Mast cells and inhalation of asbestos in rats

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ABSTRACT Mast cell counts were performed on sections taken from the lungs of rats exposed by inhalation to different UICC samples of asbestos fibres for periods ranging from a few days to two years. A comparison of mast cell counts with grades of fibrosis showed that there is a sevenfold increase when there is interlobular linking of the fibrotic lesions (grade 5). Submesothelial mast cells showed a trend of increasing numbers with increasing exposure and with increasing subpleural thickening. Each type of asbestos produced a steady increase in mast cell numbers with increasing exposure. Two samples from animals exposed to chrysotile and two from animals exposed to amphiboles (crocidolite and amosite respectively) had 10 times as many cells as the control group after six and 24 months' exposure. Another amphibole, anthophyllite, produced 50 times more cells than were present in the control specimen appropriate for the heaviest exposure. These results are briefly discussed in relation to further exposure, smoking, and characteristics of the dusts.

Inhalation of asbestos fibres is associated with production of interstitial pulmonary fibrosis in man and in animals. The role of different cells associated with inflammatory and immune processes and the order in which they appear has not been fully explored. The acute inflammatory response to a single intratracheal injection of chrysotile asbestos consists of neutrophil and eosinophil production in guinea pigs. After inhalation in rats it has been demonstrated that, firstly, alveolar macrophages are the most obvious cells in those alveoli arising from respiratory bronchioles. These cells, together with fibres, become "enmeshed in a thin reticulin network which coarsens with time and then become replaced by collagen." As more and more of these discrete lesions appear they link and coalesce.

There is a well recognised association between fibrosis and mast cells. Riley has pointed out that mast cells were described by Ehrlich in 1879 as disappearing in acute inflammation, and that they degranulate. Other authors noted that with the appearance of fibroblasts there is a progressive increase in the number of mast cells. This increase is seen in fibrotic conditions in human lungs with different pathogenesis. It has also been reported by several authors that the local mast cell population declines as the fibrous tissue becomes less cellular.

The increase in mast cells with fibrosis has been found in the fibrosis induced in rats by ionising radiation and hypoxia. Since the fibrosis that follows asbestos inhalation in rats has been graded we have undertaken a study of the number of mast cells appearing in relation to these grades, increasing exposure time, and fibre type and have investigated whether, after exposure has ceased, there is a decrease in the numbers of these cells. Mast cell mediators are responsible for and play a part in many inflammatory events, such as those concerned with vasoactive smooth muscle reactive mediators, proteolytic agents degrading ground substances, inhibition of complement, and chemotactic factors. Their presence therefore must influence the course of fibrosis in those exposed to asbestos.

Methods

This was a retrospective study performed on sections from lungs which had been fixed immediately after the animals had been killed. The specific pathogen free rats had been exposed to dusts by inhalation.

The Wistar rats had been exposed in inhalation chambers to dust clouds for seven hours a day five days a week. Five UICC (Union Internationale Contre le Cancer) standard reference samples were used—three of amphibole type (amosite, crocidolite, and anthophyllite) and two chrysotiles (A a Rhodesian and B a Canadian sample). There was
also a group of unexposed control rats. Animals were killed by chloroform, and the lungs were fixed with neutral buffered formalin. A pilot study did not show a loss of mast cells when a comparison was made between alcohol based and formalin based fixed tissue.

GRADING OF LUNG FIBROSIS
The basis for the grading of lung fibrosis was first described by Wagner et al., but an extension of this grading has been agreed internationally and the grades are now as follows: grade 1—normal lung; 2—evidence of dust inhalation in centrilobular macrophages; 3—minimal interstitial cellular reaction to the dust (macrophages containing fibres); 4—evidence of early interstitial fibrosis with discrete lesions, equivalent to those seen in early human asbestosis; 5—early interstitial fibrosis with discrete lesions, consistent with clinically recognisable asbestosis in man; 6—linkage present and collagen definitely increased; 7—most of the section affected by the interstitial fibrotic reaction; 8—whole section consisting of a dense fibrous tissue network in which occasional distorted alveoli are seen.

GRADING OF SUBPLEURAL FIBROSIS
The width of the subpleural area was measured on haematoxylin and eosin stained sections with an eyepiece graticule, calibrated by means of a stage micrometer. Pleural thickness was graded according to the following scale: A—2–5 μm; B—>5–9 μm; C—>9–14 μm; C/D—>14–18 μm (never observed); D—<18 μm. The thickening was patchy, and these measurements refer to the thickest areas observed.

