. As preliminary steps in defining the potential hazard associated with dust from the air of wool mills the ability of inspirable dust, collected from the air of wool textile mills, to cause inflammation when injected into the lungs of rats was determined. Dusts were collected from the beginning of wool processing (opening) in one factory and from the middle (combing) and late (backwinding) stages of the process in two other factories. Ability of the dusts to cause inflammation was assessed by instillation into the lungs of rats followed by bronchoalveolar lavage. All the dusts caused some inflammation which peaked on day 1 and did not persist beyond one week. A distinctive aggregation response of mononuclear cells in the lavage, however, had a different time course, peaking at day 7. An attempt was made to determine how the wool mill dusts caused inflammation and experiments showed that the dusts themselves had no inherent chemotactic activity but that they did have a pronounced ability to generate chemotaxins in serum and so could activate complement in lung fluid. In addition, dust collected from ledges in the mills had the ability to injure epithelial cells in vitro which could also contribute to inflammation. A role for endotoxin in the inflammatory activity of the dusts was not discounted and a leachate of the dust had the ability to cause inflammation when injected into the lungs of rats. Wool mill dust is likely to be a complex mixture of materials and these experiments represent a preliminary approach to understanding the biological activity of the whole unfractionated dust and further studies are in progress to define more accurately the toxic material(s) in the dust.

The Institute of Occupational Medicine studied the prevalence of respiratory symptoms in individuals employed in wool textile mills in the north of England. Clear relations have been shown between current exposures to airborne mass concentrations of inspirable dust in these mills and the prevalence of symptoms, such as chronic bronchitis, breathlessness and wheeze, rhinitis, and conjunctivitis, suggesting that inhalation of the dusts causes inflammation in the upper and lower respiratory tract. Complementary studies of the lung functional and radiographic effects of occupational exposure to wool mill dusts have also been carried out. As an adjunct to the epidemiological studies on symptoms, respirable dust was collected from the air of selected wool mills with the objective of testing the dusts in rat lung to measure their ability to cause inflammation. Similar in vitro studies have been carried out on other organic dusts associated with bronchitic symptoms. These have shown evidence of chemotactic activity, complement activating activity, and epithelial injuring activity in cotton dust components and chemotactic activity, complement activating activity, and ability to induce neutrophil chemotaxin release from macrophages in grain dust.

The present paper reports preliminary work aimed at defining the biological activity of unfractionated respirable wool mill dust and describes the leukocyte responses, assessed by bronchoalveolar lavage, to the deposition of the dust or soluble factors thereof in the lungs of laboratory rats. The ability of the wool mill dust to activate complement and cause epithelial injury has also been shown and this may explain, in part at least, how wool mill dust engenders inflammation in the lung experimentally and causes inflammation of the respiratory tract in exposed workers.
Materials and methods

Rats
Syngeneic, PVG rats, SPF maintained, were obtained from the Institute of Occupational Medicine breeding unit.

Dusts
Dust samples were collected using IOM static inspirable dust samplers from the air of three mills chosen to represent the start (S), middle (M), and end (E) of the industrial processing of wool in the vicinity of opening (carpet yarn), combing (worsted process), and backwinding (card yarn) processes respectively from three mills in west Yorkshire. Each process was sampled in a different mill (in other IOM reports these are referred to as mills 15, 4, and 11 respectively). These samples, on filters, were sent by post to the Institute of Occupational Medicine where they were stored at −20°C for up to six months before use. Samples from each mill were removed from the filter with a brush, pooled, and coarse material was removed by hand. The remaining pooled samples of fine dust were mixed overnight on a rotating mixer to ensure homogeneity and stored at −20°C. Samples of the three wool dusts were then irradiated at 150 KRad at the National Engineering Laboratory, East Kilbride, to reduce the number of live contaminating fungal spores and bacteria. Non-irradiated samples of each dust were retained as a reference. Titanium dioxide (TiO₂; rutile form; Tioxide Limited, Stockton-on-Tees) was used as an inert control dust.

