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Kinetics of the Levels of Pro- and Anti-Inflammatory Cytokines and Vascular Endothelial Growth Factor in Serum and Pleural Fluid after Major Lung Resection for Lung Cancer

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Some pro- and anti-inflammatory cytokines and angiogenesis-related factors play important roles in the inflammatory response after surgery. To study their kinetics and mutual relationships after major lung resection for lung cancer, concentrations of interleukin (IL)-6, -8 and -10 and vascular endothelial growth factor (VEGF) were measured in serum and pleural fluid. Venous blood and pleural fluid samples were collected before and up until 5 days after surgery from 10 patients with lung cancer, treated by standard lobectomy at Tottori University Hospital between 1997 and 1999. Cytokine levels after surgery were significantly higher than control levels before surgery in serum and pleural fluid. In pleural fluid, cytokines and VEGF increased after surgery 10 to 100 times than in serum. IL-6 and -10 reached their peaks in pleural fluid later than in serum, and then gradually decreased. Serum IL-8 reached its peak at 3 h, decreased until day 2 and then remained stable. Serum VEGF decreased until 3 h, and then showed little change. Pleural fluid IL-8 and VEGF reached their peaks at 3 or 6 h, decreased until day 2 and then increased up to day 5. The amount of cytokines produced per hour in drainage pleural fluid showed nearly the same kinetics as serum cytokines. Serum cytokines and VEGF showed higher coefficient values to the hourly production in pleural fluid than to the pleural fluid levels. During wound healing, IL-8 acted as a pro-inflammatory cytokine in the early phase, but as an angiogenic factor like VEGF in the late proliferative phase. The cytokine production per hour in pleural fluid is believed to be a useful marker of cytokine levels for evaluating surgical trauma after major lung resection for lung cancer.

Key words: cytokine; cytokine production per hour (hourly production of cytokine); lung cancer; vascular endothelial growth factor; wound healing

Interleukin (IL)-6 and IL-8 are known as pro-inflammatory cytokines, and IL-10 as an anti-inflammatory cytokine. There have been many reports about the kinetics of pro- and anti-inflammatory cytokine levels after surgery, including thoracic procedures (Steinberg et al., 1993; Sakamoto et al., 1994; Kawahito et al., 1995; Waller et al., 1996; Atwell et al., 1998; Liebold et al., 1999; Weissflog et al., 1999). However, most reports have described serum levels of cytokines, and few have reported their pleural fluid levels (Sakamoto et al., 1994; Weissflog et al., 1999). Thus, we thought it would be interesting to investigate the relationship of cytokine levels between serum and pleural fluid. In addition, vascular endothelial growth factor (VEGF) known as an important angiogenic factor (Fontanini et al., 1997; Inoue et al., 1997), has recently been demonstrated as acting together with pro- and anti-inflammatory cytokines (Ishimura et al., 1998; Nissen et al., 1998; Sato et al., 1999). In the present study, to clarify whe-

Abbreviations: IL, interleukin; VEGF, vascular endothelial growth factor

ther IL-6, IL-8, IL-10 and VEGF are associated after surgery for lung cancer, the kinetics of the levels of IL-6, IL-8, IL-10 and VEGF in both serum and pleural fluid were investigated.

Materials and Methods

Patients

The subjects were recruited from non-small cell lung cancer patients who underwent standard lobectomy by posterolateral thoracotomy with mediastinal nodal dissection in Tottori University Hospital between October 1997 and June 1999. We applied the following exclusion criteria for patient selection: i) incomplete resection, because the residual tumor has the potential to affect the cytokine levels (Matsuguchi et al., 1991; Yamamoto et al., 1996); ii) extended resection of the chest wall, diaphragm or mediastinum, because different degrees of surgical trauma have the potential to affect the cytokine levels (Sakamoto et al., 1994); iii) administration of perioperative ulinastatin or corticosteroid, because these agents influence cytokine release (Teoh et al., 1995; Kawamura et al., 1996) and iv) complication by pulmonary fibrosis, because this condition has the potential to affect the IL-8 level (Keane et al., 1997).

