

# Alveolar macrophage dysfunction in malignant lung tumours

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**ABSTRACT** Alveolar macrophage chemotaxis was measured in 129 individuals—13 normal volunteers, 15 tumour free patients with recent bronchopulmonary infections, 10 patients with chronic bronchitis, 29 patients with endothoracic sarcoidosis, 48 patients with primary bronchial carcinoma and 14 patients with pulmonary metastases from various origins. Chemotaxis was tested in the presence of either zymosan activated autologous serum, N-formyl-methionine-leucyl-phenylalanine (F-Met-Leu-Phe), or zymosan activated human AB serum. Alveolar macrophage chemotaxis was significantly less in patients with bronchial carcinoma than in healthy volunteers ( $p < 0.01$ ). Chemotaxis was significantly more depressed in samples obtained from the neighbourhood of the tumour than in samples from the opposite lung. Defective chemotaxis was also found in patients with sarcoidosis. In contrast, the presence of lung metastases did not affect chemotaxis. A recent bronchopulmonary infection was associated with significantly increased ( $p < 0.02$ ) chemotaxis in tumour free patients but not in patients with a primary lung tumour. The findings suggest that an intrinsic functional defect of alveolar macrophages might favour the development of bronchogenic carcinoma.

The critically important role of alveolar macrophages in the defence of the host against inhaled particles, including microorganisms and environmental toxins, has been well established.<sup>1</sup> Yet the function of alveolar macrophages in protecting against tumour growth is still poorly understood. Several reports recently reviewed by Kaltreider<sup>2</sup> give evidence for a role of alveolar macrophages within immunologically mediated host defence mechanisms, leading to either activation or suppression of mitogen induced lymphocyte proliferation, depending on the experimental conditions. It is well established also that macrophages or monocytes from sources other than the lung play an important part in antitumour immunity,<sup>3,4</sup> their chemotaxis being frequently impaired in patients with various types of neoplasia.<sup>5-9</sup> Chemotaxis along an attractant gradient is an important function of macrophages which allows them to reach the site of an infection or tumour.

We therefore thought it of interest to study the in vitro chemotactic activity of alveolar macrophages from patients with bronchial carcinoma or pulmonary metastases and compare it with that of alveolar macrophages from patients with other lung diseases and from healthy volunteers. We report here an intrinsic chemotactic defect in alveolar macrophages from patients with primary lung cancer and sarcoidosis, which was not found in cells sampled from patients with the other conditions we tested.

## Methods

### PATIENTS

Informed consent was obtained from the 129 individuals who took part in this study.

Group 1 consisted of 13 healthy volunteers: eight men and five women (mean age 30 years) with no known past history of lung disease, of whom eight were smokers and five non-smokers. Group 2 comprised 15 men (mean age 52, range 29-72 years). Twelve were smokers and three were non-smokers. All underwent bronchoscopic assessment after treatment as part of the investigation of longstand-

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ing or recent bronchopulmonary infection. Group 3 was made up of 10 men aged 37–69 years (mean 51) with non-infective chronic bronchitis. Six were smokers, four were non-smokers. Group 4 consisted of 29 untreated patients with endothoracic sarcoidosis—17 men and 12 women aged 19–56 years (mean 34). Five were smokers and 24 were non-smokers. Group 5 consisted of 48 patients—44 men and four women aged 40–73 years (mean 59) with primary bronchogenic carcinoma. Forty three were smokers and five were non-smokers. Thirty individuals had intrathoracic disease only. Eighteen patients had distant metastases. The histological types were: 28 squamous cell carcinomas, six small cell cancers, six adenocarcinomas, and eight undifferentiated tumours. Fourteen of the 48 patients had a concomitant infection or severe local inflammation. According to the diagnostic criteria for inflammation and infection, there had to be an inflamed bronchial mucosa at bronchoscopy associated with either leucocytosis (polymorphonuclear leucocytes over  $10.0 \times 10^9/l$ ), fever higher than  $38^\circ\text{C}$ , or other systemic features of inflammation (fibrinaemia  $>4.5 \text{ g/l}$ ). Group 6 comprised 14 patients: nine men and five women aged 35–70 years (mean 55) with pulmonary metastases from primary tumours originating in the breast (5), head and neck (4), testis (1), or colon (1); in three the site of the primary tumour was unknown. Six were smokers and eight non-smokers.

