Reactive pleural inflammation caused by intratracheal instillation of killed microbes

X.Y. Li*, G.M. Brown**, D. Lamb†, K. Donaldson**

ABSTRACT: To investigate the pleural leucocyte response to severe alveolar inflammation, heat-killed Corynebacterium parvum were instilled intratracheally into the lungs of PVG rats and pleural lavage was performed.

Polymorphonuclear neutrophils are not normally resident in the pleural space but were found transiently after intratracheal instillation of C. parvum. Macrophages increased gradually in the pleural space following instillation, reaching a peak at day 5. The activity of plasminogen activator inhibitor in the pleural leucocyte supernatants was increased at day 1, but returned to control levels by day 5. The activities of interleukin-1 and tumour necrosis factor secreted by pleural leucocytes were decreased compared with control pleural leucocytes at day 1 and were further reduced at day 5. The analysis of particle translocation showed that intratracheally instilled C. parvum and fluorescent beads were not retained. We hypothesize that pleural inflammation resulting from C. parvum-induced inflammation in the lung is the result of transfer of a diffusible factor from the adjacent parenchyma.

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Lung inflammation has been studied extensively, however, very few experimental studies have been carried out on pleural inflammation [1, 2]. The pleura is a structure adapted to form a lubricated surface that allows the lungs to expand and recoil without resistance, and to resist the accumulation of fluid despite negative intrapleural pressure. Pleural pathological changes, such as pleural effusions, pleural fibrosis and pleural mesothelioma may occur as a result of oral drug treatment, and also inhalation exposure to asbestos and other dusts [3, 4]. In a rabbit study, for instance, intravenous Ethchlorvynol caused a patchy, subpleural, haemorrhagic polymorphonuclear neutrophilic inflammatory response [5]. A reactive pleuritis may also occur when there is adjacent pneumonia in the underlying lung parenchyma. For example, a para-pneumonic effusion is a common complication of bacterial pneumonia [6]. Approximately 60% of patients with pneumococcal pneumonia and 40% of all bacterial pneumonias have associated pleural effusion [7].

Interleukin-1 (IL-1) [8] and tumour necrosis factor (TNF) [9] are cytokines which have effects on recruitment and activation of polymorphonuclear leucocytes (PMNs), on endothelial cell activity, fibroblast proliferation, macrophage activation and immune response. Plasminogen activator (PA) converts the abundant extracellular zymogen plasminogen into plasmin, an active protease that promotes degradation of all components of the extracellular matrix. Plasminogen activator inhibitor (PAI) [10] can influence cellular function and tissue remodelling by finely regulating the location and extent of PA-induced plasminogen activation. We have reported modulation of the production of these inflammatory mediators by pleural leucocytes following deposition of fibrous dusts in the bronchoalveolar space [11]. We previously described the inflammatory response of the lung parenchyma to a range of agents [12] and noted, in preliminary studies, that intratracheal Corynebacterium parvum caused a transient pleuritis.

In the present study, in an attempt to study reactive pleural inflammation caused by C. parvum pneumonitis, we measured the translocation of particles to the pleura following alveolar deposition. We also assessed the kinetics of the production of the cytokines, TNF and IL-1 by pleural leucocytes and determined secretion of PAI by pleural leucocytes during pleural inflammation.

Materials and Methods

Animals

Syngeneic, male and female PVG rats, aged 12 weeks or more, obtained from the Institute of Occupational Medicine Animal Unit, were used throughout.
**Intratracheal instillation of C. parvum and microspheres**

Anaesthetized rats were instilled intratracheally with 1.4 mg of heat-killed *C. parvum* (Wellcome, Beckenham, UK) in a volume of 0.2 ml. Bronchoalveolar and pleural lavage were carried out at intervals of 1–5 days thereafter. To study the possible transfer of particles from the alveolar to the pleural space, animals were intratracheally instilled with 0.4 ml of phosphate-buffered saline (PBS) containing 1:20 diluted monodispersed fluorescent carboxylated microspheres which have a geometric diameter of 0.770 μm (Polysciences Inc., Warrington, UK), since they are easily seen when viewed by UV illumination. The microspheres were injected together with *C. parvum* when required.

**Bronchoalveolar and pleural lavage**

Rats were sacrificed by intraperitoneal injection of Nembutal (Ceva Ltd, Watford, UK). The rat trachea was cannulated with a blunt, 18-gauge needle. The lungs were removed from the thorax and bronchoalveolar lavage was performed by instilling 8 ml of PBS at 37°C into the lungs via the cannula. PBS was then withdrawn after gentle massage and the entire procedure repeated 4 times.

