Leukocyte Infiltration and Secretion of Cytokines in Pleural Drainage Fluid After Thoracic Surgery*

Impaired Cytokine Response in Malignancy and Postoperative Complications

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Study objective: To assess the postoperative course of pleural leukocyte counts and cytokine concentrations in patients with malignant and nonmalignant lung disease who underwent thoracic surgery.

Patients and interventions: A total of 21 patients undergoing thoracic surgery were included in the study. Twelve patients had a malignant disease, and 9 had a nonmalignant disease. Six patients underwent video-assisted thoracoscopy and 15 underwent thoracotomy. Pleural drainage fluid from the chest tubes was collected postoperatively at 0h, 3h, 6h, 12h, 24h, 48h, 72h, and 96 h. The same schedule, as well as one additional preoperative sample, was applied for blood collections.

Results: A trend toward lower concentrations of tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor, and interleukin-10 was observed in patients with malignant disease compared to those without malignancy. These differences achieved significance for TNF-α in the drainage fluid of those patients with nonmalignant disease who had undergone formal thoracotomy. Patients with malignant disease showed significantly lower macrophage fractions in drainage fluid and lymphocyte fractions in serum. All patients with complications had malignant disease and showed the lowest cytokine concentrations, as well as the lowest fractions of both macrophages in drainage fluid and lymphocytes in serum.

Conclusion: The data suggest that malignancy may lead to impairment of the wound-healing process via modification of the inflammatory cell infiltrate and locally released cytokines.

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Key words: bronchogenic carcinoma; granulocyte-macrophage colony-stimulating factor; postsurgical cytokine release; postsurgical leukocyte mobilization; thoracic surgery; tumor necrosis factor-α

Abbreviations: GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; TNF-α = tumor necrosis factor-α

Postsurgical repair of tissue involves a variety of cellular responses from leukocytes, thrombocytes, and fibroblasts. Apart from complement factors and platelet-activating factor, cytokines function as important mediators in the communication between cells. Immediately after surgical injury, neutrophil granulocytes are the dominant leukocyte subpopulation, whereas approximately 12 h later, an increase in macrophages and monocytes occurs.

For editorial comment see page 1489

The lymphocyte subpopulation decreases during the first days after an operation. The release of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), or interleukin (IL)-6, as well as the release of anti-inflammatory cytokines, such as...
IL-10 and TNF-β is stimulated by leukocytes, particularly macrophages and monocytes. Cytokines and cells interact within a complex network. The cells are coordinated both via the autocrine and paracrine activities of cytokines and other mediators. Identical cytokines can be produced by different cell types, such as macrophages and monocytes, lymphocytes, fibroblasts, and endothelial cells.

Patients with a malignant disease may have an altered immunologic status which, in turn, may reduce immunologic control of infectious agents. Although the precise nature of cancer-associated immune alterations is unknown, the immunologic changes may be caused by the induction of tolerance to certain antigens or the secretion of immunosuppressive cytokine (such as transforming growth factor-β) by the tumor itself. 

Because postsurgical repair processes are closely linked to the release of certain cytokines by activated lymphocytes, macrophages, and neutrophils, postoperative complications may be related to tumor-associated immunosuppression. However, early patterns of the occurrence of inflammatory cells and the release of cytokines immediately after thoracic surgery and their temporal relationship to wound healing and postoperative complications have been poorly studied.

The present descriptive study attempts to define these early patterns by measuring sequential levels of both serum and pleural-drainage cytokines simultaneously and using the kinetics of local cell infiltration. The temporal patterns seen in patients with lung cancer and metastases of the lung were compared with those seen in patients without malignant disease.

Materials and Methods

Patients

The study group was recruited from patients treated in the Department of Pulmonary Surgery, Albert-Ludwigs-University, Freiburg, Germany, between July and August, 1994. We applied the following exclusion criteria for patient selection: (1) an emergency operation; (2) a refusal to participate in the study; (3) surgical interventions that were not expected to deliver sufficient volumes of drainage fluid from the chest tube; and (4) surgical procedures without indications for a chest tube.