A total of 10 patients were examined in this study. They ranged in age from 49 to 76 years (mean, 64.3 years), consisting of 5 males and 5 females. Histologically, 3 patients suffered with squamous cell carcinomas, 6 patients with adenocarcinomas and 1 patient with mucoepidermoid carcinoma. Seven patients were in Stage I, one was in Stage II and two were in Stage III. All patients gave written informed consent. After tracheal intubation (double lumen endotracheal tube), general anesthesia was applied in combination with a thoracic epidural anesthesia in all patients. The thoracic epidural catheter was used for postoperative analgesia. Two thoracic tubes were indwelled in all patients, and the pleural fluid was constantly aspirated with –10 cmH_2O pressure. The duration of the surgery was 304.9 ± 13.3 min and the bleeding volume was 181.3 ± 57.3 mL. There were no severe postoperative complications such as respiratory failure, pneumonia, liver dysfunction or renal failure in these patients.

Blood and pleural fluid collection

Samples of venous blood were collected immediately after induction of anesthesia, and served as controls. Samples of pleural fluid were collected by intrathoracic lavage with 50 mL of physiological saline just after thoracotomy, and used to determine the preoperative control data of pleural fluid.

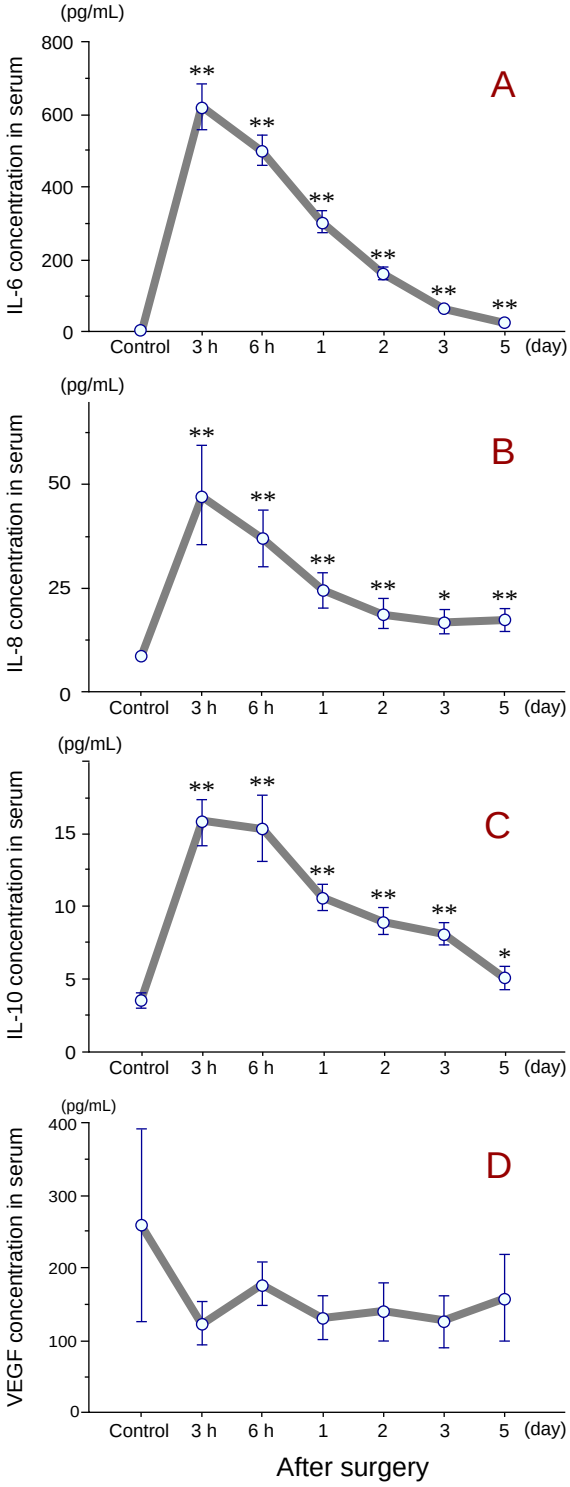
The other samples of serum and pleural fluid were simultaneously collected at 3 and 6 h and on days 1, 2, 3 and 5 after surgery. On days 1, 2, 3 and 5, the samples were collected 9:00 in the morning. All samples were centrifuged immediately at 2,500 rpm for 15 min at 4˚C. Centrifuged samples were stored at –30˚C until the assays were performed.