Pleural lavage was carried out as follows: the rat peritoneal cavity was opened and the skin covering the right chest removed. A round-ended 8-gauge cannula was inserted through the chest, between the lower two ribs, into the pleural space, for injection of 5 ml PBS at 37°C into the space, monitored via the opened peritoneal cavity. The PBS was then withdrawn after massaging the rib cage and this procedure was repeated on four occasions.

The cell suspensions were spun at 1,000 rpm for 5 min and resuspended in F10 medium (Gibco, Paisley, UK) containing 2% bovine serum albumin (BSA). (Sigma, Poole, UK). Cell viability, as judged by trypan blue exclusion, was normally more than 95%. For differential counts, cell suspensions were prepared as cytocentrifuge smears and then fixed and stained with May Grünwald-Giemsa stain.

**Mesothelial cell characterization**

To determine whether there were significant numbers of mesothelial cells present in the pleural leucocyte populations, a monoclonal antibody to rat leucocyte common antigen (CD45) (Antibody MRC OX-1, MCA 43, Serotec, Oxford, UK) was used. Pleural lavage cells obtained from control rats and rats at 16 h and 5 days after intratracheal instillation of *C. parvum* were stained with the antibody. The second antibody was a fluorescein-conjugated F(ab')2 fragment of rabbit immunoglobulins raised against mouse immunoglobulin (F313, Dako Ltd, High Wycombe, Bucks, UK). The fluorescence was determined in a Coulter Flow Cytometer (Epics-Profile II, Coulter Electronic Ltd, Luton, Beds, UK).

**Observation of beads and C. parvum in bronchoalveolar and pleural lavage leucocytes**

Following instillation of fluorescent beads, 100 PMNs and macrophages of cytospin preparation of bronchoalveolar and pleural leucocytes were observed on each smear by light microscopy under epi-fluorescent illumination. The macrophages and PMNs from both lavaged populations were divided into two groups: with or without beads. In the group with beads, the cells were divided into three subgroups: cells containing ≤5 beads, cells containing 6–10 beads and cells containing >10 beads. During bead counting, a differential count was also carried out.

In addition, following *C. parvum* exposure alone, the lavaged leucocytes were also examined for intracellular organisms. Both macrophages and PMNs were divided into one of two groups: with or without intracellular organisms.

**Preparation of supernatants**

Pleural leucocytes were adjusted to a concentration of 1x10^6·ml⁻¹ in F10 medium + 2% BSA and cultured for 24 h at 37°C in a 5% CO₂ atmosphere. For leucocyte stimulation, lipopolysaccharide (LPS) (Sigma, Poole, UK), was added to the culture at 100 ng·ml⁻¹. The supernatants were collected and centrifuged at 3,000 rpm for 10 min to remove cell debris, then stored at -70°C prior to assay.

**Assay for TNF activity**

The assay was performed according to the method of Warner and Libby [13]. In brief, murine L929 cells grown in minimal essential medium (MEM) (Gibco, Paisley, UK), containing 5% foetal calf serum (FCS) were trypsinized and suspended in assay medium (MEM + 5% FCS, no antibiotics), 100 μl aliquots containing 3x10⁶ cells were added to each well of microtitre plate and incubated at 37°C for 24 h. The supernatant was then discarded, and 100 μl of assay medium containing 1 μg·ml⁻¹ actinomycin D (Sigma, Poole, UK), 50 μl of pleural leucocyte supernatants and 50 μl of assay medium with 2 μg·ml⁻¹ of actinomycin D was added to experimental wells; then double diluted. Following further 18–20 h incubation, the wells were emptied, stained with crystal violet methanol solution, and then washed with water and allowed to dry. Optical density at 540 nm was determined with a MR650 plate reader (DYNATECH Laboratories Inc., USA). TNF activity in the supernatant was determined by comparison with a TNF-alpha standard (a gift kindly supplied by J. Symonds, Dept of Rheumatology, Northern General Hospital, Edinburgh, UK) dilution curve.
Assay for IL-1 activity

