The enhanced inflammatory response in non-small cell lung carcinoma is not reflected in the alveolar compartment

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An inflammatory response has been observed in lung cancer both locally and systemically. The aim of the present study was to investigate whether the alveolar compartment was involved in the inflammatory response in non-small cell lung carcinoma (NSCLC). Both inflammatory mediators in bronchoalveolar lavage fluid (BALF) and cytokines produced by alveolar macrophages (AM) were investigated. Twenty patients with newly detected NSCLC and nine control subjects were studied. The patients had not been treated with chemotherapy, radiotherapy or with systemic or inhaled corticosteroids. All patients and control subjects were current smokers or stopped smoking recently. BAL was performed in the affected lung as well as in the contralateral lung of NSCLC patients, and only unilaterally in control subjects. Comparable results were demonstrated for the levels of the inflammatory mediators TNF-α, Interleukin (IL)-6, IL-8, both soluble TNF receptors and the soluble adhesion molecules E-selectin and intercellular adhesion molecule (ICAM)-1 between the affected lung and the contralateral lung in the NSCLC population as well as between the NSCLC population and the control subjects. Moreover, no significant differences in cytokine profiles of AM were found between AM obtained from the affected lung and from the contralateral lung. Although BAL is a useful tool in the diagnostic procedure for NSCLC, the present findings suggest that BAL does not reflect the enhanced inflammatory state, as reported in plasma and in the interstitial compartment around the tumour cells in NSCLC.

RESPIR. MED. (1998) 92, 76-83

Introduction

Neoplastic diseases induce profound changes in the mononuclear phagocyte system: both local and systemic inflammatory responses are observed in the presence of the tumour (1–7). The local inflammatory response is characterized by the accumulation of mononuclear cells in stroma around the tumour cells (1–7). The presence of inflammatory cells that produce cytokines such as TNF-α, interleukin (IL)-6 and IL-8 can be seen as both advantageous and harmful for the tumour (8–10). The angiogenic properties of both TNF-α and IL-8 can be favourable for the growth of the tumour as well as for growth of distant metastases (11–13). However, the cytokines IL-6 and IL-8 attract other mononuclear cells that are known to interfere with tumour growth (8,9). In addition, IL-6 is well known as an inducer of the protective acute phase response of the host (14). A former study of the authors’ group, showed the presence of immunohistochemical staining patterns for TNF-α, IL-6 and IL-8 and enhanced expression of both TNF receptors in tissue samples obtained from resection specimens of non-small cell lung carcinoma (NSCLC) patients (15). These data combined with the enhanced expression of the adhesion molecules E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (16) reflect the presence of a local inflammatory response around the tumour cells in NSCLC.

The authors also observed the presence of a systemic inflammatory response in NSCLC. Enhanced levels of both soluble (s) TNF receptors, the acute-phase proteins C-reactive protein (CRP) and LPS-binding protein (LBP), and sICAM-1 were demonstrated in plasma of patients with NSCLC (7). The soluble forms of both TNF receptors, the adhesion molecules and both acute phase proteins are considered to reflect inflammatory activity (17–19). Metabolic disturbances were found to be related to the presence of these enhanced levels of inflammatory mediators in patients with NSCLC (7). The present data were supported by others who found a similar relationship between metabolic disturbances and inflammatory parameters in...
The aim of the present study was to investigate whether the inflammatory process in the interstitial compartment of the affected lung extends into the alveolar compartment of the lung in NSCLC. The bronchoalveolar lavage (BAL) is an important tool to assess the inflammatory state in the alveolar compartment of the lung. During different acute inflammatory reactions, cellular changes in BAL fluid (F) (influx of neutrophils or lymphocytes) occur and alveolar macrophages (AM), the major cellular component of the alveolar compartment, can be activated (20). Besides these cellular changes, the presence of an inflammatory state in the alveolar compartment is reflected by increased levels of the pro-inflammatory cytokines TNF-α, IL-6 and IL-8 or soluble adhesion molecules in BALF, as has been demonstrated with the alveolar compartment was involved in the inflammatory response in NSCLC. To this end, the pro-inflammatory cytokines, both soluble TNF receptors and soluble adhesion molecules were assessed in BALF and the alveolar compartment was compared with BALF obtained from the contralateral lung as well as with BALF obtained from control subjects.