#### ALVEOLAR LAVAGE

Segmental lavage through a fibreoptic bronchoscope was performed under local anaesthesia as previously described.<sup>11 10</sup> In brief, healthy volunteers of group 1 and control patients of groups 2, 3, and 4 underwent lavage in the lingular territory. In the patients with primary bronchogenic carcinoma (group 5) alveolar lavage was performed before specific treatment at the tumour site in 23 patients. A bordering territory was lavaged in the other 25 patients because bronchial obstruction by the tumour had rendered injection and aspiration difficult at the site of the tumour. In 14 cases of squamous cell carcinoma lavage was also performed in the lung opposite to the tumour site. In patients of group 6 with pulmonary metastases lavage was carried out in territories where there was radiographic evidence of tumour.

#### ALVEOLAR MACROPHAGE CHEMOTAXIS

Cells were recovered by centrifugation and then washed and resuspended in phosphate buffered saline (PBS). Total and differential cell counts of an aliquot were performed on a cytocentrifuge preparation after Wright Giemsa staining. Cells were again centrifuged for 10 minutes at 500 g, and finally

resuspended at a concentration of  $1.5 \times 10^6$  viable cells (trypan blue test) per ml of RPMI-1640 medium. Random migration and chemotaxis were measured in modified Boyden chambers with  $8.0 \mu\text{m}$  pore millipore filters.<sup>11 12</sup> The 129 samples were tested in the presence of autologous zymosan activated serum (1 mg zymosan/ml serum, one hour at  $37^\circ\text{C}$ , then heated at  $56^\circ\text{C}$  for 30 minutes and diluted 1:20 in sterile PBS) as chemoattractant. Concomitantly, alveolar macrophages from six lung cancers were tested also against human AB zymosan activated serum, and samples from five lung cancers in the presence of  $3 \times 10^{-8} \text{ mmol/l}$  of formyl-methionine-leucyl-phenylalanine (F-Met-Leu-Phe) per ml. The number of macrophages migrating across the entire thickness of the filter after 90 minutes of incubation was determined after staining with haematoxylin. All the responding cells were in one optical plane on the lower surface of the filter, and the total number could be readily determined. The results are expressed as the mean cell count (with standard deviation in parentheses) in 10 microscopic fields ( $\times 100$ ) in duplicate assays. In 14 patients with sarcoidosis purified, lymphocyte free alveolar macrophages were obtained by allowing them to adhere to glass for 60 minutes, and the data were compared with those obtained with the total, unfractionated cell population.

#### STATISTICAL ANALYSIS

Differences between groups were compared by variance analysis (*F* test). Means were compared with Student's *t* test.

#### Results

The volume of recovered fluid, total cell yield, percentage of each cell type, and viability were not significantly different between the various groups (including healthy smokers and non-smokers) with the exception of patients with sarcoidosis, where the percentage of lymphocytes was higher than in the other groups (table 1).

The results shown in table 2 indicate that random migration performed as a control test in the presence of autologous serum diluted 1:20 in PBS was no different for patients with bronchial carcinoma or metastases from the result observed for healthy controls. Alveolar macrophages from patients with longstanding or recent bronchopulmonary infections, however, showed random migration significantly ( $p < 0.02$ ) above that of healthy controls.

Neither sex nor smoking habits showed any influence on alveolar macrophage chemotaxis in the healthy volunteer group. Chemotaxis was affected

Table 1 *General characteristics of recovered lavage fluids (means with standard deviations in parentheses)*

Group	Recovery of infused lavage fluid (ml)	Total cells recovered ( $\times 10^6$ )	Alveolar macrophages (%)	Lymphocytes (%)	Polymorpho-nuclear leucocytes (%)	Cell viability (%)
1: Healthy volunteers (n = 13)						
Smokers (n = 8)	85 (20)	36 (29)	93 (6)	4 (3)	1 (1)	70 (8)
Non-smokers (n = 5)	83 (22)	15 (9)	89 (7)	12 (10)	1 (1)	70 (5)
2: Infected non-cancerous patients (n = 15)	69 (27)	30 (16)	83 (18)	15 (9)	5 (4)	70 (13)
3: Patients with chronic bronchitis (n = 10)	67 (24)	53 (29)	90 (8)	7 (5)	3 (2)	81 (13)
4: Patients with sarcoidosis (n = 29)	91 (24)	33 (28)	63 (21)	34 (20)	2 (2)	75 (14)
5: Patients with bronchial carcinoma (n = 48)						
With respiratory infection (n = 14)	64 (29)	25 (17)	91 (9)	7 (6)	2 (2)	70 (11)
Without respiratory infection (n = 34)	58 (20)	28 (20)	88 (10)	6 (5)	3 (2)	75 (12)
6: Patients with pulmonary metastasis (n = 14)	74 (23)	20 (12)	91 (6)	7 (5)	2 (2)	71 (13)