Cytokine assays

IL-6, IL-8, IL-10 and VEGF concentrations were measured using commercial enzyme-linked immunosorbent assay kits (Genzyme, Cambridge, MA for IL-6 and IL-10; Endogen, Woburn, MA for IL-8; Techne, Minneapolis, MN for VEGF).

Statistical analysis

Statistical analysis was performed with StatView 5.0 J software (Abacus, Concepts, Inc., Berkeley, CA). Differences in the kinetics of cytokine and VEGF levels were analyzed using Friedman's test and Wilcoxon's signed rank test. Correlations between the serum and pleural fluid levels of cytokines and VEGF were analyzed using Spearman's test. Results are expressed as mean ± SEM. P values of < 0.05 were considered significant.

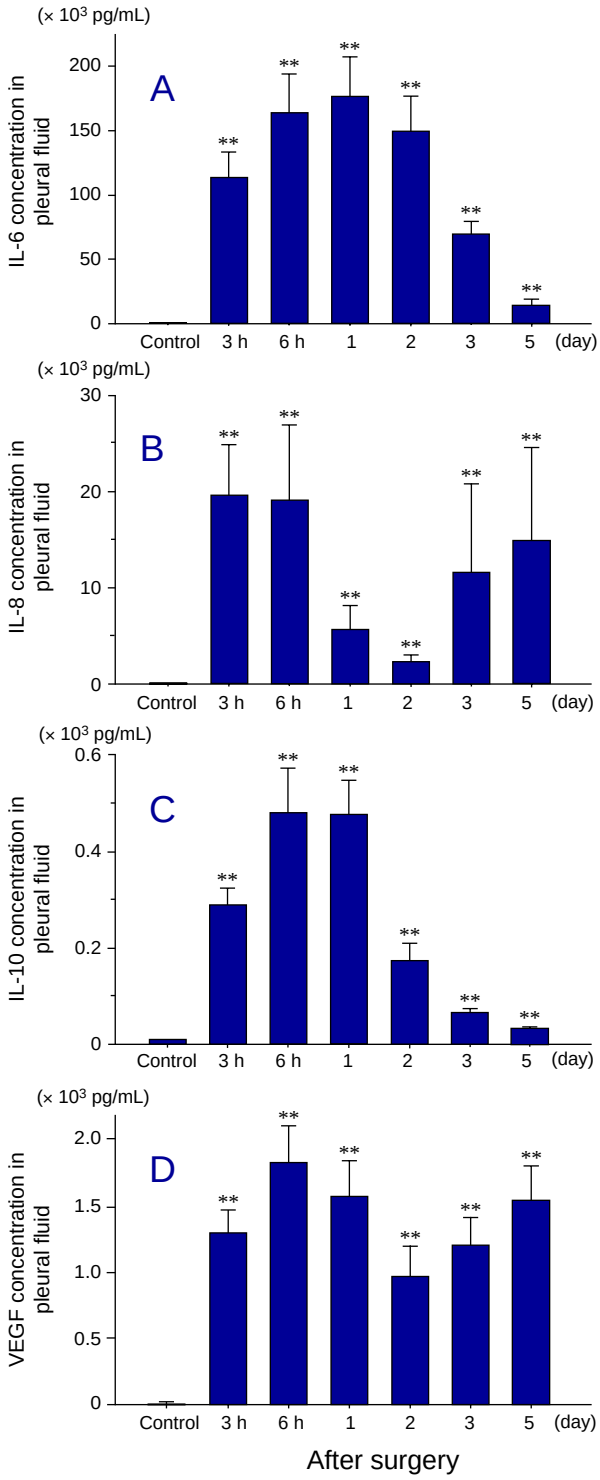


Results

Kinetics of serum cytokine and VEGF levels

Serum levels of cytokines after surgery (Figs. 1A–C) were significantly higher than control levels throughout. IL-6 (Fig. 1A) and IL-10 (Fig. 1C) reached their peaks at 3 h (IL-6: 626.8 ± 63.0 pg/mL, IL-10: 15.9 ± 1.6 pg/mL) and gradually declined with time. Serum IL-8 (Fig. 1B) showed its peak at 3 h (47.5 ± 12.0 pg/mL), declined until day 2 and then remained stable. However, serum VEGF levels (Fig. 1D) decreased until 3 h, followed by little change.

Figs. 1A–D. Kinetics of serum levels of cytokines and VEGF measured before and after lung resection. $*P < 0.05$ and $**P < 0.01$ compared with the preoperative control level. The data are presented as mean ± SEM. IL, interleukin; VEGF, vascular endothelial growth factor.