The present study investigated whether the inflammatory process in the interstitial compartment of the affected lung extends into the alveolar compartment of the lung in NSCLC. The bronchoalveolar lavage (BAL) is an important tool to assess the inflammatory state in the alveolar compartment of the lung. During different acute inflammatory reactions, cellular changes in BAL fluid (F) (influx of neutrophils or lymphocytes) occur and alveolar macrophages (AM), the major cellular component of the alveolar compartment, can be activated (20). Besides these cellular changes, the presence of an inflammatory state in the alveolar compartment is reflected by increased levels of the pro-inflammatory cytokines TNF-α, IL-6 and IL-8 or soluble adhesion molecules in BALF, as has been demonstrated in several pulmonary diseases (21–27).

The aim of the present study was to investigate whether the alveolar compartment was involved in the inflammatory response in NSCLC. To this end, the pro-inflammatory cytokines, both soluble TNF receptors and soluble adhesion molecules were assessed in BALF and the production of cytokines by AM was determined in vitro. BALF obtained from the tumour site was compared with BALF obtained from the contralateral lung as well as with BALF obtained from control subjects.

### Subjects and Methods

#### PATIENTS AND CONTROL SUBJECTS

The characteristics of the study population are shown in Table 1. Twenty patients with primary NSCLC (12 patients with squamous cell carcinoma and eight patients with adenocarcinoma) and nine control subjects were included in the study. All patients had histologically documented tumours. Fourteen tumours were located in the upper lobes, three tumours were located in the middle lobe or lingula and three tumours were located in the lower lobes. As a result of the research questions, all tumours were located peripherally in order to perform an adequate BAL. All patients and control subjects were current smokers (n=18) or had stopped smoking during the last 6 months (n=11). The patients had not been treated with chemotherapy, radiotherapy or with systemic or inhaled corticosteroids. The new international staging system for lung cancer was used to assess tumour stage (28). Control subjects were selected out of the smoking patient population who underwent a bronchoscopy for several reasons, but finally no pathology was demonstrated.

The study was approved by the medical ethical committee of the University Hospital of Maastricht. Written informed consent was obtained from all patients and control subjects.

### BRONCHOALVEOLAR LAVAGE

BAL was performed during fibre-optic bronchoscopy. After premedication (atropine 0.5 mg i.m. and diazepam 10 mg p.o. 1 h before bronchoscopy) and local anaesthesia of the larynx and bronchial tree (lidocaine 1%), a flexible, fibre-optic bronchoscope was introduced and placed in the wedge position in the segment with tumour localization as well as in either the lingula or the middle lobe of the contralateral lung. Positioning of the bronchoscope in respect to tumour localization was controlled by fluoroscopy during the endoscopic procedure. Accordingly, BAL was performed by standardized washing of these segments. Four aliquots of 50 ml sterile saline (0.9% NaCl) at 37℃ were used for each lung. In control subjects, BAL was performed unilaterally either in the lingula or right middle lobe. The first aliquot of recovered BALF was discarded and the last three aliquots were pooled. BALF was collected in polypropylene tubes (Greiner, Alphen a/d Rijn, The Netherlands) and kept on ice in order to minimize AM adherence. Cytospin slides of BALF cells were stained with May-Grünwald-Giemsa (MGG; Merck, Darmstadt, Germany) for cell differentiation. At least 1000 cells were counted. Cytologic examination of the BALF revealed no malignant cells. Next, cells were separated from BALF by centrifugation at 500 × g for 5 min after cell differentiation. Cell-free BALF was filtered through a layer of gauze to remove mucus strands. BALF was concentrated by lyophilization and resuspended in distilled water to 10% of the original volume before determinations and stored at −70℃ until analysis. No significant positive bacterial cultures were found in any of the BALF samples investigated. Blood was obtained by venepuncture and collected in evacuated blood collection tubes (Sherwood Medical, St Louis, MO, U.S.A.) containing Heparin® (Leo Pharmaceutical Products B.V., Weesp, The Netherlands).