Intratracheal injection
Wool dusts and TiO₂ were prepared at 5 mg/ml in sterile phosphate buffered saline (Gibco, Paisley) and 0.5 ml (2.5 mg) aliquots were introduced into the lungs of groups of three rats. Instillation was carried out by surgical exposure of the trachea in anaesthetised rats followed by direct injection of dust suspension into the lung through a small incision in the trachea; the overlying skin was then closed with metal clips. Animals were conscious within two minutes and completely recovered within hours; no animal died after injection of the dusts. Rats were kept for one, three, seven, or 14 days at which point the experimental group was killed and the bronchoalveolar space lavaged.

Bronchoalveolar lavage
To assess inflammation, three rats were killed by Nembutal (Ceva, Watford) overdose and the lungs were lavaged with 4 × 8 ml volumes of saline at 37°C. The cells were concentrated by centrifugation, counted, and the differential count carried out on a May-Grunwald Giemsa-stained cytospin (Shandon Runcorn) preparation. From these, the totals of each cell type were calculated. Data is presented as mean and standard error of macrophages, neutrophils, and lymphocytes.

Soluble products (leachate) from wool mill dust
A leachate was prepared, using sterile pyrogen free distilled saline, from dust M (5 mg/ml) by mixing overnight in a rotating mixer; the particulate fraction was centrifuged out to yield the soluble components, or leachate, of the dust. This was injected as 0.5 ml (equivalent to 2.5 mg of dust) as described above.

Endotoxin
Endotoxin (Gibco) was injected intratracheally into the lungs of a group of three rats as 2.5 μg in 0.5 ml of saline and the lungs lavaged three days later.

Activation of chemotaxis in serum by treatment with wool dust
Rat serum (10%) was treated with wool dust M (1 mg/ml), mixed in a water bath at 37°C for 30 minutes, and then heat inactivated (56°C for 30 minutes). Previous studies have shown that treatment of serum with particulate in this manner causes activation of complement to yield the chemotactic anaphylatoxin C5a.

Chemotaxis
Chemotaxis was carried out using conventional Blindwell chambers. Zymosan activated serum (2 μl) or serum treated with wool dust, at 5%, or leachate of wool mill dust was placed in the lower compartment of a Blindwell chamber and a 5 μm pore filter (Nuclepore, Pleasanton, California) was placed on top. In the upper compartment were placed 400 μl (6 × 10⁶ cells) of control bronchoalveolar lavage cells (>95% macrophages). Blindwell chambers were then incubated at 37°C in 5% CO₂ for 3-5 hours. Filters were removed and stained with Diff-Quik and the number of migrated cells assessed by high power light microscopy. Results are expressed as migrated cells/high power field (mean of five fields on two filters—that is, n = 10). To test for chemokinesis a modified "checkerboard" analysis was performed with 5% zymosan activated serum in both the upper and lower compartments.

Assay of injury to epithelial cells in vitro
The assay is that described previously. Briefly, cells of the alveolar epithelial cell line A549 were prelabelled with ⁵¹Cr (Amersham International, Amersham) by incubating overnight in microtitre plate wells at 5 × 10⁴ in 100 μl of MEM + 10% fetal calf serum (Gibco, Paisley). To these plates were added varying dilutions of a leachate prepared, as described above, from fine ledge dust obtained from wool mills M and S, to a total volume of 200 μl. Ledge dust was used because all the respirable...
Inflammation in the lungs of rats after deposition of dust collected from the air of wool mills

material was used for the above experiments and ledge dust was therefore the best alternative to airborne dust; it was stored as for the airborne dust. In some experiments dust was leached into lung lavage fluid as well as medium to determine whether leaching was likely to occur in the milieu of the lung. After four hours of incubation at 37°C in 5% CO₂ the wells were sampled to obtain the counts released from lysed (dead) cells and counts within cells which had detached from the substratum.

STATISTICAL ANALYSIS

Results were analysed by analysis of variance and differences between treatment groups tested for statistical significance using a Student's t test.

Results

BRONCHOALVEOLAR LEUKOCYTE RESPONSE TO DEPOSITION OF WOOL DUST

Figure 1 shows the kinetics of the bronchoalveolar leukocyte response to the injection of 2.5 mg of dust from three different wool mills and TiO₂. With two of the dusts the macrophage counts did not increase substantially, although with dust S the numbers were significantly higher than the TiO₂ response. In the case of dust M₃, however, there was pronounced recruitment on days 1 and 3. Neutrophil response shows clearly that all four dusts caused some degree of inflammation on day 1 with the following order of activity: M > S > E > TiO₂. By day 3 all dusts were producing low levels of neutrophil influx and by days 7 and 14 levels approached 0 the norm for control rats.

