Changes in alveolar macrophage, monocyte, and neutrophil cell profiles after smoke inhalation injury

B M S Riyami, R Tree, J Kinsella, C J Clark, W H Reid, D Campbell, C G Gemmell

Abstract
Thirty two fire victims with smoke inhalation, with or without burns, and 26 control subjects had bronchoalveolar lavage performed. Cell yields and differential cell counts were assessed. All patients and controls were cigarette smokers. Patients with smoke inhalation (SI) injury generally showed higher total bronchoalveolar lavage (BAL) cell yields, and this was significant on repeat lavage from 12 patients. The increase was almost entirely due to an increase in the proportion of neutrophils in patients with smoke inhalation alone (S) and those with cutaneous burns as well as smoke inhalation (S + B). On sequential lavage of 12 patients with smoke inhalation (SI) the proportion of neutrophils had increased; this was significantly higher than on initial lavage. Using various macrophage markers, the proportions of macrophage subgroups were determined. There was an increase in UCHM, and RFD, positive cells in each subgroup: the increase in UCHM, positive cells was significant in patients with burns as well as smoke inhalation, and the increase in RFD, positive cells was significant in patients with smoke inhalation alone. Assessment of the role of such cells in the development of acute lung injury (such as adult respiratory distress syndrome) may be important in our understanding of the mechanisms entailed.

Smoke inhalation has been identified as the single most important risk factor in determining mortality in fire victims. Patients who survive the acute injury, especially those that have associated cutaneous burns, often develop respiratory complications within a few days. Pulmonary complications have been identified as the single most important cause of death in these patients. In many, the clinical picture that develops is similar to that of the adult respiratory distress syndrome (ARDS). Bronchoalveolar lavage (BAL) is increasingly used to assess the effects of direct injury and cell responses in the lung. Previous studies have suggested that there is an increase in neutrophils in the lungs of smoke inhalation victims. In this study we examined BAL cell profiles from a large group of such patients.

Methods
The diagnosis of smoke inhalation was made according to previously published criteria. A cumulative score of 2 or more for each of the following criteria in a patient involved in a fire was regarded as diagnostic of clinically important smoke inhalation: history of being trapped in a fire in an enclosed space; production of carbonaceous sputum; perioral facial burns; altered level of consciousness at any time during or after the incident; symptoms of respiratory distress such as choking; signs of respiratory distress such as laboured breathing or hoarseness of voice.

The diagnosis was confirmed by the presence of an increased exposure carboxyhaemoglobin (COHb) which was estimated from the COHb concentration on admission using a nomogram. The control subjects were patients undergoing routine diagnostic bronchoscopy who had no evidence of infection, inflammation, or malignant process on clinical examination, bronchoscopy, or further investigation. The study was approved by the hospital ethical committee and all patients gave informed written consent.

Bronchoalveolar lavage was performed in the standard manner using a fibre optic bronchoscope. Buffered saline (pH 7-3) (180–240 ml) was instilled in 60 ml aliquots into the right middle lobe or lingula and immediately sucked back into a collecting pot. Twelve patients were lavaged twice, the second BAL was performed 24 hours later from the opposite lung.

The lavage fluid was pooled, excess mucus removed, and the total cell yield determined using a Fuchs-Rosenthal counting chamber (Weber Scientific International, Sussex, England). Two lots of 10⁶ cells in 0-2 ml were placed in cytocentrifuge buckets (Shandon Instruments, Runcorn) before centrifugation at 900 rpm for two minutes. The cells were air dried, heat fixed, and stained with Leishman's stain (Exogen, Clydebank). Differential counts were made from a total of 500 cells in each cytocentrifuge. In each case cell counts were made from a total of 500 cells in each cytocentrifuge. In each case the cell counts were confirmed by a non-specific esterase stain using a commercial kit (kit No 90 A-Z; Sigma).

Cytoxins were fixed in acetone:chloroform:1:1 mixture for five minutes, air dried for one hour, and then stored in slide carriers (Sterilin Ltd, Feltham) at −20°C until they were stained. An immunohistochemical method using a panel of macropage markers was used to determine cell phenotype. A
total of 500 macrophage like cells were counted and the proportions of negative and positive cells were determined.

The Mann-Whitney U test was used to compare patient groups and control patient data. Wilcoxon matched-pairs signed rank test was used to compare data from the same patient.

Results

Cell Yields

Total cell yields were increased in both patient groups and this was almost entirely due to an increase in neutrophils (figure), which were almost 10 fold higher in patients with smoke inhalation and burns (S + B) (p < 0.001). The yield of alveolar macrophages remained largely unchanged, although the proportion fell due to the increase in total numbers of neutrophils. The proportion of neutrophils was higher in the patients with smoke inhalation and burns (S + B) (table 1). The total cell yields in the second lavage were higher than those of control subjects (p < 0.01) with a further increase in the neutrophils (figure). On repeat lavage there was a further significant fall in the proportion of macrophages and an increase in the proportion of neutrophils (p = 0.007) (figure).