by clinical state (table 2); it was significantly less ( $p < 0.01$ ) in patients with bronchogenic carcinoma than in controls, and there were no differences in values between cells recovered at the tumour site (mean 4.9 (SD 2.7)) and those from the homolateral lung in a territory close to the tumour (5.4 (3.6)). The dysfunction of alveolar macrophages from patients with primary bronchial carcinoma was independent of the histological type—squamous cell carcinoma yielded 4.5 (1.7) cells per high powered field showing chemotactic migration, small cell cancers 5.1 (2.1), adenocarcinomas 6.1 (2.5), and undifferentiated tumours 7.4 (4.8). The defect in alveolar macrophage activity was similar in patients with limited disease (5.0 (2.2)) and patients with distant metastasis (6.3 (4.4)). Interestingly, a con-

comitant respiratory infection or the finding of inflammatory bronchial mucosa did not affect low chemotactic migration values associated with a primary malignant lung tumour.

The use of various chemoattractants (zymosan treated autologous serum, zymosan treated AB serum or F-Met-Leu-Phe) induced similar responses in the alveolar macrophages from patients with bronchogenic cancer (table 3). In contrast (table 2), the chemotaxis values of alveolar macrophages from patients with lung metastases of tumours from other sources (group 6) were similar to those of healthy controls (group 1). Defective alveolar macrophage chemotaxis was not, however, specific for primary bronchial carcinoma; the results for patients with endotracheal sarcoidosis (group 4) were similar to those for patients with bronchial carcinoma, showing chemotaxis significantly below ( $p < 0.01$ ) that found in healthy controls. Table 2 also shows that depletion of lymphocytes before the test did not affect the data for sarcoidosis.

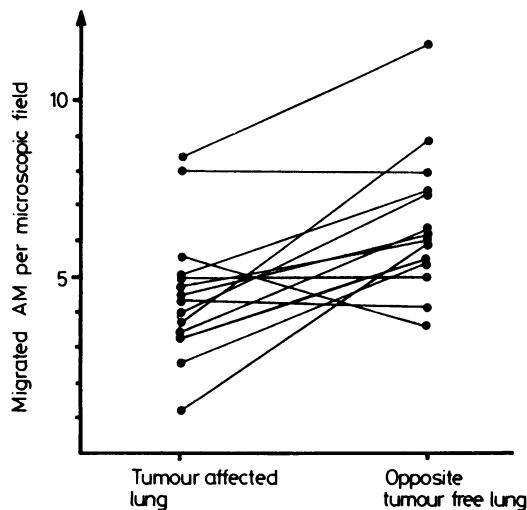
Chemotaxis was significantly more inhibited in alveolar macrophages from the tumour territory ( $p < 0.01$ ) than in macrophages from the contra-

Table 2 *Alveolar macrophage chemotaxis with autologous zymosan activated serum, expressed in terms of migrated alveolar macrophages (AM) per microscopic field (means with standard deviations in parentheses)*

Group	Random migration	Chemotactic migration
1: Healthy volunteers (n = 13)		
Smokers (n = 8)	3.4 (1.8)	12.7 (2.7)
Non-smokers (n = 5)	4.1 (1.7)	12.7 (2.3)
2: Infected non-cancerous patients (n = 15)	5.8 (5.6)	16.9 (10.0)
3: Patients with chronic bronchitis (n = 10)	3.8 (1.3)	11.7 (3.8)
4: Patients with sarcoidosis (n = 29)		
Unfractionated AM (n = 29)	2.2 (1.2)	7.2 (1.8)
Purified AM (n = 14)	1.7 (1.0)	6.3 (1.1)
5: Patients with bronchial carcinoma (n = 48)		
With respiratory infection (n = 14)	2.4 (1.9)	5.8 (3.4)
Without respiratory infection (n = 34)	2.1 (1.7)	5.3 (3.0)
6: Patients with pulmonary metastasis (n = 14)	3.9 (1.8)	11.1 (3.9)

Table 3 *Bronchial carcinoma: alveolar macrophage chemotaxis in the presence of either autologous zymosan activated serum, zymosan activated AB serum, or F-Met-Leu-Phe (means with standard deviations in parentheses)*

Chemotactic migration		
Bronchogenic cancer patients (n = 6)	Autologous zymosan activated serum	6.4 (1.3)
	zymosan activated AB serum	7.1 (2.8)
Bronchogenic cancer patients (n = 5)	Autologous zymosan activated serum	4.9 (1.9)
	F-Met-Leu-Phe	5.8 (2.3)



*Bronchial carcinoma: alveolar macrophage (AM) chemotaxis with zymosan activated serum in the lung with the tumour and in the contralateral, tumour free lung.*

lateral lung, although chemotaxis in the tumour free lung was still significantly below ( $p < 0.01$ ) that of alveolar macrophages from healthy controls.