MAST CELL IDENTIFICATION
Mast cell identification was achieved by the alcian blue-safranin method of Csaba. All the mast cells were counted in each section, with two separate subtotals—(a) for those associated with the lung interior (that is, parenchyma, blood vessels, and bronchi) and (b) for those associated with the subpleural region (that is, the basement membrane of the pleura). The sections were examined “blind.”

Tissue was available from rats exposed to dust for the following periods: 1—two to three days; 2—two months; 3—three months (1–3 killed immediately); 4—six months and killed after a further 18 months; 5—24 months and killed immediately.

Rats which were found dead or were killed because of illness were not included as the mast cells were consistently well defined by the stain only in the well preserved tissue of sacrificed rats. In a pilot study we had found that loss of mast cells was associated with poor preservation rather than with the fact that the tissue was not fixed with alcohol.

SECTION MEASUREMENT AND SCORING OF OBSERVATIONS
The section was projected on to a screen first with a magnification of 5-45 to estimate the area by a planimetric method and then with a magnification of 10-55 to obtain the pleural length by means of a map measurer. Seven rats had some bronchiolar hyperplasia and four had carcinoma of the lung, and the affected regions in these rats were excluded from the measurements.

The mast cell counts were assumed to be proportional to the sectional area (length in the pleural case) and the error variability between rats was considered likely to be proportional to the expected response. A mast cell score was therefore derived, for which simple linear techniques would be approximately valid, by using the following logarithmic transformation:

\[ \log \left( \frac{M + \frac{1}{2}}{A} \right) \]

for a count M and sectional area A (in pleural case sectional length A).

The count was increased by half to allow for possible zero counts and to reduce the bias.

From the scores obtained for a group of rats the mean and standard error were calculated. In the tables the mean scores have been converted back to counts, applicable to an “average” lung section having an area of 3-14 cm² and a pleural length of 3-26 mm; these have been presented with the standard errors just referred to, which we have called proportional errors; the standard errors of the counts can be approximated by multiplying the counts themselves by the corresponding proportional errors (hence the term). For example (table 1), the cells for grade 1 fibrosis give an estimated (geometric) mean total lung count of 62, with a proportional error of 0.23; the product of these gives an approximate standard error of 14· and hence approximate 95% confidence limits of 34- and 90; calculating limits for the mean score (that is, log (62)) and transforming these limits back to counts yields the more valid estimated 95% confidence limits 39- and 98. Formal statistical testing has been limited because material could not be analysed from a large proportion of the original rats, who either had died before their term, in which case the material was unsuitable for staining, or had already been used for quantification of the dust.
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Results

DISTRIBUTION OF MAST CELLS
Normal distribution of mast cells was confined to the subpleural region, around bronchi and small blood vessels. In the exposed rats the increase in number of the mast cells was found within the interstitial fibrous lesions, although some were found in alveoli nearby. As fibrosis increased and fibrotic areas were found surrounding blood vessels and near to bronchi, it was no longer possible to distinguish those associated only with these latter structures—"lung interior" therefore consists of lung parenchyma, bronchi, and blood vessels. A thin rim of mast cells was occasionally distributed round both bronchial carcinoma and areas of bronchoalveolar hyperplasia. They were very rarely found infiltrating these lesions, and therefore the distribution was quite different from the distribution in the interstitial fibrosis.

FIBROSIS
The mast cells in the total lung sections showed a steady increase with the severity of fibrosis (table 1). Between grade 1 and grades 6–7 there was a 20 fold increase. There were insufficient rats for us to comment on the higher count for grade 6 than for grade 7 and the extremely high count for grade 8. In the interior of the lung there is at least a 100 fold increase from grade 1 to grades 5–8, with a sevenfold increase between the two adjacent grades of 4 and 5. This latter increase can be compared with the approximately fivefold increases observed between grades 2 and 4 and between grades 5 and 8 (although formal significance tests would be inappropriate here). This is highlighted when the percentage of the total lung mast cell counts, which are in the lung interior, are considered. This might suggest that with regard to mast cells the grades 4–5 group could be usefully expanded into finer divisions if an appropriate way could be found.

In contrast, the mast cells in the subpleural region show a small, steady increase with grade of interstitial fibrosis. A steady increase in these cells also occurs with grade of subpleural thickening, which is a more appropriate measure here (table 2). Only four rats showed the grade D fibrosis (three of these had been exposed to anthophyllite and one to chrysotile A, all for 24 months).