### ALVEOLAR MACROPHAGES CULTURE

After centrifugation, cells obtained from BALF were washed twice and filtered through a modified transfusion

#### Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>NSCLC (n=20)</th>
<th>Control subjects (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>14/6</td>
<td>12/2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63 ± 2</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>43 ± 3</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>88 ± 5</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>IVC (%)</td>
<td>105 ± 3</td>
<td>103 ± 5</td>
</tr>
<tr>
<td>Tumour stage (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I and II</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Stage IIIA</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Stage IIIB</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>2</td>
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</tbody>
</table>

Data are expressed as mean ± standard error of the mean. No significant differences have been found between the lung cancer population and control subjects. FEV₁, forced expiratory volume in 1 s; IVC, inspiratory vital capacity; n, number; NSCLC, non-small cell lung cancer.
system (N.P.B.I., The Netherlands). Cells were cultured in medium consisting of RPMI 1640 (Gibco, Paisley, U.K.) supplemented with antibiotics and 10% bovine calf serum (Hydolone, Logan, UT, U.S.A.). Bovine calf serum was heated at 56°C for 30 min before storage at 4°C and contained <5 pg ml⁻¹ endotoxin as determined in the Limulus assay (Coatest, Kabi Vitrum, Stockholm, Sweden). Cells, consisting for more than 90% of AM, were cultured at a number of 5 x 10⁶ ml⁻¹ in 24-well plates (Costar, Cambridge, MA, U.S.A.) and stimulated in duplo with LPS (E. coli 055:B5, Sigma, St. Louis, MO, U.S.A.); in concentrations of 0, 10 ng ml⁻¹, 1 µg ml⁻¹ and 100 µg ml⁻¹. After 20 h of stimulation, which was found to be the optimum for TNF-α, IL-6 and IL-8 production by AM as determined (data not shown), plates were centrifuged at 500 x g, supernatant was collected and stored at -70°C until analysis.

INFLAMMATORY MEDIATORS

Inflammatory mediators were assessed in both plasma and BALF using sandwich ELISA as described previously (29–33). In short, the TNF-α ELISA consisted of 61E71 as coating mAb and polyclonal rabbit anti-human TNF-α Abs. 1NF-α could be detected with a lower detection limit of 20 pg ml⁻¹. For measurement of sTNF-R55 and sTNF-R75, MAbs MR1-1 and MR2-2 were used for coating, respectively. Specific biotin labelled polyclonal rabbit anti-human sTNF-R IgG were used as detector reagents. The detection limit of both assays was 50 pg ml⁻¹.

IL-6 was caught by mAb 5El and detected by polyclonal rabbit anti-human IL-6 Abs. IL-6 could be detected with a lower detection limit of 10 pg ml⁻¹. The IL-8 ELISA consisted of HM5 as coating mAb and biotinylated polyclonal rabbit anti-human IL-8 Abs. The detection limit of IL-8 was 20 pg ml⁻¹. For sICAM-1 ELISA, mAb HM.2 was used for coating. Biotinylated mAb HM.1 was used for detection. The detection limit of the assay was 400 pg ml⁻¹. For sE-selectin ELISA, plates were coated with mAb ENA1 and biotin labelled mAb ENA2 was used as detector reagent. Since binding of ENA1 and ENA2 to sE-selectin is Ca-dependent, 4 mM Ca²⁺ and 2.5 mM Mg²⁺ were added to the buffers in this assay. The detection limit of the assay was 100 pg ml⁻¹.