In contrast to the depressed alveolar macrophage chemotaxis associated with primary lung cancer or endothoracic sarcoidosis, chemotactic values were significantly greater in patients with a recent bronchopulmonary infection ( $p < 0.02$ ) than in controls. Patients with chronic bronchitis without evidence of infection did not show any alteration in alveolar macrophage chemotaxis.

## Discussion

When alveolar macrophages from healthy donors were tested for response to chemoattractants, only 10–15% migrated. This contrasts sharply with results from mouse macrophage cell lines, where nearly 100% migration has been demonstrated.<sup>13</sup> The lack of migration was not due to loss of cell viability as recovered non-migrating cells excluded trypan blue. Neither was it due to the chemoattractant, as the results were comparable in assays with zymosan activated autologous serum, zymosan activated human AB serum, and F-Met-Leu-Phe. The non-migrating population could not be explained by cell crowding or competition for pores on the filter. The finding suggests that alveolar macrophages could be made up of various subsets, although the migrating subset appeared to be independent of the chemotaxis agent, in contrast to blood monocyte subpopulations.<sup>14 15</sup> In the present

series using alveolar macrophages from 32 women and 97 men, of whom 82 were smokers and 47 non-smokers, no difference in responses were found that could be attributable to smoking habits or sex associated influences.

The data presented here indicate that alveolar macrophage chemotaxis was reduced in the presence of primary lung cancer and of sarcoidosis, in which cases inhibited alveolar macrophage responses were found to be independent of the presence or absence of lymphocytes in the sample studied in the chemotaxis chamber. This confirms a previous report.<sup>16</sup> In contrast, normal values for alveolar macrophage chemotaxis were found in cells from patients with lung metastases, previously reported by Sone and Fidler.<sup>17</sup> The finding might be related to the anatomical position of metastases.

Alveolar macrophages are situated at the air-tissue interface, strategically located for initial contact with particulates and providing non-specific defence of the lung. Alveolar macrophages behave differently from blood monocytes and peritoneal macrophages in most activities,<sup>18</sup> and may possess an autocontrol mechanism for local recruitment.<sup>19</sup> Possibly metastases, made up of cells that have escaped capture by monocytes or macrophages in blood stream and lymph node and remaining separated from alveolar macrophages by a normal tissue barrier, are unable to deliver suppressive factors to the bronchial or alveolar lumen, whereas in a primary lung cancer developing on the bronchial epithelium there is cell to cell contact and free excretion of suppressive factors. Nevertheless, a study on a larger group of patients would be of interest, to confirm that alveolar macrophages from patients with lung metastases show a normal chemotactic response.

Alveolar macrophage chemotaxis was increased after a recent bronchopulmonary infection. This suggests an augmented mobility of alveolar macrophages induced by bacterial antigens. Augmented mobility was not seen in patients with bronchial carcinoma who had had recent bronchopulmonary infection. Mobility was presumably impaired by the concomitant presence of suppressor factors. Inhibitory factors released by tumour cells from sources other than the lung and impairing macrophage chemotaxis have been described<sup>6 20 21</sup> and a low molecular weight inhibitory factor has been isolated from growing neoplasia.<sup>22</sup> A low molecular weight suppressor factor with prostaglandin like activity has, however, also been isolated from alveolar macrophages.<sup>23 24</sup> Nearby lymphocytes might also be affected by reduced alveolar macrophage chemotaxis. It seems probable that suppressive factors are locally produced as chemotaxis was more inhibited in samples from the tumour territory than



in samples from other lung areas.

Chemotactic responsiveness favours the accumulation of macrophages near the site of infection. It seems possible that the high incidence of bronchopulmonary infection in patients with lung cancer could be related to impaired alveolar macrophage chemotaxis. Accumulation of macrophages near the tumour is generally thought also to reflect participation in the defence against neoplasia, as impaired monocyte chemotaxis is found in about 60% of patients with cancers other than bronchogenic ones, and surgical removal is followed by restoration of chemotaxis to normal.<sup>5,9</sup> It is tempting to speculate that an intrinsic functional defect of alveolar macrophages could favour the development and spread of primary lung cancer. The assay of alveolar macrophage chemotaxis could turn out to be a valuable diagnostic aid, especially when a peripheral tumour is not readily accessible to fiberoptic examination and biopsy, and perhaps particularly if it were associated with determination of the concentration of carcinoembryonic antigen in the lavage supernatant.<sup>25</sup>

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