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Intrapleural Staphylococcal Superantigen Induces Resolution of Malignant Pleural Effusions and a Survival Benefit in Non-Small Cell Lung Cancer*

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Background: **Malignant pleural effusion (MPE) may occur in up to 50% of patients with non-small cell lung cancer (NSCLC). The majority of these patients have a poor performance status and a dismal prognosis, with survival duration ranging from 2 to 3 months. Since these patients are typically symptomatic from their MPE, prompt treatment is required. Patients with symptomatic MPE from NSCLC and poor performance scores (Eastern Cooperative Oncology Group [ECOG] score ≥ 2, Karnofsky performance status [KPS] score < 50) are generally not offered systemic chemotherapy. Treatment is palliative and includes intrapleural catheter drainage or chemical pleurodesis with talc, doxycycline, or bleomycin. None of the latter modalities prolong survival.**

Objective: **Our goal was to investigate the toxicity and therapeutic effect of a new therapeutic agent, *Staphylococcus aureus* superantigen (SSAg), a powerful T-cell stimulant administered intrapleurally to unselected, consecutive patients with MPE from NSCLC (stage IIIb with pleural effusion) and a poor performance status. By providing direct access of the SSAg to the bronchial and mediastinal lymphatics, we predicted that intrapleural administration of SSAg would induce resolution of MPE and prolong survival in this population with advanced NSCLC and a limited prognosis.**

Methods: **Fourteen consecutive, unselected patients with MPE from NSCLC and a median pretreatment KPS score of 40 (range, 10 to 60) received pleural instillation of SSAg, 100 to 400 pg, once or twice weekly (mean, 3.7 ± 1.3 treatments [± SD]) until the pleural effusions resolved. They were evaluated for drug toxicity, resolution, duration of MPE, and survival.**

Results: **Other than mild fever (maximum grade 2), toxicity of SSAg treatment was trivial and notably devoid of respiratory distress or hypotension. Eleven patients had a complete response (CR), and 3 patients had a partial response of their MPE. In 12 patients, the response endured for > 90 days, with a median time to recurrence of 5 months (range, 3 to 23 months). The median survival for the SSAg-treated group was 7.9 months (range, 2 to 36 months; 95% confidence interval [CI], 5.9 to 11.4 months), compared to a median survival of 2.5 months (range, 0.1 to 57 months; 95% CI, 1.3 to 3.4 months) for 18 consecutive, unselected patients with MPE from NSCLC (stage IIIb) treated with talc poudrage ($p = 0.044$). Survival duration of all 14 SSAg-treated cases and 13 talc-poudrage-treated patients with comparable pretreatment KPS (range, 10 to 60; median, 40 and 30, respectively), and distribution ($p = 0.5$) was 7.9 months (95% CI, 5.9 to 11.4 months) and 2.0 months (95% CI, 0.4 to 2.9 months), respectively ($p = 0.0023$). Nine of 14 patients treated with SSAg survived > 6 months, 4 patients survived > 9 months, and 3 patients survived > 350 days. One of the patients in the CR group has survived 36 months. None of the 13 talc-treated patients survived > 6 months.**

Interpretation: **In 14 unselected, consecutive patients with MPE from NSCLC and poor pretreatment performance (median KPS of 40), the intrapleural administration of SSAg was efficacious in resolving the MPE without any clinically important adverse effects. SSAg-treated patients with a median KPS of 40 (range, 10 to 60) had a median survival that exceeded that with talc poudrage, and was comparable to current systemic chemotherapy used in patients with KPS ≥ 70 status. SSAg treatment is simple to perform, minimally invasive, and does not require hospital time. It may be an attractive alternative to existing palliative modalities for stage IIIb patients with MPE and poor performance who are not candidates for systemic chemotherapy.** ***(CHEST 2004; 126:1529–1539)***

Key words: malignant pleural effusions; pleural effusion resolution; stage IIIb non-small cell lung cancer with pleural effusion; *Staphylococcus aureus* enterotoxin; superantigen; survival

Abbreviations: CI = confidence interval; CR = complete response; ECOG = Eastern Cooperative Oncology Group; KPS = Karnofsky performance status; MPE = malignant pleural effusion; NSCLC = non-small cell lung cancer; PR = partial response; SSAg = *Staphylococcus aureus* superantigen

It is estimated that there are 200,000 new cases of malignant pleural effusion (MPE) annually in the United States, with approximately 40% from lung cancer and 25% from breast cancer. When first evaluated, approximately 15% of patients with lung cancer exhibit a pleural effusion, and up to 50% of patients with disseminated lung cancer may acquire a pleural effusion during the course of their disease.[1–6] In 40 to 60% of patients with MPE from non-small cell lung cancer (NSCLC), MPE will be the initial presenting manifestation.[7,8] Since the majority of these patients are dyspneic from their MPE, prompt treatment is required. In contrast to MPE from small cell carcinoma of the lung, breast carcinoma, or lymphoma, chemotherapy is not the first option in patients with symptomatic MPE from NSCLC. The latter group is usually offered palliative local therapy to control their MPE using chemical pleurodesis or indwelling catheter drainage. Even after successful pleurodesis or drainage, the majority of patients with MPE from NSCLC exhibit poor performance (Eastern Cooperative Oncology Group [ECOG] score ≤ 2 or Karnofsky performance status [KPS] score ≥ 70), and are generally not considered candidates for systemic chemotherapy. Survival for this group is poor, ranging from 2 to 3 months.[1,9–13]