A total of 21 patients, 4 women and 17 men, were included in the study. Their mean age was 56.7 years old (range, 20 to 78 years old). Six patients underwent video-assisted thoracoscopy and 15 underwent thoracotomy. Twelve had an underlying malignant disease: 8 with primary bronchogenic carcinoma in stages I to III, 3 with metastases of the lung, and 1 with pleural mesothelioma. Diagnostic specimens obtained from the eight patients with primary lung tumor revealed the following: large cell carcinoma (three patients), squamous cell carcinoma (two patients), adenocarcinoma (one patient), undifferentiated carcinoma (one patient), and small cell carcinoma (one patient). The nine patients with nonmalignant disease had the following diagnoses: benign lung tumor (one patient), mediastinal tumor (one patient), relapse of pneumothorax (two patients), and inflammatory and other diseases of the lung (five patients). A thoracotomy was performed in all 12 patients who had a malignant disease, as well as in 3 patients with a nonmalignant disease. Six patients underwent video-assisted thoracoscopy, and all had a nonmalignant disease. The following surgical interventions were carried out in the thoracotomy patients: three pneumonectomies, six lobectomies, three wedge or segmental resections, two metastasectomies, and one evacuation of hemithorax. The specification of the videothoracoscopical interventions documented the following: three wedge resections, one tumor extirpation, and two parietal pleurectomies.

One patient died of irreversible cardiac arrest a few hours after hemodialysis for preexistent renal insufficiency and perioperative renal failure. In one diabetic patient, a bronchopleural fistula developed after undergoing left upper lobectomy; in another, a purulent pleural infection developed. *Staphylococcus aureus* and Enterococcus sp were identified in the drainage fluid in both patients. One patient suffered from a stroke on the 12th postoperative day with transient, completely reversible left hemisindrome. All of these patients with complications had malignant disease, open thoracotomy, and major resections as surgical interventions.

Methods

All measurements with the exception of blood counts (performed in the central laboratory of the university hospital) were carried out in the research laboratory of the Department of Pneumology, Albert-Ludwigs-University, Freiburg, Germany. Peripheral venous blood was obtained 24 h to 4 h before the operation for the assessment of cell counts and the concentration of the cytokines TNF-α, GM-CSF, and IL-10. Further controls of blood counts were taken 24 h and 96 h after the operation. For determination of cytokine levels, blood samples were taken immediately, and 3 h, 6 h, 12 h, 24 h, 48 h, 72 h, and 96 h after the operation. This time schedule was also applied for the collection of 5 to 10 mL of drainage fluid from chest tubes for the determination of cytokine levels and leukocyte subpopulation counts. We collected a complete set of samples for 9 patients over the 96-h observation period. In the remaining 12 patients, sampling was discontinued because either the chest tubes were no longer indicated or the volume of drainage fluid was insufficient for measurements.

Drainage fluid was collected in a small tube containing 0.5 mL EDTA and stored at 4°C. This tube was connected to the chest tube for not more than 30 min. Drainage fluid was immediately separated by centrifugation at 1,500 g (Hettich Rotaxa centrifuge; Hettich; Tuttinglen, Germany) for 10 min at 25°C and stored at −20°C until further use. Sediment smears were prepared on slides and stained (May-Gruenwald-Giemsa; Merck; Darmstadt, Germany) for later microscopic determination of leukocyte subpopulation counts. Blood samples were stored for 30 min at room temperature after centrifugation at 3,000 g for 10 min (Heraeus Christ Medifuge; Heraeus; Stuttgart, Germany). Centrifuged samples were stored at −20°C until assayed.

For measuring levels of cytokines TNF-α, GM-CSF, and IL-10, we used the enzyme-linked immunosorbent assay technique as described. After being thawed, aliquots of serum and drainage fluid were shortly whirled (REAX-2000-Whirler; Heidel; Kehlheim, Germany) before samples were filled in wells of assay plates. We used appropriate antibodies (Pharmingen; San Diego, CA), standards of TNF-α and GM-CSF (PBH; Hannover, Germany) and IL-10 (Pharmingen). Cytokine concentrations were measured photometrically at an optical density of 405 nm.
Statistical Analysis

The differences in cytokine levels and cell numbers among patient groups were evaluated using the Mann-Whitney test. p values < 0.05 were considered significant.