Immunobead plates (Nunc-Imuno Plate Maxisorp, Roskilde, Denmark) were used for the ELISA assays. Biotinylated samples were detected with streptavidin-peroxidase conjugate (Dako, Glostrup, Denmark), while peroxidase-conjugated goat anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA, U.S.A.) was used in the TNF-α and IL-6 ELISAs. 1MB (3,3',5,5'-tetramethylbenzidine, Kirkegaard & Perry Lab., Gaithersburg, MD, U.S.A.) was used as a substrate. Photospectometry (450 nm) was performed using a micro ELISA autoreader.

BIOCHEMICAL PARAMETERS

Serum urea and albumin have been determined on a Synchro CX-7 analyser (Beckman Instruments Inc, U.S.A., California) using test kits from Beckman Instruments Inc. For the determination of urea in both serum and BALF, an enzymatic conductivity rate method was used. For the determination of serum albumin, the bromcresol purple method was used. In BALF, albumin was determined on an Array 360 System for immunochemistry (CA, U.S.A.), using a micro-albumin method. BALF total protein was measured with the pyrogallol method described by Watanabe et al. (34). Lactate dehydrogenase (LDH) activity was measured by an enzymatic rate method, using pyruvate as a substrate. Alkaline phosphatase (ALP) was measured by an enzymatic rate method using p-nitrophenylphosphate as a substrate.

STATISTICS

Data are expressed as mean ± standard error of the mean (SEM) and, if appropriate, as median with range. Groups were statistically compared using analysis of variance or the Mann–Whitney U-test. The BALF/blood ratios were calculated for albumin and urea in order to get insight in possible leakage between the alveolar compartment and the blood compartment. BALF/blood ratios for albumin and urea were calculated as follows: BALF/blood ratio for albumin = [albumin level in BALF]/[albumin level in blood]. The same calculation was used for urea. Probability values less than 0.05 were considered to be significant. The statistical analyses were performed using the SPSS/PC+ 4.0 package (35).

Results

First the systemic and local inflammatory response were assessed in the study population. Thirteen out of the 20 NSCLC patients demonstrated an acute phase response (CRP level: 20 ± 18 µg ml⁻¹, range 7–57 µg ml⁻¹), while no enhanced levels of CRP were detected in control subjects (CRP <5 µg ml⁻¹). The mean values (±SEM) of the other inflammatory mediators in plasma of the lung cancer patients compared to controls were as follows: sTNF-R55 1.3 ± 0.2 vs 1.1 ± 0.4 ng ml⁻¹ (not significant), sTNF-R75 2.0 ± 0.3 vs 1.3 ± 0.4 ng ml⁻¹ (P<0.01), sICAM-1 66.9 ± 6.0 vs 40.3 ± 5.4 ng ml⁻¹ (P<0.05), s-selectin 29.1 ± 3.0 vs 33.4 ± 5.6 (not significant). IL-6 was not detectable in controls and IL-6 and IL-8 were only present in very small amounts in plasma of the lung cancer patients. 

In order to investigate the inflammatory response in the alveolar compartment in NSCLC, the cellular profile of BALF was assessed (Table 2). The data show that the total cell count and the differential cell count did not show significant differences between the BALF obtained from patients suffering from NSCLC and control subjects. In line with these data, also no differences in cellular profile...
ENHANCED INFLAMMATORY RESPONSE IN NSCLC

Table 2. Cellular profile of the bronchoalveolar lavage fluid in NSCLC and control subjects

<table>
<thead>
<tr>
<th></th>
<th>NSCLC patients (n=20)</th>
<th>Control subjects (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumour site</td>
<td>Contralateral</td>
</tr>
<tr>
<td>Recovery (ml)</td>
<td>60.3 ± 4.5</td>
<td>71.6 ± 5.1</td>
</tr>
<tr>
<td>Total cell count (× 10⁴ ml)</td>
<td>33.6 ± 5.9</td>
<td>31.8 ± 5.3</td>
</tr>
<tr>
<td>AM (%)</td>
<td>89.3 ± 2.7</td>
<td>91.1 ± 2.3</td>
</tr>
<tr>
<td>PMN (%)</td>
<td>2.5 ± 0.9</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>8.0 ± 2.3</td>
<td>7.8 ± 2.3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of the mean. AM, alveolar macrophages; PMN, polymorphonuclear neutrophils; NSCLC, non-small cell lung cancer.