The lymphocyte response was in keeping with the general pattern of the leukocyte response but it was noticeable that by day 14 the lymphocytes present in wool exposed lung exceeded those present in TiO₂ exposed lung.

The total number of cells present in the lavage is not completely reflected in the free cells described above in view of the fact that a small proportion of

![Graphs showing leukocyte response](https://via.placeholder.com/150)

Figure 1  (a) Mean number of macrophages lavaged from lungs of three rats injected with 2.5 mg of indicated dusts; bars represent SEM. Significant (p < 0.05 – 0.01) increases compared with TiO₂ with following; one day M, S; three days M. (b) Mean number of neutrophils lavaged from lungs of three rats injected with 2.5 mg of indicated dusts; bars represent SEM. Significant (p < 0.05 – 0.01) increases compared with TiO₂ for following; one day M, S; three days M, S. (c) Mean number of lymphocytes lavaged from lungs of three rats injected with 2.5 mg of indicated dusts. Significant increases compared with TiO₂; three days M, S; seven days S, E.
In all cases the number of aggregates had fallen substantially by day 14 except in the case of dust M where the number remained high.

ROLE OF DIFFUSIBLE COMPONENTS IN THE WOOL DUST
To test whether soluble components of wool dust were diffusing from the particles and causing inflammation, a particulate free leachate of dust M (2.5 mg of dust equivalent) was injected intratracheally into the lungs of rats and the rats lavaged three days later. As shown in table 1 some, but not all, of the inflammatory potential of whole wool dust could be reproduced with a particle free extract, strongly suggesting that soluble factors do have a role in causing inflammation. It was noticeable, however, that these diffusible components did not have the ability to produce clumps.

Endotoxin (2.5 μg) caused only moderate neutrophil recruitment and had little effect on macrophage recruitment (table 1).

EFFECTS OF IRRADIATION ON THE ABILITY OF WOOL DUST TO CAUSE INFLAMMATION
Irradiation of wool mill dusts did not substantially affect their ability to generate inflammation in rat lungs. The irradiated dusts did, however, cause slightly more macrophage and neutrophil recruitment than the non-irradiated: results given are (mean ± SEM) of cells × 10^6 for groups of three rats, three days after instillation—non-irradiated macrophages 4.5 ± 0.5; neutrophils 3.7 ± 0.7; irradiated macrophages 7.8 ± 1.1, neutrophils 5.1 ± 0.9.

GENERATION OF CHEMOTAXINS BY WOOL DUST
As shown in table 2 the leachate of pooled dust M had no chemotactic activity so precluding the possibility that products released by the wool dust itself were directly attractive to leukocytes and could contribute to the recruitment of inflammatory cells. As shown in table 3, however, wool dust was highly active in generating chemotaxins in rat serum and, by analogy, in alveolar fluid. The modified “checkerboard” analysis (table 3) showed that although some

Table 1  Inflammation assessed by bronchoalveolar lavage three days after intratracheal instillation of soluble components derived from wool dust M and by endotoxin. Significantly (p < 0.001) increased numbers of both macrophages and neutrophils with all treatments compared with saline

<table>
<thead>
<tr>
<th>Cells (× 10^6)</th>
<th>Macrophages</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2.6 (0.4)*</td>
<td>0.1 (0.0)</td>
</tr>
<tr>
<td>Leachate (equiv to 2.5 mg wool dust M)</td>
<td>7.2 (0.7)</td>
<td>1.3 (0.4)</td>
</tr>
<tr>
<td>Wool dust M (2.5 mg)</td>
<td>10.3 (1.2)</td>
<td>3.0 (0.4)</td>
</tr>
<tr>
<td>Endotoxin (2.5 μg)</td>
<td>5.5 (0.5)</td>
<td>0.6 (0.1)</td>
</tr>
</tbody>
</table>

*SEM; m = three rats.

 Donald, Brown, Brown, Slight, Cullen, Love, Soutar
chemokinetic activity was present in the activated serum most of the migration was true chemotaxis.