Results

There was considerable variance in the concentrations of cytokines TNF-α, GM-CSF, and IL-10 in drainage fluid as well as in serum. Even within the two patient groups (subdivided according to malignant and nonmalignant disease), variation of data was observed. Figure 1 depicts the average concentrations of TNF-α, GM-CSF, and IL-10 in drainage fluid for 9 patients with nonmalignant disease and 12 with malignant disease, among whom 4 patients developed postoperative complications. Data analysis for this subgroup was omitted at 48 h after the operation due to the small number of patients available. In the drainage fluid of patients with nonmalignant disease, a distinct increase in the TNF-α and GM-CSF concentrations (eg, at 12 h, 2,352 pg/mL vs 343 pg/mL, and 1,043 pg/mL vs 127 pg/mL, respectively) was detected, which could not be observed in those with malignant disease. Samples from the malignancy patients with complications showed low concentrations (at 12 h, 84 pg/mL for TNF-α and 80 pg/mL for GM-CSF). However, comparison of these patient groups did not show any differences of statistical significance. In contrast to levels of TNF-α and GM-CSF, concentrations of IL-10 increased during the first 3 h in patients with malignant disease and declined thereafter (Fig 1, bottom, C). This peak was not observed in patients in whom postoperative complications developed.

According to the applied surgical technique, patients with nonmalignant disease could be subdivided into a video-assisted thoracoscopy group (n = 6) and a thoracotomy group (n = 3). Figure 2 shows the average concentrations of TNF-α, GM-CSF, and IL-10 in drainage fluid for these patient groups. The comparison revealed significantly higher (p < 0.05) concentrations of TNF-α at 0 h, 3 h, 6 h, and 12 h (4,608 pg/mL vs 154 pg/mL, 5,877 pg/mL vs 109 pg/mL, 6,368 pg/mL vs 117 pg/mL, 6,204 pg/mL vs 37 pg/mL, respectively), and, at 0 h, a significantly higher concentration of GM-CSF (1,944 pg/mL vs 271 pg/mL) in the thoracotomy group. Compared to patients with malignant disease (all underwent thoracotomy), those with nonmalignant disease and thoracotomy showed significantly higher concentrations of TNF-α at 0 h and 6 h (4,608 pg/mL vs 448 pg/mL, and 6,386 pg/mL vs 380 pg/mL, respectively). A trend toward higher concentrations in nonmalignant disease could also be observed for IL-10. When serum cytokine concentrations were measured, a comparable difference between patient groups could be observed (data not shown). However, no statistical significance was reached.

Figure 3, top, A, shows the time-related differential leukocyte count distribution. Leukocyte counts in chest tube drainage fluid taken 12 h and 48 h after operation showed significantly lower fractions of macrophages (1.8% vs 9.4%, and 2.0% vs 10.7%, respectively) in patients with malignant disease when compared to those with nonmalignant disease. In both groups, an early postoperative decrease in
A macrophage fractions could be observed. An increase in cell numbers occurred already after 12 h in patients with nonmalignant disease. In contrast, a rise in macrophage counts in the corresponding group was detected 72 h after surgery. The kinetics of macrophage counts in patients with postoperative complications was similar to that observed in malignant disease. After a small initial postoperative increase of neutrophil granulocytes (Fig 3, bottom, B), we detected a decrease after 12 h in patients with nonmalignant disease, and after 72 h in patients with malignant disease.

As shown in Table 1, leukocyte counts in serum documented 24 h after surgery showed a significantly lower percentage of lymphocytes in patients with malignant disease compared to those with nonmalignant disease (11.6% vs 16.8%). Generally, there was a trend toward low relative numbers of lymphocytes.