IL-1 activity in the supernatant was quantified using the C3H/HeJ mouse thymocyte assay [14]. Briefly, thymus glands of mice killed by ether were removed and single cell suspensions of thymocytes were prepared by homogenization. The cells were washed and suspended at $6 \times 10^6$ ml$^{-1}$ in RPMI-1640 medium (Gibco, Paisley, UK) with 10% FCS and 20 µM 2-mercaptoethanol (Sigma, Poole, UK). One hundred µl of the cell suspension was added to each well, together with 50 µl of RPMI medium containing 20 µg ml$^{-1}$ of phytohaemagglutinin (PHA) (Sigma, Poole, UK) and 50 µl of dilutions of test pleural leucocyte supernatants (PLS). The cells were then incubated at 37°C in 5% CO$_2$ for 48 h and pulsed with 0.25 µCi 3H-methyl-thymidine per well (Amersham International, Amersham, UK). After one more day of culture, the cells were harvested onto glass filters, and 3H incorporation was quantitated by scintillation spectrophotometry.

Assay for plasminogen activator inhibitor activity

PAI activity was assayed in 125I-fibrin coated tissue culture wells as described previously [15]. Briefly, dissolved fibrinogen (Sigma, Poole, UK) was labelled with Na$^{125}$I (Amersham International, Amersham, UK). Free $^{125}$I was removed by chromatography on a Sephadex G25 column (Pharmacia, Milton Keynes, UK). $^{125}$I-labelled fibrinogen was diluted with PBS and added to microtitre plates so that each well contained $1 \times 10^5$ cpm; the plates were then dried at 45°C for 3 days. The fibrinogen was converted to fibrin by treatment with F10 medium containing 2.5% freshly thawed FCS. The plates were washed twice with PBS prior to assay. To determine the PAI activity in the supernatants, the concentrations of urokinase (kindly provided by I. McGregor, Blood Transfusion Centre, Edinburgh, UK) which caused 50% of total 125I-fibrin lysis when cultured with an excess of plasminogen (Kabi, Stockholm, Sweden and a gift from I. McGregor), were used. The activity of PAI in the PLS was determined by titration against urokinase and plasminogen. Solubilized 125I-fibrin degraded by urokinase in the culture was measured after incubation for 24 h at 37°C, 5% CO$_2$. The PAI activity was expressed as percentage inhibition of plasminogen-dependent fibrinolysis according to the following equation:

\[
\text{Percentage inhibition} = \frac{(\text{Fib without PLS} - \text{Blank}) - (\text{Fib with PLS} - \text{Blank})}{\text{Fib without PLS} - \text{Blank}} 
\times 100
\]

Where units of fibrinolysis = cpm; and Fib = fibrinolysis.

Statistical analysis

All experiments were repeated at least twice and details of experiments are given in legends to figures and tables. Results of repeated experiments were subjected to analysis of variance. When there was a significant F value for the effect of treatment, individual means were compared for significance using t-test [16].

Results

Alveolar and pleural leucocyte populations after intratracheal instillation of C. parvum

Normal rat alveolar leucocyte populations contained more than 95% macrophages with a total number of less than $5 \times 10^5$. One day after intratracheal instillation of C. parvum, large number of PMN were recruited to the bronchoalveolar space and this increased the total cell number to around $30 \times 10^6$, although the macrophage number remained at the same level (fig. 1). Three days after instillation, the number of PMNs had decreased dramatically and was minimal by day 5. The number of macrophages was markedly increased by day 3 and remained elevated at day 5.

![Fig. 1. Bronchoalveolar and pleural leucocyte populations at various times after intratracheal instillation of C. parvum. Each point shows means of 2-13 separate experiments. For clarity, sum which varies from 8 to 30% of mean, is not presented in the graph. Asterisks denote a significant difference from control: *: p < 0.05; **: p < 0.01; ***: p < 0.001. --: total cell number; --: macrophage; --: PMN; --: lymphocyte; --: mast cell; --: eosinophil.](image-url)
There were no pleural PMNs detectable in normal pleural leucocyte populations. One day after intratracheal instillation of C. parvum there was a slight increase in number of macrophages but a marked increase in number of PMNs in the pleural space (fig. 1). The peak of PMNs at day 1 disappeared quickly and no PMNs were found by day 5 after C. parvum instillation. However, number of macrophages increased gradually from day 3 and this was sustained up to day 5. When the components of pleural leucocytes were compared with those of alveolar leucocytes, the pattern of change in the macrophage and PMN populations was similar.

**Analysis of beads and C. parvum organisms in alveolar and pleural leucocytes**

When beads were instilled intratracheally alone, they produced a moderate alveolitis characterized by 24% PMNs in the bronchoalveolar lavage (table 1).