Table 3. Non-cellular constituents of the bronchoalveolar lavage fluid in NSCLC and controls

<table>
<thead>
<tr>
<th></th>
<th>NSCLC patients (n=20)</th>
<th>Control subjects (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumour site</td>
<td>Contralateral</td>
</tr>
<tr>
<td>Albumin (mg I⁻¹)</td>
<td>33.3 ± 4.2</td>
<td>27.6 ± 3.6</td>
</tr>
<tr>
<td>Total protein (mg I⁻¹)</td>
<td>27.0 ± 13.0–75.0</td>
<td>22.0 8.0–58.0</td>
</tr>
<tr>
<td>Urea (mmol I⁻¹)</td>
<td>50 ± 10</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>BALF/blood ratio for albumin</td>
<td>0.46 ± 0.05</td>
<td>0.48 ± 0.04</td>
</tr>
<tr>
<td>BALF/blood ratio for urea</td>
<td>0.50 10.0–70</td>
<td>0.50 10.0–70</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of the mean; the median is given with the range in parentheses. No significant differences were found between the different sites investigated. BALF, bronchoalveolar lavage fluid; NSCLC, non-small cell lung cancer.

between the affected lung and the contralateral lung of the NSCLC patients were observed. In case of damage to the alveolo-capillary membrane and oedema, one would expect increased levels of the non-cellular constituents total protein, albumin and urea in BALF. Equal levels of the non-cellular constituents were demonstrated in BALF obtained from the lung cancer population and from control subjects (Table 3). Moreover, the levels were also similar between the affected lung and the contralateral lung in NSCLC patients. In order to assess the degree of dilution of BALF, BALF/blood ratios for albumin and urea were determined. The data showed that these ratios were similar for both the tumour lung and contralateral lung and for the lung cancer population and control subjects. No differences in degree of dilution could therefore be detected among the three groups.

Next, the levels of inflammatory mediators in BALF of both NSCLC patients and control subjects were determined. The data are summarized in Table 4. Comparable results were found for both soluble TNF receptors, sICAM-1, sE-selectin and the pro-inflammatory cytokines IL-6 and IL-8 between patients suffering from NSCLC and control subjects. TNF-α was not detectable in any of the BALF samples investigated (data not shown). Only small amounts of sTNF-R55, sE-selectin and IL-6 were present in BALF. The mean levels were near the detection limit of the ELISA which explains the narrow range observed for these parameters. In accordance with other studies, larger ranges were detected for the other inflammatory mediators. In order to investigate whether tumour characteristics could influence levels of inflammatory mediators in BALF, patients were divided according to tumour stage and histology of the tumour. In accordance with the study by others, larger ranges were detected for the other inflammatory mediators. No differences were found between the different sites investigated. BALF, bronchoalveolar lavage fluid; NSCLC, non-small cell lung cancer.

Next, the levels of inflammatory mediators in BALF of both NSCLC patients and control subjects were determined. The data are summarized in Table 4. Comparable results were found for both soluble TNF receptors, sICAM-1, sE-selectin and the pro-inflammatory cytokines IL-6 and IL-8 between patients suffering from NSCLC and control subjects. TNF-α was not detectable in any of the BALF samples investigated (data not shown). Only small amounts of sTNF-R55, sE-selectin and IL-6 were present in BALF. The mean levels were near the detection limit of the ELISA which explains the narrow range observed for these parameters. In accordance with other studies, larger ranges were detected for the other inflammatory mediators. The analyses revealed no differences in levels of inflammatory mediators (data not shown).