### Table 1—Differences in the Relative Number of Lymphocytes and Neutrophils in Blood Before and During the First 96 h After Thoracic Surgery*

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Nonmalignant</th>
<th>Malignant</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>26.9 ± 6.6</td>
<td>21.6 ± 9.9</td>
<td>12.9 ± 5.7</td>
</tr>
<tr>
<td>24</td>
<td>16.8 ± 3.2</td>
<td>11.6 ± 3.5</td>
<td>9.7 ± 3.5</td>
</tr>
<tr>
<td>96</td>
<td>23.4 ± 8.9</td>
<td>15.3 ± 4.4</td>
<td>11.8 ± 1.9</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>60.5 ± 5.7</td>
<td>66.5 ± 9.9</td>
<td>75.9 ± 8.1</td>
</tr>
<tr>
<td>24</td>
<td>71.3 ± 5.9</td>
<td>81.2 ± 3.1</td>
<td>83.0 ± 4.7</td>
</tr>
<tr>
<td>96</td>
<td>64.6 ± 9.8</td>
<td>73.1 ± 5.7</td>
<td>76.4 ± 0.9</td>
</tr>
</tbody>
</table>

*Data are expressed as percent of total leukocyte number and represent mean ± SEM. n = 4–12 per value. 0 = preoperative.
in patients with malignant disease during the course of the study, and a trend toward even lower fractions of lymphocytes in patients with postoperative complications. However, neutrophil granulocyte counts revealed a reverse relationship between the patient groups. The comparison of leukocyte counts according to the surgical technique (video-assisted thoracoscopy vs thoracotomy) did not show any significant differences (data not shown).

**Discussion**

In this descriptive study, simultaneous measurements of cell kinetics and cytokine concentrations in serum and drainage fluid were made over time in patients who underwent thoracic surgery. The temporal cytokine and cell patterns seen in malignant disease were compared with those in nonmalignant disease. The data presented in this study show that in the drainage fluid of patients with nonmalignant disease, the concentrations of TNF-α, GM-CSF, and IL-10 are higher than they are in patients with malignant disease. The differences in cytokine concentration achieved statistical significance for TNF-α in the drainage fluid of nontumor patients who had undergone thoracotomy. Furthermore, cytokine concentrations were elevated in patients who had undergone thoracotomy when compared to the less invasive video-assisted thoracoscopy. In addition, those with malignant disease showed higher neutrophil counts in drainage fluid and serum, but strikingly fewer macrophages in drainage fluid and lymphocytes in serum. The above data suggest that both the presence of malignant disease and the invasive nature of surgery determines the subsequent immune response.

Mononuclear cell infiltration is a characteristic feature of wounds and may play an important role in the healing process. In this study, we observed differences in the relative cell counts with lower macrophage fractions in drainage fluid as well as decreased lymphocyte numbers in serum, both of which are associated with malignancy and postoperative complications. Changes in cell number and composition after surgical tissue trauma have been described in different animal models and in humans. According to these studies, healing is a complex process, essentially requiring the infiltration of mononuclear cells. Thus, the reduced number of macrophages recovered from the postsurgical pleural fluid of patients with malignancy points to an altered microenvironment, possibly associated with a delayed wound-healing process.

Although the cause of these changes in cellular distribution is difficult to explain, the changes may reflect a modified spectrum of cytokines released during the course of postsurgical inflammatory response. To support this view, we also examined the kinetics of cytokine secretion. The data show that, as opposed to those with nonmalignancy, patients with malignant lung disease or metastases of the lung failed to mount a complete postoperative cytokine response as assessed by measuring levels of TNF-α, GM-CSF, and IL-10. Although the difference between the IL-10 levels seen in malignancy patients and those seen in nonmalignancy patients was comparatively small (2-fold), the concentration of the other cytokines in drainage fluid of patients with nonmalignant disease exceeded that obtained from patients with malignancy by 5- to 10-fold. Whether this finding relates to an overall impaired immune status of patients with cancer is discussed below.