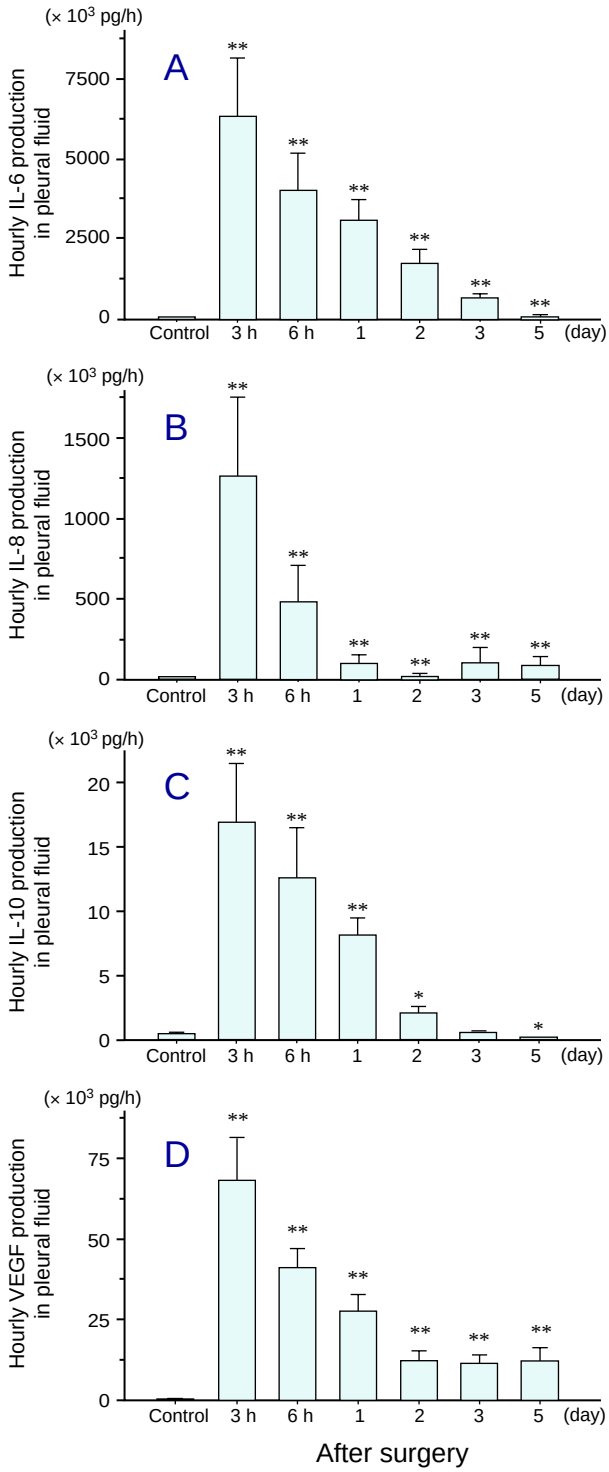


Kinetics of drainage fluid cytokine and VEGF levels

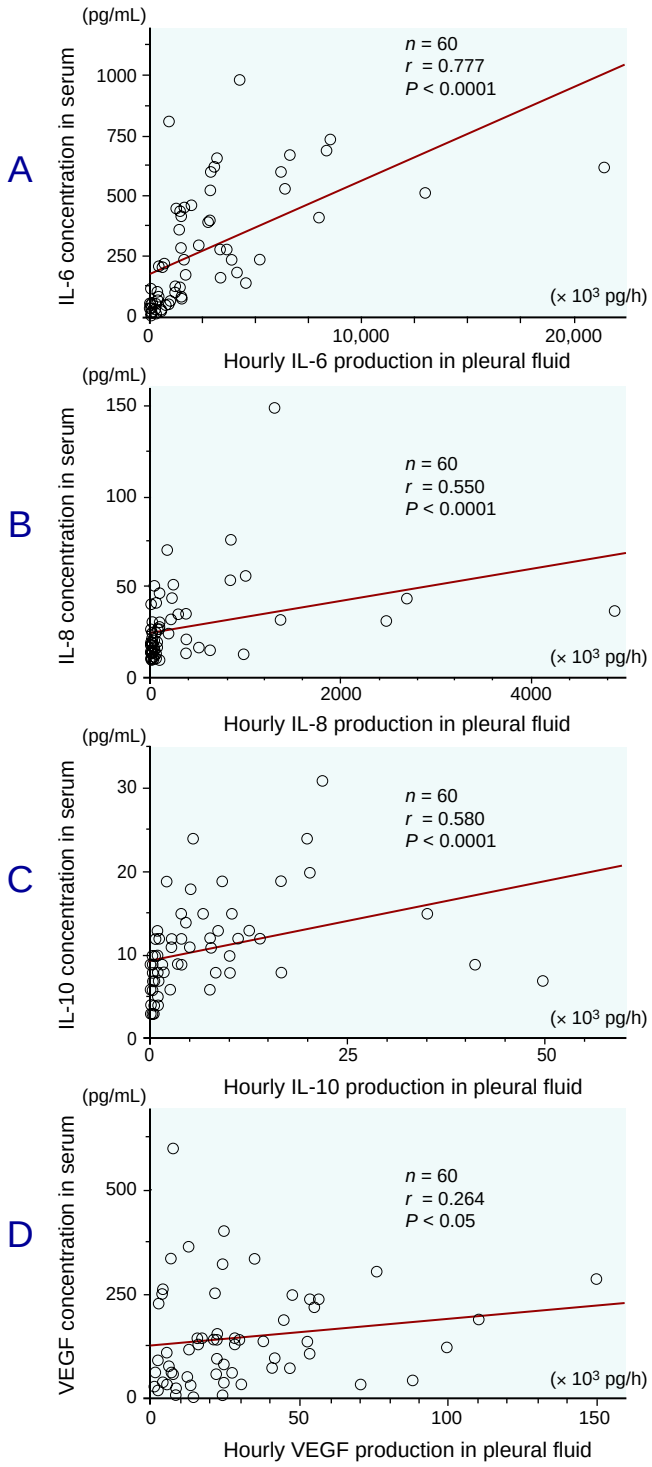
Pleural fluid levels of cytokines and VEGF (Figs. 2A–D) after surgery were significantly higher than control levels throughout. IL-6 and IL-8 were about 100-fold and IL-10 and VEGF were about 10-fold higher in concentration than in serum. Concerning level changes, IL-6 (Fig. 2A) and IL-10 (Fig. 2C) reached their peaks (IL-6: 177,000 ± 30,600 pg/mL, IL-10: 481.0 ± 91.8 pg/mL) 6 h or 1 day after surgery, later than in the patterns seen in serum. In pleural fluid, however, IL-8 (Fig. 2B) and VEGF (Fig. 2D) levels showed their peaks in the early phases, at 3 or 6 h (IL-8: 19,700 ± 5030 pg/mL, VEGF: 1838 ±