The local inflammatory response was further investigated by measuring LDH and ALP in BALF as indicators of cell death and injury (36,37). Mean levels for LDH in the lung cancer population were 28 ± 3 U I⁻¹ in the affected lung and 29 ± 2 U I⁻¹ in the contralateral lung while mean level for LDH in control subjects was 31 ± 4 U I⁻¹ (normal range 10–50 U I⁻¹). ALP was neither detectable in BALF of patients suffering from NSCLC nor in BALF of control subjects (normal range 0–25 U I⁻¹).
TABLE 4. Inflammatory mediators in the bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th>NSCLC patients (n=20)</th>
<th>Tumour site</th>
<th>Contralateral</th>
<th>Controls (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTNF-R55 (ng ml⁻¹)</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>0.04 (0.01-0.40)</td>
<td>0.04 (0.01-0.48)</td>
<td>0.03 (0.01-0.27)</td>
</tr>
<tr>
<td>sTNF-R75 (ng ml⁻¹)</td>
<td>0.37 ± 0.07</td>
<td>0.30 ± 0.05</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>0.32 (0.01-1.10)</td>
<td>0.26 (0.02-0.87)</td>
<td>0.11 (0.07-0.48)</td>
</tr>
<tr>
<td>sICAM-1 (ng ml⁻¹)</td>
<td>103.6 ± 19.6</td>
<td>87.0 (20.6-410.2)</td>
<td>94.0 (28.0-462.0)</td>
</tr>
<tr>
<td></td>
<td>66.9 (19.0-326.0)</td>
<td>0.10 ± 0.10</td>
<td>0.10 (0.10-0.25)</td>
</tr>
<tr>
<td>sE-selectin (ng ml⁻¹)</td>
<td>0.12 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.10 (0.10-0.31)</td>
<td>0.10 (0.10-0.14)</td>
<td>0.10 (0.10-0.25)</td>
</tr>
<tr>
<td>IL-6 (ng ml⁻¹)</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.05 (0.01-0.13)</td>
<td>0.03 (0.01-0.11)</td>
<td>0.04 (0.02-0.08)</td>
</tr>
<tr>
<td>IL-8 (ng ml⁻¹)</td>
<td>0.36 ± 0.16</td>
<td>0.14 ± 0.03</td>
<td>0.26 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0.15 (0.01-3.1)</td>
<td>0.11 (0.02-0.39)</td>
<td>0.11 (0.01-0.93)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of the mean; the median is given with the range in parentheses. No significant differences were found between the different sites investigated, s, soluble; TNF-R55, TNF receptor 55; ICAM-1, intercellular adhesion molecule-1; NSCLC, non-small cell lung cancer.

Finally, the ability of AM to produce cytokines in vitro was determined. AM were obtained from both the affected and the contralateral lung of the lung cancer population and were activated in culture with different concentrations of LPS. Comparable results were demonstrated in cytokine production between AM obtained from the tumour lung and AM obtained from the contralateral lung either with or without stimulation of LPS (Fig. 1).

Discussion

Nowadays, BAL is a widely accepted tool for both diagnostic and research purposes to investigate the alveolar compartment. Enhanced levels of inflammatory mediators in BAL reflect the inflammatory state in the alveolar compartment of several pulmonary diseases such as pulmonary sarcoidosis, idiopathic pulmonary fibrosis, HIV-infected patients, chronic obstructive pulmonary disease, adult respiratory distress syndrome and pneumoconiosis (21-27). Previously, the authors demonstrated increased plasma levels of inflammatory mediators in NSCLC patients (7). In addition, a local inflammatory response around the tumour cells was demonstrated in tissue samples obtained from resection specimens of NSCLC patients (15,16). In the present study, however, the levels of inflammatory mediators in BALF of NSCLC patients were comparable with those in control subjects. In addition, no differences in levels of inflammatory mediators in BALF and cytokine profiles of AM were found between the affected and the contralateral lung in patients with NSCLC. The present data are in contrast with Arias-Diaz et al. (38) who found increased levels of TNF-α and IL-6 in BALF obtained from 22 patients with bronchogenic squamous carcinoma. Plumb et al., however, supported the present data (39); they reported no differences in cytokine profiles (IL-1 and TNF-α) in BALF between lung cancer patients and control subjects. Further, the present authors observed that the levels of the chemotactic cytokines IL-6 and IL-8 in BALF were similar between the NSCLC patients and control subjects. Since the expression of both TNF receptors on NSCLC cells was enhanced (15), both sTNF receptors were determined in BALF. Comparable results for both sTNF receptors were demonstrated in BALF obtained from the tumour lung and the contralateral lung as well as from the lung of control subjects. In addition, the soluble adhesion molecules E-selectin and ICAM-1 in BALF were determined. An enhanced expression of these adhesion molecules was observed in NSCLC (16). The soluble forms of these adhesion molecules are considered to represent inflammatory activity as well (19). No differences were found for sE-selectin and sICAM-1 in BALF between NSCLC and controls either.