RESPONSE TO DIFFERENT DUSTS

Total lung (table 3) There is no consistent evidence to suggest that there is any difference between the two chrysotile samples, and in the comparisons made these two dusts have been considered together. The same applies to two of the amphibole dusts, amosite and crocidolite; but the third, anthophyllite, was completely different. There is a considerable rise in numbers of mast cells with increasing exposure to each dust (only one rat was exposed to amosite for six months). Up to three months, there is little difference between unexposed and exposed animals owing to increasing numbers of these cells with age. Among rats of the same age (24 months) but exposed to dust for different lengths of

Table 1 Mast cell counts* and grades of lung parenchymal fibrosis

<table>
<thead>
<tr>
<th>Grade of fibrosis</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of rats</td>
<td>20</td>
<td>32</td>
<td>16</td>
<td>19</td>
<td>11</td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Total lung</td>
<td>62</td>
<td>93</td>
<td>133</td>
<td>307</td>
<td>920</td>
<td>1636</td>
<td>1333</td>
<td>3975</td>
</tr>
<tr>
<td>Prop error</td>
<td>0.23</td>
<td>0.13</td>
<td>0.18</td>
<td>0.22</td>
<td>0.14</td>
<td>0.58</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Lung interior</td>
<td>9</td>
<td>24</td>
<td>40</td>
<td>115</td>
<td>770</td>
<td>1482</td>
<td>1174</td>
<td>3737</td>
</tr>
<tr>
<td>Prop error</td>
<td>0.32</td>
<td>0.27</td>
<td>0.26</td>
<td>0.47</td>
<td>0.16</td>
<td>0.62</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>% mast cells</td>
<td>14</td>
<td>26</td>
<td>30</td>
<td>38</td>
<td>84</td>
<td>90</td>
<td>88</td>
<td>94</td>
</tr>
<tr>
<td>Lung interior</td>
<td>38</td>
<td>44</td>
<td>69</td>
<td>108</td>
<td>187</td>
<td>352</td>
<td>233</td>
<td>381</td>
</tr>
<tr>
<td>Prop error</td>
<td>0.21</td>
<td>0.14</td>
<td>0.17</td>
<td>0.15</td>
<td>0.13</td>
<td>0.15</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

* Mast cell counts adjusted to refer to an average lung section of area 3-14 cm².
Prop err—proportional error: standard error of mast cell score (log (mast cell count +½, divided by sectional area)).

Table 2 Mast cell counts* and grades of subpleural fibrosis

<table>
<thead>
<tr>
<th>Grade of fibrosis</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of rats</td>
<td>39</td>
<td>41</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Subpleural</td>
<td>39</td>
<td>84</td>
<td>124</td>
<td>323</td>
</tr>
<tr>
<td>Proportional error</td>
<td>0.14</td>
<td>0.13</td>
<td>0.15</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*Mast cell counts adjusted to refer to an average lung section of pleural length 3-26 mm.
Standard error of mast cell score (log (mast cell count +½, divided by sectional length)).

Thorax: first published as 10.1136/thx.39.7.539 on 1 July 1984. Downloaded from http://thorax.bmj.com/ on November 1, 2023 by guest. Protected by copyright.
time, only the counts for anthophyllite appear to be of a different order.

Lung interior (table 4) As early as three months the anthophyllite exposed and the two chrysotile exposed rats showed five times more mast cells than the control rats and those exposed to the other two amphibole dusts. Among the rats killed at 24 months, those exposed to amosite and crocidolite for only six months show slightly more cells than those exposed for the full period, but for the animals exposed to the two chrysotile dusts the reverse was true. The increase for anthophyllite is again of a different order.

Subpleural region (table 5) Amosite and crocidolite exposure gave a small, steady rise from two months onwards, which was nevertheless greater than that for the chrysotile dusts. The 24 month old rats with both short and long exposure showed changes similar to those found in the lung interior. The third amphibole, anthophyllite, did not produce any rise with less than six months' exposure, and there was a bigger increase with the longer period than with six months' exposure.