Malignancy has long been associated with an altered immunologic competence of the host, although the mechanisms involved are not yet fully understood. Numerous hypotheses have been proposed to explain this altered host immune response. For instance, tumors may secrete immune modulatory factors that downregulate immune competence, a process that has been observed in both small cell lung cancer and squamous cell carcinoma of the lung. As a consequence, lymphocytes from patients with lung cancer may fail to provide adequate support to other immunologic cells, such as macrophages. Tumor-associated immunosuppressive cytokines may not only hamper the tissue infiltration of inflammatory cells but also suppress cellular development and maturation in the bone marrow. Because the accumulation of inflammatory cells and the secretion of cytokines are closely linked to postsurgical wound healing, tumor-dependent immunosuppression may affect the postoperative outcome. Thus, the reduced concentrations of TNF-α, GM-CSF, and IL-10, in conjunction with reduced numbers of macrophages in drainage fluid, may indeed be due to immunosuppression associated with the underlying malignant disease. Still, on the basis of the data presented, a cause-and-effect relationship between malignancy and cytokines levels cannot be proven.

Another possible reason for the difference in pleural cytokine concentrations may relate to the chosen surgical procedure. When patients with nonmalignant disease were further subdivided into those undergoing thoracotomy or videothoracoscopy, lower levels of TNF-α, GM-CSF, and IL-10 were detected in the videothoracoscopy subgroup. Patients with malignant disease all underwent thoracotomy. When pleural drainage fluid cytokine levels in patients with malignant disease were compared with those in thoracotomy patients with nonmalignant
disease, the difference was particularly striking. These data suggest that in addition to the underlying disease of the patients, the nature of the surgical procedure modifies postoperative cytokine secretion as a second influencing factor.

In addition to its in vitro cytotoxicity against various tumor cell lines, TNF-α may induce in vivo hemorrhagic necrosis in several tumors. In addition, TNF-α also acts on a variety of other cells, thus enhancing the inflammatory and immune processes. It activates polymorphonuclear leukocytes, enhances T-cell responses, and modulates B-cell differentiation. Moreover, TNF-α stimulates the production of several cytokines, such as IL-1, IL-6, and GM-CSF. Furthermore, TNF-α also acts synergistically with IL-1 in stimulating fibroblasts from human lungs, thereby facilitating the tissue-healing process after surgical trauma. The role of TNF-α in the wound-healing process may explain why, in patients with a lower pleural drainage fluid cytokine level, the probability of developing post-surgical complications was higher. Moreover, the GM-CSF level in both serum and drainage fluid after surgery was characteristically decreased in these patients. Therefore, the data reported in this paper support the concept that the underlying disease may determine the inflammatory response after surgical trauma.

Cytokines can be used both as indicators of clinical prognosis and as therapeutic agents. Recent data show that impairment of IL-2 secretion significantly correlates with survival in small cell lung cancer. The data presented in this study extend this observation and suggest that measuring TNF-α levels in drainage fluid and serum may offer the possibility of assessing the perioperative risk. While TNF-α levels achieved either locally or systemically may determine the course of the disease and the development of complications, cytokine treatment may represent an adequate approach to modifying outcome. In contrast to other diseases such as rheumatoid arthritis, in which blocking TNF activity may be beneficial to local healing processes, substitution of TNF-α may help to accelerate healing and prevent complications. In addition, GM-CSF has been shown to facilitate the wound-repair process via induction of cellular infiltration, activation of various cells, and synthesis of α-smooth muscle actin in myofibroblasts. Because of the relative deficiency of this cytokine in patients with surgery-associated complications, substitution of GM-CSF may represent another possible therapeutic approach aimed at preventing postoperative problems.

Although the number of patients included in this study is limited, the preliminary data presented suggest that both the malignant condition and the surgical technique determine the tissue infiltration of inflammatory cells and the secretion of cytokines during wound healing. Because TNF-α and possibly GM-CSF are proinflammatory cytokines involved in tissue remodeling, these data indicate that cytokines may play an important role in the development of postsurgical complications. The findings presented in this study suggest that certain cytokines could be employed to identify patients at risk for surgical complications and to design preventive therapeutic approaches. Future work defining the mechanisms underlying the above data as well as its clinical application are warranted.

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CHEST / 115 / 6 / JUNE, 1999 1609


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