A high percentage (72%) of alveolar macrophages engulfed beads and most macrophages (>70%) were found to contain more than 10 beads (table 2). One day after intratracheal C. parvum plus beads, the total number of alveolar leucocytes increased up to eight times compared with bead instillation alone. A severe inflammatory response was observed with PMNs accounting for more than 80% of the total alveolar population (table 1). The distribution of beads in the macrophages changed with most macrophages (>80%) containing less than 10 beads. The numbers of PMN with beads reduced from 33 to 4% (table 2).

In the pleural space, little effect on cell components was seen following alveolar deposition of beads alone and beads were negligible in the pleural leucocytes. Intratracheal instillation of C. parvum and beads together caused recruitment of PMNs into the pleural space (29% of total population) and the total cell number increased nearly threefold (table 1). In spite of such severe pleural inflammation resulting from

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**Table 1. – Alveolar and pleural leucocyte populations after intratracheal instillation of beads and/or C. parvum**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total cell no. ( \times 10^6 )</th>
<th>Differential percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar leucocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B alone</td>
<td>7.36 (1.26)</td>
<td>75 (3.5)</td>
</tr>
<tr>
<td>B+Cp (day 1)</td>
<td>58.97 (2.95)</td>
<td>16 (2.1)</td>
</tr>
<tr>
<td>B+Cp (day 5)</td>
<td>13.87 (1.76)</td>
<td>81 (9.5)</td>
</tr>
<tr>
<td>Pleural leucocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B alone</td>
<td>4.10 (0.70)</td>
<td>76 (6.4)</td>
</tr>
<tr>
<td>B+Cp (day 1)</td>
<td>11.80 (3.38)</td>
<td>60 (0.7)</td>
</tr>
<tr>
<td>B+Cp (day 5)</td>
<td>9.47 (0.55)</td>
<td>86 (0)</td>
</tr>
</tbody>
</table>

Results are presented as mean (sd) of two separate experiments. B: beads; Cp: C. parvum; Mac: macrophages; PMN: polymorphonuclear leucocytes; Lymph: lymphocytes; Mast: mast cells; Eos: eosinophils.

**Table 2. – Analysis of beads in the alveolar and pleural leucocytes, following intratracheal instillation of beads and/or C. parvum**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of cells with beads</th>
<th>Distribution of beads in cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B alone</td>
<td>72 (7.1)</td>
<td>12 (8.5)</td>
</tr>
<tr>
<td>B+Cp (day 1)</td>
<td>37 (6.4)</td>
<td>57 (7.1)</td>
</tr>
<tr>
<td>B+Cp (day 5)</td>
<td>15 (1.5)</td>
<td>52 (8.7)</td>
</tr>
<tr>
<td>Alveolar PMNs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B alone</td>
<td>33 (18.4)</td>
<td>90 (2.8)</td>
</tr>
<tr>
<td>B+Cp (day 1)</td>
<td>4 (0.7)</td>
<td>100 (0)</td>
</tr>
<tr>
<td>B+Cp (day 5)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Pleural macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B alone</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>B+Cp (day 1)</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>B+Cp (day 5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pleural PMNs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B+Cp (day 1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B+Cp (day 5)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are presented as mean (sd) of two separate experiments. For abbreviations see legend to table 1.
C. parvum in the lung, negligible numbers of beads could be observed in the pleural leucocytes. We found that the beads seen very occasionally in cytospins of pleural lavage leucocytes were extracellular. In another study (data not shown), we demonstrated that even one month after intratracheal instillation of beads alone, or together with mineral dusts, there was no detectable translocation of beads from airspace to pleural space.

Our results suggest that there is no direct communication of particles between the alveolar space and the pleural space, even during acute inflammation. To confirm this hypothesis, we extended our observations to assess numbers of cells containing C. parvum organisms in both spaces. Table 3 shows that one day after intratracheal instillation of C. parvum, numbers of macrophages and PMNs containing organisms intracellularly in the alveolar space, were 26 and 34%, respectively. By day 5, the cells with organisms were negligible in the alveolar space and the percentages of macrophages and PMNs containing identifiable organisms had dropped to 1 and 0%. No organisms could be found in either macrophages or PMNs in the pleural space at any time.