EPITHELIAL INJURY CAUSED BY DUST FROM WOOL MILLS

Figure 3 shows that the leachate from wool mill ledge dust was capable of causing direct epithelial injury of both the lytic and detachment type. Lysis was present to the greatest extent in neat and 1/10 dilutions whereas detachment was present at all concentrations even down to 1/100, with some samples of dust E showing pronounced activity at this dilution. Detachment and lysis were also caused, whether the dust was leached into fluid lavaged from rat lung or into medium. On the whole, dust M caused more measurable detachment to epithelial cells than dust E with an average of 5.1-fold more detachment with undiluted leachate compared with control (p < 0.05); dust E by comparison showed an average of 2.1-fold more detachment than the medium control. Detachment is underestimated in cases where there are high concentrations of lysis as with dust E, since lysed cells are not available for detachment. Dust E was most toxic causing more lysis, 2.9-fold more than control with the undiluted leachate (p < 0.05) whereas this figure was 1.9 for dust M. Significant increases in detachment were also caused by dust E at 1/10 and 1/100 dilution (p < 0.05).

Discussion

The most obvious response in rat lungs to instilled wool mill dust was acute inflammation. The inflammation was short lived, since, despite the fact that a considerable insult of 2.5 mg of dust was used, the bronchoalveolar lavage cellular profile had returned to near normal by day 7. Presumably daily inhalation exposure to wool dust would result in chronic inflammation, though we have not investigated this. In addition to inflammation an immune response was suggested by the presence of aggregates of leukocytes, peaking at seven days after exposure. We have not observed this response with any of the mineral dusts we have tested previously using a similar assay system. A continuing immune response in the alveolar region could contribute to chronic inflammation through immune complex mediated injury in the case of humoral immunity or, in the case of a cell mediated immune response, lymphokine mediated inflammation. The presence of persistently, although modestly, increased free lymphocytes in the bronchoalveolar lavage tends to support the idea that the immune system is affected. The type(s) of cells comprising the clumps was not ascertainable but is to be the object of further study. A parallel study of the effects of wool dust on the immune system will be reported separately.

The inflammation caused by the wool dusts was not due simply to a phagocytic burden since TiO₂ was substantially less active than wool mill dust. We investigated two possible mechanisms whereby wool mill dusts could have generated inflammation; (a) endotoxin, or some other component within the dust, could possess chemotactic activity or directly activate the complement cascade to generate chemotaxins; (b)

Table 2  Chemotactic activity in wool dust M leachate. No significant increase in chemotaxis with leachate compared with saline alone

<table>
<thead>
<tr>
<th>Chemotaxis (Migrated cells/high power field)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Wool dust leachate</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>0.4 (0.7)*</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>1.2 (1.2)</td>
</tr>
</tbody>
</table>

*Zymosan activated serum.
†(SEM) of 10 high power fields on two filters.

Table 3  Generation of chemotactic activity by the action of wool dust on rat serum. Significant (p < 0.01) increase in chemotaxis with wool treated serum compared with control serum

<table>
<thead>
<tr>
<th>Chemotaxis (Migrated cells/high power field)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control serum</td>
<td>Wool treated serum</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>23.9 (3.2)*</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>31.9 (5.7)</td>
</tr>
</tbody>
</table>

*Zymosan activated serum.
†‡ (SEM) of 10 high power fields on two filters.
component(s) of the wool mill dust could injure epithelial cells. Wool mill dust samples possessed no intrinsic chemotactic activity but did activate complement and injure epithelial cells in vitro. The potential role of endotoxin in causing these inflammatory/immune effects in the lungs of workers and rats exposed to wool dust deserves attention because of its inflammatory activity and because endotoxin was present in other samples of wool mill dust collected from the air of the mills studied here.

Endotoxin may contribute to the respiratory symptoms observed in workers from a variety of industries exposed to airborne dusts and has been implicated in symptoms such as those of byssinosis in workers in a wool carpet factory in Turkey.

Some, but not all, of the inflammatory activity of the wool dust could be accounted for by soluble components that could be leached into saline. Some of this leached material could have been endotoxin but a relatively large dose of endotoxin (2.5 μg) was not very active in causing inflammation in the rat lung. This was a commercially obtained sample of endotoxin, however, and may not have been representative of the endotoxin in the samples of the wool mill dust. Endotoxin may also have been involved in the generation of chemotaxins in serum. Since alveolar fluid is, in part, a transudate of plasma and contains complement, then wool dust depositing in the lung could activate complement leading to inflammation.