The present study did not demonstrate an enhanced inflammatory state in the alveolar compartment of NSCLC patients. The data suggest that no damage of the alveolar membrane was present and, as a consequence, no leakage of inflammatory mediators occurred. This hypothesis is supported by the absence of signs of cell damage in BALF; no enhanced levels of LDH or ALP, known as parameters for cell damage or cell death, were detected. Comparable results were found in cellular distribution of BALF samples obtained from the three groups. Moreover, no malignant cells were found in BALF obtained from the tumour site.

Another explanation for the absence of an inflammatory state in the alveolar compartment might be that the tumour itself or the infiltrating cells produce immunosuppressive molecules that affect the production of cytokines by AM in the alveolar compartment.
Immunosuppressive molecules such as IL-10, nitric oxide, prostaglandin E2, and sTNF-receptors are produced by tumour cells and macrophages in the environment of the tumour (40–46). TNF receptors can have an immunosuppressive function by binding to biologically active TNF-α. However, the authors observed an enhanced expression of both TNF receptors in the interstitial compartment but not in the alveolar compartment. Increased levels of nitrite/nitrate and prostaglandin E2 have been demonstrated in BALF of patients with primary lung cancer (38, 46).

Beside these possible explanations for the absence of an enhanced inflammatory state (as reflected by BALF analysis) in the alveolar compartment next to the tumour, smoking behavior needs to be taken into account as well. Smoking can affect the total and differential cell count of the BALF and changes the viability and cytokine production of AM. Reduced production of TNF-α, IL-1β and IL-6 by AM has been demonstrated in smokers by several authors (47–49). In the present study, all patients and control subjects were smokers or stopped smoking recently, and no significant differences were detected in smoking behavior between patients suffering from NSCLC and control subjects. Although possible differences in BALF analysis could be hypothesized in non-smoking patients, the dominant role of cigarette smoking in lung cancer causation evidenced the study of inflammatory changes in smoking or ex-smoking lung cancer patients.

In conclusion, comparable results in levels of inflammatory mediators in BALF were found between NSCLC patients and control subjects. In addition, no differences in cytokine profiles of AM were found between the affected lung and the contralateral lung in patients with NSCLC. Although the BAL is a useful tool in the diagnostic procedure for NSCLC, the present findings suggest that BAL does not reflect the enhanced inflammatory state as reported in plasma and in the interstitial compartment around the tumour cells in NSCLC.

Acknowledgements

The authors thank Celltech, Slough, U.K. for providing the NSO-10 and NSO-23 cells producing, respectively, the extracellular parts of both TNF-R55 and TNF-R75; NSO cells designated sICAM-1/2, producing sICAM-1 and CHO-cells producing SE-selectin. Human recombinant IL-6 was kindly provided by Prof. W. Sebald, Physiologisch-Chemisches Institut der Universität, Würzburg, FRG. Human recombinant TNF-α was kindly provided by BASF/Knoll, Ag Ludwigshafen, FRG.

The authors thank Prof. Dr M. van Dieijen-Visser for performance of the biochemical analyses of the BALF.

References