Table 3. - Analysis of C. parvum organisms in the alveolar and pleural leucocytes after intratracheal instillation of C. parvum

<table>
<thead>
<tr>
<th>Cells</th>
<th>% of cells with organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar macrophages</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>26 (3.1)</td>
</tr>
<tr>
<td>Day 5</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Alveolar PMNs</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>34 (5.6)</td>
</tr>
<tr>
<td>Day 5</td>
<td>0</td>
</tr>
<tr>
<td>Pleural macrophages</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0</td>
</tr>
<tr>
<td>Day 5</td>
<td>0</td>
</tr>
<tr>
<td>Pleural PMNs</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0</td>
</tr>
<tr>
<td>Day 5</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are presented as mean (sd) of 3 to 5 separate experiments. PMNs: polymorphonuclear leucocytes.

Characterization of pleural leucocytes

In all treatments the number of leucocyte common antigen positive cells was around 95% (mean (sd) of two experiments: Control: 96.6 (1.2)%; 16 h after intratracheal instillation of C. parvum: 94.9 (3.5)%; and 5 days after C. parvum instillation: 95.9 (0.9)%). The remainder of the cells (around 5%) was assumed to be exfoliated mesothelial cells. Any difference between the pleural populations was very likely, therefore, to be a result of change in leucocytes and their functions and not the population of mesothelial cells.

Secretion of cytokines by pleural leucocytes in vitro: effect of intratracheal instillation of C. parvum

Normal pleural leucocytes produced high levels of IL-1 and TNF in culture and this production was dramatically enhanced whilst the leucocytes were stimulated with LPS (fig. 2). One day after intratracheal instillation of C. parvum, the activities of both IL-1 and TNF released by pleural leucocytes decreased compared to control levels. Five days after instillation, this reduction had become even more marked (fig. 3).
PAI produced by pleural leucocytes in vitro after intratracheal instillation of C. parvum

Figure 4 shows that the production of PAI by pleural leucocytes lavaged from rats one day after C. parvum instillation had increased significantly. However, five days after instillation of C. parvum, PAI activity produced by pleural leucocytes had returned to normal levels.

![Graph showing fibrinolytic inhibition caused by pleural leucocytes supernatants](image)

**Fig. 4.** - Fibrinolytic inhibition caused by pleural leucocytes supernatants at various times after intratracheal instillation of C. parvum. Results are presented as mean and SEM of 3-8 separate experiments performed in triplicate. The dilution of pleural leucocyte supernatant is 1:32. ***: p<0.001, significantly different from control.

**Discussion**

This study has shown that intratracheal instillation of C. parvum induces severe alveolar inflammation, characterized by transient recruitment of large numbers of PMN. The C. parvum-elicited macrophage alveolitis was a relatively long-lived process. The same inflammatory pattern, but of a much lesser magnitude, was found in the pleural space after intratracheal instillation of C. parvum. The very similar inflammatory patterns in both alveolar and pleural spaces indicated that there may be some communication of inflammatory messages between the lung and the pleural space. To address this question, we investigated the possibility that particles might be translocated from the lung to the pleural space.

The present study on the translocation of particles (i.e. beads and C. parvum organisms) indicated that, following deposition in the lung, those particles were rapidly engulfed by alveolar macrophages and PMNs. Meanwhile, in the pleural space, no macrophages or PMNs were found to contain beads. Analysis of macrophages and PMNs containing C. parvum organisms also confirmed that no microbial particles reach the pleural space. These findings are consistent with the study of Lehnhert et al. [17] which showed that, after instillation of particles with diameter of 2 μ into the lung, there was no detectable translocation of particles to the pleural spaces.

Although there is no translocation of particles to the pleural space, this region is, nevertheless, influenced by inflammation in the lung as shown here by the fact that one day after intratracheal instillation of C. parvum, there was an increase in the number of pleural PMNs. The visceral pleura is thick and composed of five different layers, including mesothelial layer, submesothelial layer, external elastic layer, interstitial layer and internal elastic layer [4]. This represents a substantial barrier to passive movement of particles from alveolar to pleural spaces and could explain absence of particle transfer. Since particles do not cross the pleura, we presume that pleuritis is due to the transfer of a diffusible signal(s) to the pleural space from the parenchyma.