Phenotypic Analysis

Patients with smoke damage alone (S) had some increase in UCHM1 positive cells but patients with smoke inhalation and burns (S + B) had a larger, significant increase in UCHM1 positive cells (p < 0.02) (table 2). The proportions of RFD4 positive cells were higher in both patient groups compared with controls, and this was significant in the patients with smoke inhalation alone (p < 0.01). There were no significant differences in the proportions of RFD-positive cells between smoke inhalation patients (SI) and control subjects. On repeat lavage there was no significant change in any of the macrophage subgroups (table 3).

Discussion

Several studies have documented changes in BAL cell profiles following smoke inhalation. These studies have suggested that there is an influx of neutrophils into the lung after smoke inhalation. This study confirms that finding, and, furthermore, shows that patients with smoke inhalation and burns (S + B), who as a group have the highest mortality, have a much higher increase in the neutrophil population.

In patients with smoke inhalation a progressive increase in neutrophil count was observed. The observed progression in the influx of neutrophils and the higher counts in those with more serious injuries may be related to the subsequent development of respiratory complications. Respiratory complications in

Table 1 Cell yields

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Mean (SEM) cell x 10^6/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macrophages</td>
</tr>
<tr>
<td>Controls</td>
<td>3.67 (0.65)</td>
</tr>
<tr>
<td>(n = 26)</td>
<td></td>
</tr>
<tr>
<td>Smoke alone</td>
<td>4.44 (0.60)</td>
</tr>
<tr>
<td>(n = 15)</td>
<td></td>
</tr>
<tr>
<td>Smoke and burns</td>
<td>6.05 (2.07)</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
</tr>
<tr>
<td>Repeat BAL, smoke with or</td>
<td>8.80 (4.00)</td>
</tr>
<tr>
<td>without burns</td>
<td>(n = 12)</td>
</tr>
<tr>
<td>*p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>†p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>
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### Table 2 Percentage of macrophage subgroups determined by macrophage markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Mean % (SEM) positive for:</th>
<th>RFD, RFD, UCHM,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>43-05 (6-05) (0-05)</td>
<td>0-99</td>
</tr>
<tr>
<td>(n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke alone</td>
<td>40-0 (77-79) (3-64)</td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke and burns</td>
<td>44-5 (67-85) (5-25)</td>
<td></td>
</tr>
<tr>
<td>(n=14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.02

### Table 3 Macrophage subgroups in initial and repeat lavage (matched pairs) in cases of smoke inhalation with or without burns (n=6)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Mean % (SEM) positive for:</th>
<th>RFD, RFD, UCHM,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial lavage</td>
<td>44-14 (70-16) (0-95)</td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td>(6-46) (3-41) (0-35)</td>
<td></td>
</tr>
<tr>
<td>Repeat lavage</td>
<td>49-30 (71-03) (2-60)</td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td>(6-89) (6-73) (1-43)</td>
<td></td>
</tr>
</tbody>
</table>

patients with smoke inhalation injury resemble that seen in ARDS, and the accumulation of neutrophils in the lungs of patients with ARDS is well recognised. Inhaled smoke debris has been shown to be phagocytosed by alveolar macrophages. Phagocytosis of particulate matter by these macrophages has been shown to stimulate the release of neutrophil chemotactic factor derived from alveolar macrophages.

Systemic complement activation, which occurs after cutaneous burns, may also have a role in pulmonary neutrophil sequestration in these patients. Activation of alveolar macrophages after smoke inhalation and the systemic complement activation after cutaneous burns may explain the considerable neutrophil accumulation following the combined injury (S+B).

Changes in macrophage subpopulations after smoke inhalation have not been previously reported, although influx of monocytes into the lungs after smoke inhalation has been suggested. The monoclonal antibody UCHM1 marks peripheral blood monocytes. A small percentage of alveolar macrophages are positive for this antibody in healthy subjects (Table 2). The increase in numbers of UCHM1 positive cells seen after smoke inhalation and burns (S+B) suggests that there is an influx of monocytes as well as neutrophils. After five to seven days UCHM1 positive cells in vitro express the RFD1 marker. Our finding of a lack of increase in RFD1 positive cells is in keeping with this observation. RFD1 positive cells are mature macrophages that are primarily phagocytic. RFD2 positive cells are "tangible macrophages" and have been found to occur in normal lungs. The clinical importance of the increase in the patients with smoke inhalation alone (SO) is unclear.

The observed changes in macrophage subgroups suggest that in addition to the changes in the numbers of neutrophils there are changes within the subpopulations of macrophages. The changes in macrophage subgroups reported in this study may explain some of the changes in macrophage function that are seen after smoke inhalation. Generalised cell activation is recognised and may be attributable in part to the changes in cell profile seen in the present study. To understand this further it will be necessary to separate each cell population and follow their individual activity. Our studies have shown significant alveolar cell changes which may have important implications in the development of adult respiratory distress syndrome. The methods used may be helpful in the understanding of the mechanisms and management of acute lung injury in fire victims.

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