Figs. 2A–D. Kinetics of pleural fluid levels of cytokines and VEGF measured before and after lung resection. **P* < 0.05 and ***P* < 0.01 compared with the preoperative control level. The data are presented as mean ± SEM. IL, interleukin; VEGF, vascular endothelial growth factor.

182 pg/mL), decreased until day 2 (IL-8: 2286 ± 787 pg/mL, VEGF: 980 ± 223 pg/mL) and elevated again during the late phase up to day 5 (IL-8: 14,900 ± 9580 pg/mL, VEGF: 1553 ± 261 pg/mL). When the amount of cytokines produced per hour (hourly production) in pleural fluid was calculated by multiplying the concentration by drainage volume per hour, the hourly cytokine production in pleural fluid (Figs. 3A–C) showed nearly the same kinetics as serum concentration kinetics (Figs. 1A–C).



Figs. 3A–D. Kinetics of hourly production in pleural fluid of cytokines and VEGF measured before and after lung resection. **P* < 0.05 and ***P* < 0.01 compared with the preoperative control level. The data are presented as mean ± SEM. IL, interleukin; VEGF, vascular endothelial growth factor.



Relationships of cytokine and VEGF levels between serum and pleural fluid

All factors showed significant correlations between serum levels and hourly productions in pleural fluid (IL-6: $r = 0.777$, IL-8: $r = 0.550$, IL-10: $r = 0.580$, VEGF: $r = 0.264$) (Figs. 4A–D). These values of coefficients were higher than those between serum levels and pleural fluid levels for all factors (IL-6: $r = 0.517$, IL-8: $r = 0.494$, IL-10: $r = 0.564$, VEGF: $r = 0.235$).

Discussion

Various cytokines with a wide range of biological activities play the role of controlling inflammatory and immunological reactions to surgical trauma. In the present study, the concentrations of cytokines in pleural fluid were about 100-fold greater than those in serum. Previous researchers also reported that the IL-6 and IL-8 levels in thoracic drainage fluid were about 100- to 1000-fold greater than those in peripheral blood (Sakamoto et al., 1994; Krohn et al., 1998). Therefore, it is very clear that the cytokine response is much stronger in the local pleural cavity than in the systemic blood.

Figs. 4A–D. Relationships between serum levels and hourly productions in pleural fluid studied for cytokines and VEGF. IL, interleukin; VEGF, vascular endothelial growth factor.

In our present study, the kinetics of cytokine levels in serum and pleural fluid showed significant correlations. In particular, the correlation between the hourly cytokine production in pleural fluid and the serum cytokine level was higher than that between the pleural fluid cytokine level and the serum cytokine level. Sakamoto et al. (1994) reported that the messenger RNAs of cytokines could not be detected in leukocytes from the peripheral blood but could be demonstrated in leukocytes from drainage fluid: they suggested that cytokines are induced and secreted mainly in the surgical field and simultaneously appear in the blood stream. Asadullah et al. (1995) reported data from neurosurgery supporting this hypothesis. Regarding the mechanism of this effect, we consider it possible that pleural cytokines spill over into the peripheral blood through pleural absorption. Therefore, when the concentrations of cytokines in pleural fluid are markedly higher than serum cytokine levels, such as after thoracic surgery, serum cytokine levels are strongly influenced by the amount of cytokines produced per hour in pleural fluid. Pleural fluid can be collected because the thoracic tube is always indwelled after thoracic surgery. Thus, the hourly production of cytokines in pleural fluid appears to be a new useful marker of cytokine levels for evaluating the degree of trauma caused by thoracic procedures.