Asbestos and other fibres have been found at visceral and parietal sites of the pleura, and in pleural fluid after deposition in airspaces [18, 19], but failure to find fibres has also been reported [20]. Miller et al. [5] found that intravenous injection of Ethchlorvynol (ECV) produced a PMN-predominant exudative alveolitis and pleural effusion in rabbits, and they proposed that ECV-induced lung injury results in migration of PMN into the alveolar and interstitial space. Furthermore, damaged lung tissue was also suggested to stimulate PMN infiltration into and across the adjacent visceral pleura. Flenley [6] also suggested that pneumonia, in a subpleural location with an abundance of PMNs, causes increased pulmonary and pleural capillary permeability resulting in a leak of protein-rich fluid into the pleural space. Once the pulmonary endothelium becomes more permeable, the pleura is likely to offer less of a barrier to fluid, protein, and cellular movement, promoted by a pulmonary-pleural pressure gradient that is enhanced by the lung leak [5].

IL-1 levels normally increase during inflammation [21]. Our present study indicated that IL-1 activity released by pleural leucocytes one day after C. parvum instillation was decreased and the reduction was even more dramatic by day 5 compared with control levels. Presumably, the initial reduction in IL-1 activity may be due to the accumulation of PMNs in the pleural space, since IL-1 inhibitor is constitutively present in normal PMNs [22]. During the later stages after C. parvum instillation, when PMN numbers decreased, the further reduction of IL-1 activity might result from the additional secretion of IL-1 inhibitor by newly recruited macrophages [23]. The same explanation may be extended to the reduction in TNF activity [24]. Our findings, which indicate the existence of IL-1 and TNF inhibitor activity in the normal pleural lavage fluid (data not presented), favour this hypothesis.

Rich et al. [25] found that in vitro cultivation of blood monocytes resulted in decreased expression of TNF in response to LPS, which suggests that maturation of mononuclear phagocytes leads to a phenotype capable of producing higher levels of TNF. From this point of view, it is likely that the decrease in TNF production by the pleural leucocytes may partly result from the observed recruitment of blood monocytes to
the pleural space. However, IL-1 elaboration does not show this pattern, and in fact decreases with mono­cyte maturation [25, 26].

Decreased fibrinolytic activity has been reported in oleic acid-induced lung injury [27] and in quinacrine pleurodesis treatment of malignant pleural effusion [28]. We also demonstrated an increased PAI production by rat pleural leukocytes after intratracheal instillation of crocidolite asbestos [11]. The newly recruited PMNs may be activated and release products, such as oxidants, bioactive lipids and proteases, which influence the function of macrophages [29] including production of PAI. The similarity of changes of pleural PMN numbers and PAI production during the study period favours this hypothesis.

An altered balance of coagulation and fibrinolysis, caused by increased PAI, may establish local conditions which could promote acute fibrin deposition in the pleura. This is supported by the findings of Wiedström et al. [30], that fibrin deposition is associated with increased formation of thrombin in pleural fluid after intrapleural BCG. Fibrin is not a normal protein constituent of either the pulmonary interstitium or the alveolar space. Fibrin deposition commonly accompanies acute lung injury as well as influencing the subsequent repair processes [31], including the formation of a scaffold for fibroblast proliferation leading to fibrosis. Intrapleural urokinase [32] inhibited the development of pleural fibrosis in rabbits treated with high-dose tetracycline, implying that enhanced pleural fluid clotting and inhibition of fibrinolysis are important in drug-induced pleural fibrosis. The rapid return of the PAI levels to normal in the present study supports the observation that pleural fibrosis rarely results from acute, resolving pneumonia.

Our findings suggest that inflammatory pleural macrophages may produce cytokine inhibitors. In addition, their production of PAI, which may cause fibrin deposition and promote an inflammatory reaction in local connective tissue, is also reduced to normal levels as soon as PMN recruitment passes. These findings may represent an important protective role exerted by pleural macrophages. The phenomenon of a mild, long-standing pleural macrophage accumulation in our study may represent a beneficial reaction to the PMN recruitment engendered in the pleural space by inflammation in the adjacent lung parenchyma. By recruiting monocytes, tissue damage may be restricted through their ability to control the production of a series of inflammatory regulators.

In conclusion, after intratracheal instillation of C. parvum or beads, there was no penetration of particles from the airspaces to the pleural space. The PMN-macrophage inflammation occurring in the pleura, characterized by egress of PMNs into the pleural cavity, followed by an increase in macrophage numbers, is therefore presumed to result from transfer of a signal(s) from the parenchyma. The effects on the pleural leukocytes are a generalized decrease in cytokine activities and temporary enhancement of PAI activity. The role that these changes play in pleural effusion is not clear but this rat model of reactive pleural inflammation caused by intratracheal instillation of C. parvum may be useful in explaining some pleural events resulting from infection in the airspaces or deposition of agents such as asbestos.

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Reference