Discussion

This study has shown that mast cells are associated with the interstitial fibrosis induced by asbestos fibres in rats, and it pinpoints the greatest increase in the interstitium as occurring at the time of transition from pathological evidence of minimal asbestosis to asbestososis associated with disease that is clinically apparent. Mast cells appear with hypoxia and after pulmonary oedema, either or both of which might be present locally with grade 5 fibrosis. Partial degranulation has been described in patients with fibrotic lung disorders, which might result in increased concentrations of histamine, as already noted in bronchiolar lavage fluid from patients with cryptogenic fibrosing alveolitis. Smoking can cause this degranulation, as can asbestos fibres in vitro (RE Edwards, unpublished observations). These cells release many other mediators, including proteases capable of activating procollagenase, with increased concentrations of collagenase possibly appearing in bronchiolar lavage fluid. Phagocytosis of these released granules by fibroblasts may be
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Table 5  Mast cell counts for subpleural area (standard errors in parentheses applying to mean log count—that is, the untransformed score)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Length of exposure with numbers of rats</th>
<th>n 2-3 days</th>
<th>n 2 months</th>
<th>n 3 months</th>
<th>n 6 months (+18 m)*</th>
<th>n 24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Control</td>
<td></td>
<td>3</td>
<td>31 (0-49)</td>
<td>4</td>
<td>29 (0-60)</td>
<td>5</td>
</tr>
<tr>
<td>2 Asbestos</td>
<td></td>
<td>4</td>
<td>22 (0-38)</td>
<td>3</td>
<td>73 (0-17)</td>
<td>2</td>
</tr>
<tr>
<td>3 Crocidolite</td>
<td></td>
<td>4</td>
<td>33 (0-15)</td>
<td>2</td>
<td>72 (0-31)</td>
<td>2</td>
</tr>
<tr>
<td>4 Anthophyllite</td>
<td></td>
<td>8</td>
<td>27</td>
<td>5</td>
<td>72</td>
<td>6</td>
</tr>
<tr>
<td>5 Chrysotile</td>
<td></td>
<td>12</td>
<td>26</td>
<td>2</td>
<td>33 (0-006)</td>
<td>12</td>
</tr>
<tr>
<td>6 Chrysotile</td>
<td></td>
<td>4</td>
<td>19</td>
<td>3</td>
<td>36 (0-32)</td>
<td>6</td>
</tr>
<tr>
<td>Agents 2, 3, 4</td>
<td></td>
<td>12</td>
<td>26</td>
<td>7</td>
<td>56</td>
<td>12</td>
</tr>
<tr>
<td>5 Chrysotile A</td>
<td></td>
<td>2</td>
<td>19 (0-06)</td>
<td>3</td>
<td>61 (0-32)</td>
<td>6</td>
</tr>
<tr>
<td>6 Chrysotile B</td>
<td></td>
<td>4</td>
<td>53 (0-55)</td>
<td>3</td>
<td>57 (0-52)</td>
<td>6</td>
</tr>
</tbody>
</table>

*Six months' exposure but killed at 24 months;
†The same five control rats are regarded as belonging to both 24 month groups.

associated with further recruitment of fibroblasts, and the fibres may inhibit the amount of collagen produced. The collagen laid down in areas of grades 4 and 5 fibrosis may be different types. Established scar tissue has been shown to be associated with type I collagen, and early active fibrosis (usually containing variable numbers of fibroblasts and chronic inflammatory cells) with an increased proportion of type III. Thus there are many reasons why those with moderate asbestososis may have disorganised collagen synthesis as a result of the release of mediators from mast cells caused by further exposure or by smoking.

With the exception of those for anthophyllite, these results in general are in agreement with those found with the grading—that is, there is little variation among the different dusts. Nevertheless, there are small differences which are consistent with the characteristics of the fibres. Chrysotile is associated with greater cytotoxicity and loss of lysosomal enzymes from macrophages than is crocidolite, for example. This would give rise to more widespread fibrosis and the recruitment of mast cells to the lung parenchyma, while leaching of the chrysotile fibres may be responsible for lower counts (compared with those for amosite or crocidolite) seen in rats exposed for six months and examined 18 months later. Anthophyllite, which tends to produce the increase later, the cell counts then rising so abruptly with the larger doses, is a more friable amphibole and probably has a greater diameter (V Timbrell, unpublished observations). The shorter fibres may at first be cleared from the lung, while the diameter size is associated with greater profusion (V Timbrell, unpublished observations), many more mast cells, and also pleural thickening. Clinical evidence in support of this comes from the Finnish anthophyllite mines, where there is much fibrosis and pleural plaques are very common.

The identification of other specific cells, the order in which they appear, and thus their relative importance in the production of fibrosis need to be investigated. Histological and immunohistochemical methods are now available to study many of these cells. In addition, the presence or absence of mast cells may assist in differentiating tumours and hyperplasia from fibrosis.

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References