IL-8 is a pro-inflammatory cytokine and has a potent chemoattractant activity for neutrophils (Baggiolini et al., 1989). Recent studies have shown that the serum IL-8 level peaked within 24 h after surgery and decreased thereafter (Sakamoto et al., 1994; Atwell et al., 1998; Liebold et al., 1999). However, few studies have examined the kinetics of IL-8 later than 3 days after surgery, particularly after major lung surgery. In the present study, the kinetics of the IL-8 level in pleural fluid showed 2 significant peaks postoperatively, at 3 or 6 h and again on day 5. Until day 2 after surgery, IL-8 functioned as a pro-inflammatory cytokine. However, re-elevation of IL-8 later than day 3 did not necessarily indicate its function as a pro-inflammatory cytokine at that time. Koch et al. (1992) reported that IL-8 has another function; it induces neovascularization without inflammation. During wound healing, the period until day 3 after surgery corresponds to an inflammatory phase, while the period later than day 3 corresponds to a proliferative phase (Fine and Mustoe, 1997) during which angiogenesis occurs at the wound site (Adzick, 1997). Grad et al. (1998) reported that, in multiple trauma patients, serum VEGF was elevated from day 3 after injury. Angiogenesis-related factors such as VEGF are induced in hypoxia (Shweiki et al., 1992; Steinbrech et al., 1999). Hangai (1996) reported that the tissue oxygen tension at the anastomosis site reached its lowest level on day 3 after tracheoplasty. Therefore, this condition may re-elevate VEGF levels after day 3. Because both IL-8 and VEGF showed the same kinetics in pleural fluid, we figured that the function of IL-8 later than day 3 after surgery could act as an angiogenic factor rather than as a pro-inflammatory cytokine.

In the present study, IL-8 and VEGF were produced at high levels in pleural fluid after lung surgery. Angiogenesis is required for the growth and metastasis of solid tumors (Folkman et al., 1989; Bicknell and Harris, 1991; Imoto et al., 1998), and anti-IL-8 and anti-VEGF therapy can inhibit both primary tumor growth and metastasis (Asano et al., 1995; Warren et al., 1995; Arenberg et al., 1996; Rowe et al., 2000). Moreover, the inflammatory cytokine level in serum was reported to be higher after lobectomy of the lung than by gastrectomy and colorectal resection (Sakamoto et al., 1994). Pro-inflammatory cytokines such as IL-6 and IL-8 stimulate VEGF expression (Hanahan and Folkman, 1996; Risau, 1997). It has been reported that excess surgical stress from thoracotomy facilitates metastasis (Hattori et al., 1980; Hirai et al., 1997). Furthermore, Gabrilovich et al. (1996) recently reported that in vivo VEGF can inhibit the functional maturation of dendritic cells which play a critical role in antitumor immune responses. Based on the above, lobectomy by standard thoracotomy for lung cancer may likely induce proliferation and metastasis of the tumor not only due to angiogenesis but also to tumor immunity because VEGF was produced at high levels after lung resection.

Our study examined only a small number of patients who underwent elective surgery for lung cancer. Also, because none of our patients had severe postoperative complications, our findings only reflected normal cytokine kinetics after major lung resection for lung cancer. Therefore, further studies are needed to investigate whether our findings are applicable to wedge resection, to lobectomy under video-assisted thoracic surgery regarded as minimally invasive for lung cancer, to extended procedures such as pneumonectomy and combined resection of other involved organs for primary lung cancer, and to lung surgery for conditions other than malignancy. If the cytokine cascade producing the pro- and anti-inflammatory and angiogenic reactions were balanced, normal healing after surgical trauma could be achieved. However, overexpression of angiogenic factors such as IL-8 and VEGF might promote recurrence or metastasis of cancer cells. Therefore, studies of cytokine kinetics will facilitate understanding of the normal biological response and provide reference information for optimal perioperative patient management.

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