Our finding that a soluble product from wool mill dust caused both lysis and detachment of epithelial cells indicates another possible mechanism by which wool dust could contribute to inflammation. Epithelial injury is a powerful stimulus to inflammation, since dead cells are chemotactic and exposed basement membrane activates complement.

The dust collected from the carding room (M) was the most active of the dusts in causing inflammation; the backwinding dust the least. Since the three dusts were collected from different factories we cannot be sure that these differences are related to the stages of the manufacturing process rather than the differences between the factories. Size distribution could be important in determining what mass of dust reaches the alveoli and is phagocytosed. In this respect the ranking of activity exactly follows the ranking of the dusts with regard to particle size. The proportions by mass of fine (respirable) dust in each sample were M—21.8%, S—16.6%, and E—10.4%. Since the dusts in this study were instilled, however, aerodynamic behaviour is unlikely to influence deposition of the dusts and increased particle number per unit mass in the finer dusts is a more likely explanation for the differences in inflammatory response.

This predominantly inflammatory response to wool mill dust is broadly consistent with what is known of dust related illness in wool textile workers. The symptoms we found to be associated with exposure to dust in workers in the industry included conjunctivitis, rhinitis, chronic bronchitis, and wheeze. There was little evidence that wool dust caused asthma, the most dramatic allergic reaction in the lung, and atopic individuals were not more susceptible to symptoms related to wool dust than other subjects. There was no evidence on the chest radiographs of lung fibrosis or alveolitis. Lung functional deficits have not so far been convincingly shown to be related to exposure to wool dust, though...
we do not consider this possibility to have been excluded.3

Thus the symptoms conform to what is called “non-specific” lung disease, analogous to the chronic bronchitis associated with smoking, air pollution,15 and exposure to coalmine dust16 and some other industrial dusts, including organic dusts such as grain dust.17 The mechanisms of this non-specific response are not well understood, but inflammatory cell recruitment has been reported in the lung lavage of subjects with chronic bronchitis18 and in smokers19 and rats exposed experimentally to coalmine dust.20 Experimental studies similar to those described in the present paper have been carried out with grain dust in view of its ability to cause bract extract has been found pattern of activity shown wool dust may be fibrosis, neutrophils to in the

The dust. 

emphysema) while wool

in the whole lung or (b) the ability to approach the alveoli while wool dust is inhaled regularly causing increased leukocyte activation (TiO2).

Inflammation in the lungs of rats after deposition of dust collected from the air of wool mills could cause epithelial injury in the airways or respiratory zone so contributing to inflammation. In addition complement could be activated by the dust, causing some recruitment via C5a and also coating the wool dust particles with C3b. Macrophages may thereby be stimulated through their complement receptors to release chemotaxins and other mediators leading to further leukocyte recruitment. A combination of epithelial injury generated inflammation and leukocyte recruitment via complement activation could lead to further epithelial injury due to the injurious effects of the leukocytes themselves. The involvement of the specific immune response could also be a factor in enhancing inflammation.

No attempt has been made to characterise this complex mixture of materials likely to be present in the dust but further work is in progress.

This research was funded by the Health and Safety Executive.


16 Rae S, Walker DD, Atfield MD. Chronic bronchitis and dust
Vancouver style

All manuscripts submitted to the Br J Ind Med should conform to the uniform requirements for manuscripts submitted to biomedical journals (known as the Vancouver style).

The Br J Ind Med, together with many other international biomedical journals, has agreed to accept articles prepared in accordance with the Vancouver style. The style (described in full in Br Med J, 24 February 1979, p 532) is intended to standardise requirements for authors.

References should be numbered consecutively in the order in which they are first mentioned in the text by Arabic numerals above the line on which each occasion the reference is cited (Manson¹ confirmed other reports² ₃ ...). In future references to papers submitted to the Br J Ind Med should include: the names of all authors if there are six or less or, if there are more, the first three followed by et al; the title of journal articles or book chapters; the titles of journals abbreviated according to the style of Index Medicus; and the first and final page numbers of the article or chapter.

Examples of common forms of references are: