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INCREASED IL-6 AND TGF-β_1 CONCENTRATIONS IN BRONCHOALVEOLAR LAVAGE FLUID ASSOCIATED WITH THORACIC RADIOTHERAPY

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Abstract:

Purpose: To assess, in lung cancer patients, the effects of thoracic radiotherapy (RT) on the concentrations of transforming growth factor-β_1 (TGF-β_1) and interleukin-6 (IL-6) in the bronchoalveolar lavage (BAL) fluid.

Methods and Materials: Eleven patients with lung cancer requiring RT as part of their treatment were studied. BAL was performed bilaterally before, during, and 1, 3, and 6 months after RT. Before each BAL session, the patient's status was assessed clinically using pulmonary function tests and an adapted late effects on normal tissue-subjective, objective, management, analytic (LENT-SOMA) scale, including subjective and objective alterations. The National Cancer Institute Common Toxicity Criteria were used to grade pneumonitis. The TGF-β_1 and IL-6 levels in the BAL fluid were determined using the Easia kit.

Results: The TGF-β_1 and IL-6 concentrations in the BAL fluid recovered from the irradiated areas were significantly increased by thoracic RT. The increase in TGF-β_1 levels tended to be greater in the group of patients who developed severe pneumonitis. In the BAL fluid from the nonirradiated areas, the TGF-β_1 and IL-6 concentrations remained unchanged.

Conclusion: The observed increase in TGF-β_1 and IL-6 concentrations in the BAL fluid recovered from the irradiated lung areas demonstrated that these cytokines may contribute to the process leading to a radiation response in human lung tissue.

Keywords : Lung ; Radiotherapy ; BAL ; IL-6 ; TGF-β_1.

INTRODUCTION

Lung carcinoma is the leading cause of cancer mortality in industrialized countries (1). Although treatment of 30-40% of patients with lung cancer includes radiotherapy (RT), lung radiation can be associated with toxicity that may threaten a patient's quality of life and limit the dose that can be delivered. Currently, about 20% of patients treated with thoracic RT will develop pulmonary symptoms secondary to radiation-induced lung injury such as radiation pneumonitis or pulmonary fibrosis (2, 3).

In lung carcinoma patients, the delivery of high doses of radiation is essential to improve local control and survival (4). In addition, RT for lung cancer often requires the radiation of large volumes of normal tissue, making dose escalation even more difficult. The maximal tolerated dose of radiation that can be safely delivered to the lungs is limited by the tolerance of the healthy pulmonary tissue.

The response of normal tissue to radiation is a complex process. Recent advances in molecular biology have

made it possible to investigate the cellular and molecular mechanisms involved in the radiation-induced response of healthy tissues. Radiation is associated with immediate cellular damage by generation of reactive oxygen species, inducing cytokine-mediated multicellular interactions that ultimately result in lung fibrosis. In animal models, pulmonary radiation is followed by early and persistent elevation of cytokines production. This supports the concept of a perpetual cascade of cytokines that prompts collagen genes to activate (5). This cascade of cytokines persists until the expression of late effects becomes apparent pathologically and clinically. According to recent human studies, changes in the blood levels of cytokines such as transforming growth factor-β_1 (TGF-β_1) or interleukin-6 (IL-6) may serve as a predictor of radiation pneumonitis (6-8). The influence of thoracic RT on the production of cytokines in the human lung, however, has never been examined. The present study was designed to assess, in lung cancer patients, the effects of RT on the concentrations of a proinflammatory cytokine, IL-6, and of a pro-fibrogenic cytokine, TGF-β_1 in the bronchoalveolar lavage (BAL) fluid collected before, during, and after thoracic RT.

METHODS AND MATERIALS

Patients

The institutional ethics committee approved the study protocol, and 14 patients undergoing RT for histologically proven lung cancer, without metastasis at diagnosis, and having a life expectancy of at least 1 year, gave their informed consent to participate in the study. Three patients were excluded from the final analysis because of inadequate BAL sampling (n = 1) or persistent right lung atelectasis (n = 1), or because the patient underwent surgery before RT completion (n = 1). All protocol procedures were performed in accordance with the ethical standards on human experimentation and the Helsinki Declaration of 1975, as revised in 1983.

RT description

All patients underwent CT-based treatment planning using a three-dimensional computerized RT system (ISIS, Institut Curie, Paris, France). The doses and target volume delineations were prescribed according to International Commission on Radiation Units and Measurements Report 50 recommendations. They were based on the gross tumor volume, clinical target volume, planning target volume, tolerance of critical organs, and the patients' condition. The percentage of lung volume that received doses >20 Gy (V_{20}) or 30 Gy (V_{30}) of the right, left, and both lungs was obtained from dose-volume histograms using three-dimensional treatment planning. In 6 patients, 60 Gy was administered in 2-Gy fractions, five times weekly. Two other patients received 54 Gy in 2-Gy fractions, five times weekly. The last 3 patients received a total dose of 48 Gy in 4-Gy fractions, three times weekly, in two series of six fractions given 2 weeks apart. The quoted doses were corrected for lung heterogeneity. The treatment was carried out within a mean period of 51 ± 11.2 days.

Clinical, functional, and radiographic evaluations

Medical histories and physical examinations emphasizing the respiratory system were performed before, during (i.e., after 50-66% of the total dose had been delivered), and 1, 3, and 6 months after RT completion. Pulmonary function tests, chest X-rays, and thoracic CT scans were performed at the same intervals. Clinical and radiographic evaluations of pulmonary injury were recorded using the late effects on normal tissue-subjective, objective, management, analytic (LENT-SOMA) lung grading system. Two experienced chest radiologists independently graded the radiographs and CT scans. The National Cancer Institute Common Toxicity Criteria (NCI-CTC) were used to divide patients into two groups according to the pneumonitis grade. Group 1 included patients with NCI-CTC Grade 0 (no radiographic change) or 1 (radiographic changes but asymptomatic or symptoms not requiring steroids). Group 2 included patients with NCI-CTC Grade 2 (radiographic changes and requiring steroids or diuretics), NCI-CTC Grade 3 (radiographic changes and requiring oxygen), or NCI-CTC Grade 4 pneumonitis (radiographic changes and requiring assisted ventilation). Pneumonitis, thoracic CT scans, and chest X-rays were graded before the cytokine levels were determined.

Measurements of lung volumes and ventilatory mechanics were performed using a pneumotachograph and body plethysmograph. The pulmonary diffusing capacity was assessed by the single breath carbon monoxide test. The forced expiratory volume in 1 s, vital capacity, carbon monoxide diffusing capacity, and single breath carbon monoxide were reported as the percentage of the predicted value for normal subjects with identical characteristics.

Bronchoalveolar lavage

BAL fluid specimens were obtained bilaterally before RT, during RT, and 1,3, and 6 months after RT completion, at the same intervals as the clinical and radiologic evaluations. To obtain BAL fluid, the fiberoptic bronchoscope was wedged into a bronchus and three aliquots of 50 mL of 0.9% sterile sodium chloride were successively infused. The fluid of each aliquot was recovered by gentle aspiration. The fluid recovered from the first aliquot was discarded, and the liquid recovered after the last two aliquots was pooled. The tubes were immediately immersed in a slurry of ice and transferred to the laboratory where they were centrifuged for 10 min at 700g. The supernatants were separated into aliquots and kept frozen at -70°C until assay. The cell pellet was processed and analyzed as described below. For each patient, the same sites of sampling were used throughout the study. In the lung with tumor, BAL was performed in an area free of tumor and receiving high doses of radiation. This area was in the upper lobe in 3 patients, middle lobe in 6, lingula in 1, and lower lobe in 1. In the opposite unaffected lung, BAL was performed in the middle lobe in 2 patients, lingula in 4, and upper lobe in 5.

Cell counts and cytologic typing

The cell pellet obtained after centrifugation was used for a total cell count under a microscope using a Thoma plaque and for cell typing after Papanicolaou staining. A differential cell count was performed on at least 300 cells per sample.

TGF-β_1 and IL-6 assay

Concentrations of IL-6 were measured in BAL superna-tants using the enzyme-linked immunoabsorbent assay with the Medgenix Easia Kit according to the protocol recommended by the manufacturer (Biosource Europe S.A., Fleurus, Belgium). Aliquots of BAL fluid were concentrated 10-fold, and the TGF-β_1 concentration was determined using the TGF-β_1 Easia kit from the same manufacturer. The concentrations were calculated as the mean of duplicate assays.

Statistical analysis

The results are expressed as the mean and standard deviation (SD) or as the median and range. The groups were compared using the Mann-Whitney *U* test. Repeated TGF-β_1 and IL-6 values were analyzed on a log-scale using the general linear mixed model to test for group differences while accounting for the time effect and intraindividual variability. The results were considered statistically significant at the 5% critical level.

RESULTS

Patient characteristics

All patients were men, with a mean age of 59 ± 10 years. The patients' clinical characteristics are summarized in Table 1. Three patients underwent surgery before starting RT. RT was combined with chemotherapy in 10 patients: simultaneously in 5 patients and sequentially in 5. Chemotherapy consisted of a combination of some of the following drugs: cisplatin, carboplatin, ifosfamide, mitomycin C, gemcitabine, etoposide, and/or vindesine.

The median clinical follow-up after the first session of RT was 16 months (range 4-39). All but 2 patients had a clinical follow-up of at least 12 months; 1 patient died of sudden hemoptysis and another 1 of cardiac failure and complications of steroid therapy. The mean percentage of lung volume that received a dose >20 Gy was 45.1% ± 12.3%, 9.4% ± 4.8%, and 26.8% ± 5.6% of the lung with tumor, the unaffected lung, and both lungs, respectively. The corresponding values for the percentage of lung volume that received a dose >30 Gy were 40.4% ± 12.4%, 7.1% ± 3.9%, and 23.4% ± 5.3%.

Table 1. *Clinical characteristics*

Variable	Patients (*n*)
ECOG performance status	
0	5
1	5
2	1
UICC clinical stage	
IIB	1

IIIA	5
IIIB	5
Histologic type	
Squamous cell carcinoma	5
Adenocarcinoma	4
Mixed	1
Small cell carcinoma	1
Tumor location	
Right lung	9
Left lung	2
Upper lobe	8
Middle lobe	3

Abbreviations: ECOG = Eastern Cooperative Oncology Group; UICC = International Union Against Cancer.

Radiation pneumonitis

In Group 1, 5 patients had NCI-CTC Grade 0 (n =1) or 1 (n = 4) pneumonitis. In Group 2, 6 patients had NCI-CTC Grade 2 (n = 4) or Grade 3 (n = 2) pneumonitis. No patient had Grade 4 pneumonitis. The median time to the onset of pneumonitis was 3.1 months (range 1-8) after the beginning of RT. No statistically significant differences were observed in the percentage of lung volume that received a dose >20 Gy or >30 Gy between Groups 1 and 2.

As shown in Table 2, the symptoms related to radiation-induced lung toxicity significantly ($p < 0.0001$) increased throughout the study period, especially in Group 2 but not Group 1. Similarly, radiation-induced alterations on the chest radiographs increased significantly with time, and the slope was more marked in Group 2. The thoracic CT scans also revealed significantly increased radiation-induced alterations with time ($p < 0.0001$), but no difference was observed between the two groups (Table 3). The pulmonary function tests showed that the forced vital capacity remained unchanged ($p = 0.28$) and the forced expiratory volume in 1 s and diffusing capacity of the lung for carbon monoxide decreased significantly with time (Table 4). Again, no group effect was discerned.

Table 2. *Effects of radiotherapy on subjective signs*

			Follow-up (mo)		
Signs	Before RT	During RT	1	3	6
Dyspnea*					
All patients	0.7 ±0.9	0.7 ± 1.2	1.3 ± 1.0	1.2 ± 1.1	1.4 ± 1.1
Group 1	0.6 ±0.5	0.4 ± 0.5	0.8 ± 0.8	0.3 ±0.5	0.5 ± 0.6
Group 2	0.8 ± 1.2	1.0 ± 1.5	1.7 ± 1.0	2.0 ±0.7	2.2 ± 0.8
Dyspnea, cough, thoracic pain†					
All patients	2.4 ± 1.3	1.8 ± 1.8	2.5 ± 1.8	2.7 ±2.6	2.9 ±2.6
Group 1	2.8 ± 1.6	1.6 ± 1.5	2.0 ± 2.0	0.5 ±0.6	0.5 ± 0.6
Group 2	2.0 ±0.9	2.0 ±2.1	3.2 ± 1.7	4.4 ±2.3	4.8 ± 1.8

Abbreviations: RT = radiotherapy; NCI-CTC = National Cancer Institute-Common Toxicity Criteria; LENT-SOMA = late effects on normal tissue-subjective, objective, management, analytic.
* Time effect ($p < 0.001$), group effect on slope ($p = 0.0025$).
† Time effect ($p = 0.0098$), group effect on slope ($p < 0.0001$).
Group 1 patients ($n = 5$) had NCI-CTC Grade 1 or less; Group 2 patients ($n = 6$) had NCI-CTC Grade 2 or more; signs graded according to LENT-SOMA scale.
Data presented as the mean ± SD.

Table 3. *Radiologic alterations associated with radiotherapy*

Radiology			Follow-up (mo)		
parameter	Before RT	During RT	1	3	6
Chest X-ray*					
All patients	0.0 ± 0.0	0.3 ± 0.5	0.7 ± 1.0	1.4 ± 1.4	2.1 ± 1.3
Group 1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.8 ± 1.0	1.5 ± 1.3

Group 2	0.0 ± 0.0	0.5 ± 0.5	1.2 ± 1.2	2.0 ± 1.6	2.6 ± 1.1
Chest CT[†]					
All patients	0.0 ± 0.0	0.5 ± 0.6	1.0 ± 0.9	1.5 ± 0.9	2.3 ± 0.9
Group 1	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.5	1.3 ± 0.6	2.0 ± 1.2
Group 2	0.0 ± 0.0	0.8 ± 0.5	1.3 ± 1.0	1.6 ± 1.1	2.6 ± 0.5

Abbreviations as in Table 2.
* Time effect ($p < 0.0001$), group effect on slope ($p = 0.043$). † Time effect ($p < 0.0001$), group effect on slope ($p = 0.30$). Data presented as the mean ± SD.
Group 1 patients ($n = 5$) had NCI-CTC Grade 1 or less; Group 2 patients ($n = 6$) had NCI-CTC Grade 2 or more; alterations graded according to LENT-SOMA scale.

Table 4. *Effects of radiotherapy on VC, FEV_1, and DLCO*

Pulmonary function test	Before RT	During RT	Follow-up (mo) 1	3	6
VC*					
All patients	84 ± 16	90 ± 22	87 ± 17	85 ± 16	85 ± 11
Group 1	81 ± 18	92 ± 23	88 ± 18	88 ± 2	85 ± 13
Group 2	87 ± 16	88 ± 23	86 ± 18	82 ± 10	85 ± 10
FEV[†]					
All patients	71 ± 15	76 ± 21	74 ± 21	68 ± 21	65 ± 19
Group 1	68 ± 17	77 ± 23	72 ± 22	74 ± 23	65 ± 23
Group 2	74 ± 15	75 ± 21	75 ± 21	63 ± 20	65 ± 19
DLCO[‡]					
All patients	59 ± 19	59 ± 30	52 ± 19	52 ± 18	47 ± 21
Group 1	66 ± 18	55 ± 7	56 ± 18	58 ± 18	47 ± 14
Group 2	53 ± 20	62 ± 42	49 ± 21	47 ± 18	48 ± 27

Abbreviations: VC = vital capacity; FEV_1 = forced expiratory volume in 1 s; DLCO = diffusing capacity of the lung for carbon monoxide; other abbreviations as in Table 2.
* Time effect ($p = 0.43$), group effect on slope ($p = 0.52$).
† Time effect ($p < 0.0001$), group effect on slope ($p = 0.30$).
‡ Time effect ($p = 0.0031$), group effect on slope ($p = 0.23$).
Data presented as the mean ± SD, expressed as percentage of predicted value.
Group 1 patients ($n = 5$) had NCI-CTC Grade 1 or less; Group 2 patients ($n = 6$) had NCI-CTC Grade 2 or more.

BAL fluid characteristics and cell count

On average, 31% ± 16% and 32% ± 14% of the injected volume was recovered from the irradiated and nonirradiated areas of the lung, respectively. Before RT, the protein concentration was similar in the BAL fluid collected from both lungs in Groups 1 and 2. The protein concentrations in BAL fluid recovered from both lungs remained unchanged throughout the study period in both groups. Before RT, the total number of cells collected in the BAL fluid from the lung with tumor did not differ from the cellularity of the BAL fluid collected from the contralateral lung. It did not change during the study. Macrophages accounted for 80% of the collected cells, and the remaining 20% were lymphocytes or neutrophiles. No statistically significant alteration of this distribution was observed during or after treatment in either lung. The lymphocyte concentration was higher in the BAL fluid recovered from the radiated areas in Group 2 patients, but this difference did not reach statistical significance ($p = 0.087$; Table 5).

Table 5. *Cell counts and typing in BAL fluid recovered from nonirradiated and irradiated areas*

Cytologic findings	Nonirradiated areas	Irradiated areas
Total cell number (10^4/mL)		
Group 1	280 ± 534	174 ± 126
Group 2	262 ± 311	297 ± 326
Macrophages (%)		
Group 1	87 ± 18	87 ± 21
Group 2	75 ± 19	72 ± 24
Lymphocytes (%)		
Group 1	6 ± 8	4 ± 5

Group 2	12 ± 12	13 ± 17
Neutrophiles (%)		
Group 1	7 ± 14	9 ± 17
Group 2	13 ± 17	15 ± 18

Abbreviations: BAL = bronchoalveolar lavage; other abbreviations as in Table 2.
Data presented as the mean ± SD for Group 1 (n = 5; NCI-CTC Grade 1 or less) and Group 2 (n = 6; NCI-CTC Grade 2 or more).

IL-6 concentrations in BAL fluid

Before treatment, the IL-6 level was similar in the BAL fluid recovered from nonirradiated and irradiated areas (15 ± 11 vs. 17 ± 12 pg/mL, respectively). RT significantly increased the IL-6 levels in the BAL fluid recovered from the irradiated areas (p = 0.012) but IL-6 levels remained unchanged in nonirradiated areas (Table 6 and Fig. 1). Moreover, the IL-6 concentrations in the BAL fluid recovered from irradiated areas were similar in both groups throughout the study period, as revealed by longitudinal data analysis (p = 0.34).

TGF-β_1 concentrations in BAL fluid

Before RT, the TGF-β_1 levels were comparable in the BAL fluid recovered from nonirradiated and irradiated areas (5.9 ± 1.7 vs. 5.5 ± 1.2 pg/mL, respectively). During the study period, the TGF-β_1 levels increased significantly in the irradiated areas (p = 0.0053) and remained unchanged in the other side (Table 7 and Fig. 2). When considering the irradiated areas only (Fig. 3), the TGF-β_1 levels tended to be greater (p = 0.058) in the group of patients with moderate to severe pneumonitis (Group 2).

***Table 6**. IL-6 levels in BAL fluid recovered from nonirradiated and irradiated areas as a function of time*

			Follow-up (mo)		
Location*	Before RT	During RT	1	3	6
Nonirradiated areas					
All patients	15 ± 11	17 ± 25	15 ± 10	20 ± 17	24 ± 32
Group 1	13 ± 4.4	9.2 ± 4.3	17 ± 6.5	16 ± 13	32 ± 41
Group 2	16 ± 14	26 ± 35	12 ± 12	24 ± 20	2.6 ± 1.1
Irradiated areas					
All patients	17 ± 12	22 ± 32	121 ± 283	110 ± 226	47 ± 45
Group 1	18 ± 9.9	12 ± 3.1	26 ± 25	28 ± 23	52 ± 62
Group 2	16 ± 14	31 ± 43	200 ± 380	175 ± 300	42 ± 36

Abbreviations: IL-6 = interleukin-6; BAL = bronchoalveolar lavage; other abbreviations as in Table 2.
* Time effect (p = 0.17), radiation effect on intercept (p = 0.012), group effect on intercept (p = 0.34).
Data presented as the mean ± SD, in picograms per milliliter.
Group 1 patients (n = 5) had NCI-CTC Grade 1 or less; Group 2 patients (n = 6) had NCI-CTC Grade 2 or more.

***Table 7.** TGF-β_1 levels in BAL fluid recovered from nonirradiated and irradiated areas as a function of time*

			Follow-up (mo)		
Location*	Before RT	During RT	1	3	6
Nonirradiated areas					
All patients	5.9 ± 1.7	5.8 ± 1.8	5.5 ±2.1	5.4 ± 1.1	5.9 ± 1.8
Group 1	5.4 ± 1.3	5.9 ± 2.0	5.9 ± 1.5	5.1 ± 1.2	4.8 ± 0.8
Group 2	6.3 ± 1.9	5.7 ± 1.8	5.1 ±2.7	5.6 ± 1.1	6.7 ± 2.0
Irradiated areas†					
All patients	5.5 ± 1.2	5.3 ± 1.0	9.4 ± 8.4	6.7 ± 2.8	28 ±49
Group 1	5.5 ± 1.2	5.4 ± 1.1	5.6 ± 1.4	6.5 ±3.1	6.8 ± 3.3
Group 2	5.5 ± 1.2	5.2 ± 0.9	13 ± 11	6.8 ± 2.9	49 ± 68

Abbreviations: TGF-β_1 = transforming growth factor-β_1; BAL = bronchoalveolar lavage; other abbreviations as in Table 2. Group 1 patients (n = 5) had NCI-CTC Grade 1 or less; Group 2 patients (n = 6) had Grade 2 or more.
* Time effect (p = 0.0090), radiation effect on slope (p = 0.0053).
† Time effect (p = 0.0036), group effect on slope (p = 0.058).
Data presented as the mean ± SD, in picograms per milliliter.

Fig. 1. *Comparison of IL-6 levels in BAL fluid (mean ± SEM) between irradiated and nonirradiated areas before, during, and after radiotherapy.*

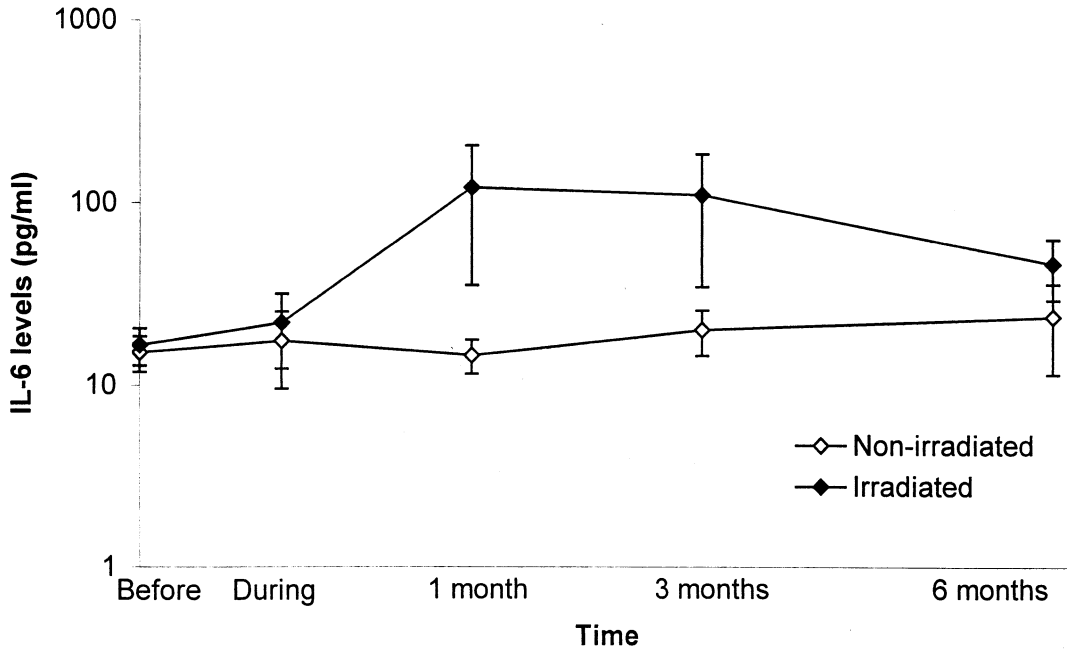


Fig. 2. *Comparison of TGF-β_1 levels in BAL fluid (mean ± SEM) between irradiated and nonirradiated areas before, during, and after radiotherapy.*

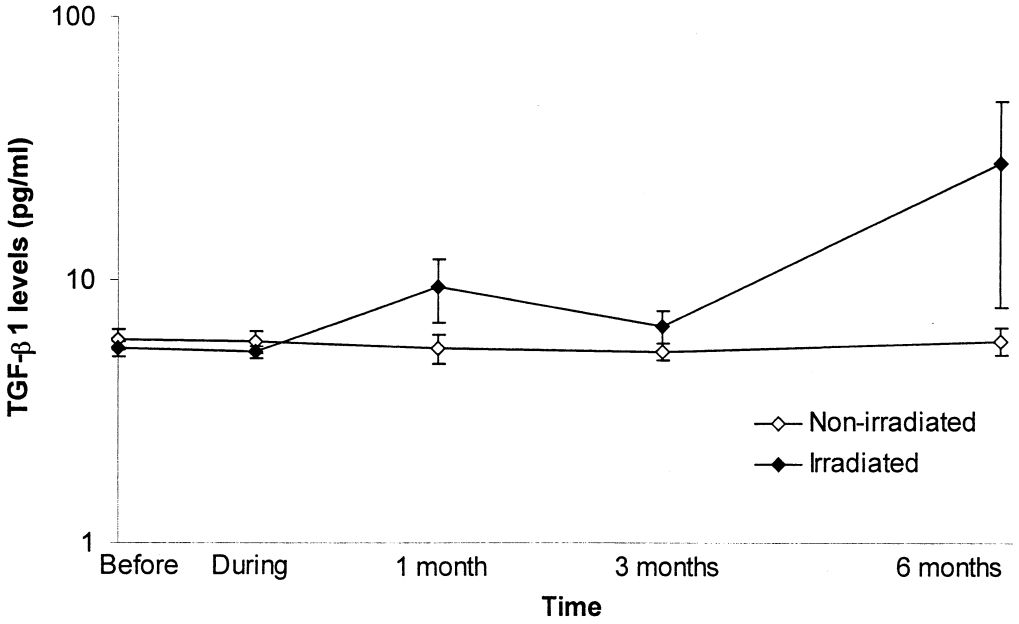
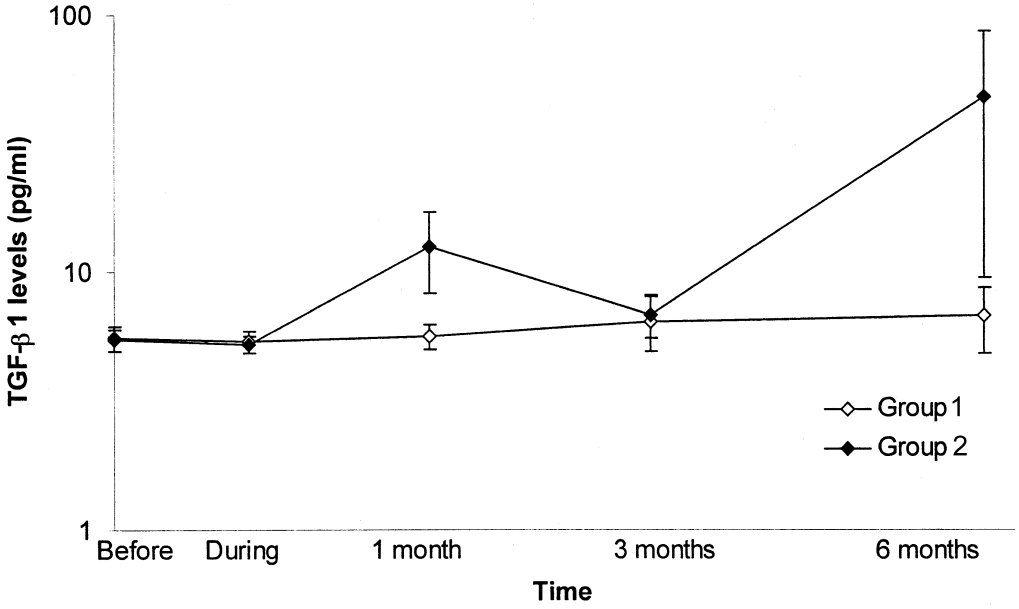


Fig. 3. *Comparison of TGF-β_1 levels in BAL fluid recovered from irradiated areas (mean ± SEM) between Group 1 (NCI-CTC Grade 1 or less) and Group 2 (NCI-CTC Grade 2 or more) patients before, during, and after radiotherapy.*



DISCUSSION

To our knowledge, this is the first study investigating, in humans, the effects of lung radiation during lung cancer treatment on the cytokine levels in BAL fluid. BAL is a minimally invasive procedure that offers the opportunity to investigate the local tissue alterations associated with lung disease. In this study, two biologic mediators were examined, a proinflammatory cytokine, IL-6 and a pro-fibrotic cytokine, TGF-β_1. Our results clearly showed that the IL-6 and TGF-β_1 levels in the BAL fluid collected from irradiated areas increased progressively during lung radiation. Moreover, we found the increase in TGF-β_1 concentrations tends to be larger in the group of patients with moderate to severe pneumonitis (Group 2). Cellularity was not altered during the study period.

IL-6 is a pleiotropic cytokine implicated in the regulation of many inflammatory and immunologic processes (9, 10). Several cells in the lung parenchyma can synthesize this proinflammatory cytokine. These include Type II pneumocytes, T lymphocytes, alveolar macrophages, and lung fibroblasts (11-15). Increased IL-6 blood levels have been found in several inflammatory lung diseases such as allergic asthma (16), mycoplasma pneumonia (17), acute exacerbation of chronic obstructive pulmonary disease (18), severe adult respiratory distress syndrome (19, 20), and lung cancer (21). Also, the lung appears to be a producer of IL-6 in patients with an active inflammatory lung process (22). Several pulmonary diseases are associated with increased IL-6 levels in BAL fluid, including acute infectious pneumonia, interstitial pneumonia, acute complications of lung transplantation, acute respiratory distress syndrome, chronic obstructive pulmonary disease, and lung cancer (23-31).

In the present study, thoracic RT was associated with increased IL-6 levels in the BAL fluid recovered from irradiated areas, although they remained unchanged in the BAL fluid from the other areas. This is in agreement with *in vitro* studies demonstrating that ionizing radiation induces IL-6 mRNA in macrophages (32) and the trend toward increased IL-6 plasma concentrations after thoracic RT, as recently reported (8). Throughout the present study, the IL-6 levels were similar in patients who developed severe pneumonitis (Group 2) and in those who did not (Group 1). This contrasts with recent studies that found greater plasma IL-6 levels before, during, and after RT in patients who went on to develop pneumonitis (7, 8). This suggests that the IL-6 produced by the lung is not a major determinant of circulating IL-6 levels. It has been suggested that the increased circulatory level of IL-6 observed in lung cancer patients could be a part of the systemic inflammatory response syndrome (21). This discrepancy may also be related to differences in patient population or in sample size.

TGF-β_1 plays a central role in fibrotic diseases, contributing to both the influx and the activation of inflammatory

cells, as well as to activation of fibroblasts, to elaborate excessive extracellular matrix. Regarding pulmonary diseases, TGF-β_1 has been shown to be involved in the process leading to idiopathic pulmonary fibrosis, bronchiolitis obliterans syndrome after lung transplantation, lung complications in scleroderma, pneumoconiosis or sarcoidis-associated pulmonary fibrosis, and airway wall remodeling in asthma (33-37). In addition, it has been proposed that TGF-β_1 could be involved in the process leading to radiation-induced lung injury, because pneumonitis is associated with increased TGF-β_1 plasma levels at the end of RT (38). Elevated TGF-β_1 plasma levels at the end of RT would be a risk factor for symptomatic radiation-induced lung injury (39, 40). In contrast, patients with low TGF-β_1 plasma levels could benefit from radiation dose escalation (6). The increased TGF-β_1 plasma levels observed in some lung cancer patients whose treatment included thoracic RT would be related to an increased local production of TGF-β_1 resulting from the exposure of the tumor and its environment to radiation (41). The radiation-induced elevation of TGF-β_1 levels in the BAL fluid observed in our study supports this hypothesis. However, a recent study failed to find any significant change in TGF-β_1 plasma levels associated with RT (8). Mechanisms potentially responsible for this discrepancy include sample size, population, and methodologic differences (42).

The increase in TGF-β_1 levels in the BAL fluid recovered from the irradiated areas is in agreement with animal studies showing that lung radiation is associated with an increased secretion of this cytokine. In a fibrosis-prone mice strain, thoracic radiation induced an acute and a long-lasting increase in the expression of TGF-β_1 in pulmonary tissue (5, 43, 44). Thoracic radiation to rats caused an increase in TGF-β_1 protein concentration in BAL fluid that reached a maximum at between 3 and 6 weeks (45). This increase is paralleled by enhanced TGF-β_1 mRNA expression, as shown by whole lung Northern blot assay. It precedes histologically detectable pulmonary fibrosis, which is never apparent until 8 weeks after radiation (45). *In vitro* radiation of cultured rat lung fibroblasts increased the amount of TGF-β_1 in the culture medium, and TGF-β_1 would have an important role in triggering radiation-induced inhibition of clonogenic activity and terminal differentiation of rat lung fibroblasts (46). Furthermore, TGF-β_1 has recently been demonstrated to be a major autocrine regulator of intrinsic radiation sensitivity of mouse lung fibroblasts (47).

A significant finding of the present clinical study was that TGF-β_1 levels in the BAL fluid tended to increase more in the group of patients who developed more severe radiation pneumonitis (Group 2), suggesting that it could causally contribute to the radiation-induced lung injury. Such a role has been previously demonstrated in experimental studies. In mice sensitive to radiation-induced pulmonary fibrosis, the mRNA abundance of TGF-β_1 increased after radiation but its level in radioresistant mice was unaffected (48). Furthermore, after radiation, TGF-β_1 was found in more cells in the lungs of fibrosis-prone mice than in the lungs of non-fibrosis-prone mice (49).

In our study, TGF-β_1 remained elevated in the BAL fluid recovered from the irradiated areas for several months after RT completion. This finding suggests that the molecular events leading to radiation-induced lung injury persist for a long time after treatment.

It would be interesting to confirm, in a larger cohort of patients, the kinetics of cytokine concentration changes and to test the value of IL-6 and/or TGF-β_1 levels in BAL fluid from irradiated areas in predicting radiation-induced lung injury such as radiation-induced pneumonitis or fibrosis.

Elevated TGF-β_1 plasma levels occur frequently in patients with lung cancer (42, 50, 51). TGF-β_1 could be produced in the lung by the tumor-associated stromal cells more than by the malignant cells themselves (51). In our study, however, TGF-β_1 levels in the BAL fluid from the tumor side and from the opposite side were similar before treatment. This discrepancy can be explained by the choice of the site of BAL, highly irradiated but free of tumor. Furthermore, one cannot rule out that TGF-β_1 is secreted by the tumor exclusively in the circulation and not in the alveoli or airways. Finally, a β-type error cannot be excluded because of the limited number of patients included in the present study.

CONCLUSION

We performed a pilot study on a limited number of lung cancer patients to evaluate the significance of two cytokines, IL-6 and TGF-β_1, as biologic markers useful for the prediction of radiation-induced fibrosis. We showed that RT increases IL-6 and TGF-β_1 levels in BAL fluid collected from irradiated areas. In addition, the increase in TGF-β_1 levels tended to be greater in patients with more severe pneumonitis (Group 2). These findings suggest that IL-6 and TGF-β_1 are involved in the process leading to a radiation response in human lung tissue.

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