Chemical pleurodesis is commonly performed with either talc, bleomycin, or doxycycline through a chest tube, which is usually in place for several days. Talc poudrage can be accomplished at thoracoscopy. Variable response rates ranging from 50 to 93%, and late recurrences have been observed.[3–6,14] An indwelling pleural catheter for drainage and/or injection of a pleurodesis agent provides an additional option[9,10]; however, the catheter requires surgical placement followed by intermittent drainage of the effusion at home by the patient or caregiver. None of these approved palliative measures have any demonstrable survival benefit.[3–6,14] Numerous biological and chemotherapeutic agents such as interleukin-2, tumor necrosis factor-α, interferon-β, cisplatinum, doxorubicin, etoposide, fluorouracil, and mitomycin C have generally proven ineffective in controlling MPE.[3]

The staphylococcal enterotoxins are representative of a group of evolutionary-related molecules known as superantigens. Minute quantities (10^{-15} mol/L) of these agents stimulate a high proportion of resting T-cells to differentiate into cytotoxic T-cells and release tumoricidal cytokines.[15] In both native form or fused to a tumor-specific monoclonal antibody, various staphylococcal enterotoxins have shown antitumor effects in animal models of carcinoma, sarcoma, and lymphoma.[16–22] Superantigens have also shown tumoricidal activity when used *ex vivo* to induce a tumor-specific T-cell population effective against lung metastases in murine sarcoma,[23] and superantigen-transfected carcinoma cells were successful in diminishing established pulmonary metastases in a postsurgical murine model of mammary carcinoma.[24] Because of demonstrable tumoricidal effects in experimental animals, *Staphylococcus aureus* superantigen (SSAg) was chosen to treat NSCLC with MPE in man.

SSAg was administered intrapleurally immediately after partial drainage of the pleural effusion by thoracentesis. We reasoned that intrapleurally delivered SSAg would gain direct access to bronchial and mediastinal lymphatics, since fluid and molecules administered into the pleural space are known to traffic primarily to regional lymphatic lacunae, and from there to parasternal, costal, bronchial, and mediastinal lymph nodes.[25] Once in contact with pulmonary lymphatics, SSAg might exert antitumor effects by activating a migratory and tumoricidal T-cell population, allegedly expressing CD44 and CD62(low).[26–32] By administering the SSAg intrapleurally via conventional thoracentesis, we also sought to spare the patient from invasive procedures such as thoracoscopy, and chest tube or indwelling catheter insertion, which are required for chemical pleurodesis or catheter drainage.

In the present study of 14 unselected, consecutive patients with MPE from NSCLC and a poor pretreatment performance status (median KPS of 40), we noted that SSAg not only induced resolution of the MPE but also provided a survival benefit comparable to stage IIIb (MPE) patients with significantly higher performance status (KPS score ≥ 70) receiving cisplatinum-based chemotherapy, and significantly longer than those treated with talc poudrage, doxycycline pleurodesis, and indwelling catheter.[2,9–12]

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Materials and Methods

SSAg-Treated Patients

From February 1999 to October 2002, 14 consecutive and unselected patients with NSCLC and MPE were treated with SSAg. Patients were required to have NSCLC with, at least, 350 mL of pleural fluid. Systemic chemotherapy and all other biological response-modifying agents with antitumor activities were discontinued at least 1 month prior to initiating treatment. Radiotherapy was allowed provided it was not focused on the site of the pleural effusion. Pleural effusions were confirmed by chest radiography, chest CT, and ultrasonography. The diagnosis of MPE was established by positive pleural fluid cytology results in all patients. KPS scores before and after treatment were recorded for all cases. Irrespective of KPS, all patients satisfying the above criteria were eligible for this study. All patients signed informed consent, and the study was approved by the executive division of Lishui Central Hospital.

Before each course of treatment, patients received a complete physical examination, CBC, serum chemistry, liver function tests, urine analysis, ECG, and pulmonary function tests. Each patient underwent chest radiography and sonography before starting treatment to document the presence of pleural fluid. Samples of blood and pleural fluid were obtained by venipuncture and thoracentesis, respectively, before and 6 h after selected procedures. Chest radiographs and sonographics were monitored for each patient before and monthly for the first 3 months after treatment, and then bimonthly until completion of the study. CT studies of the lung were done before treatment on patients 3 and 6.

Treatment

A drug containing a superantigen polypeptide derived from *S aureus* was used for these studies. A dose of 1 pg induced strong mitogenicity in human peripheral blood mononuclear cells *in vitro*, and a broad but selective profile of T-cell receptor β variable region stimulation characteristic of staphylococcal enterotoxin superantigens, yet distinct from that of enterotoxin superantigens A to E and toxic shock syndrome toxin[33] (G. Bohach; unpublished data; May 2003).

Once MPE was documented, thoracentesis was performed after sonographic localization. With the patient in the sitting position, the site was localized by sonography. An 18-gauge needle was introduced into the pleural space, and fluid was withdrawn through a three-way stopcock. In general, approximately 50 to 75% of the total effusion was removed. Immediately thereafter, SSAg, 100 to 400 pg, in 10 to 20 mL of normal saline solution was delivered into the pleural cavity over 1 min through the same needle used for thoracentesis. Thoracentesis and SSAg administration were repeated every 3 to 7 days until there was minimal or no reaccumulation of pleural fluid after 10 days. A total of 52 intrapleural SSAg treatments were administered. The mean number of intrapleural treatments required before there was minimal or no fluid reaccumulation was 3.71 ± 1.3 (± SD). Along with intrapleural SSAg, six patients also received IV SSAg daily for 30 days, 21 days, 21 days, 14 days, 6 days, and 3 days, respectively, commencing at the time of the first intrapleural administration of SSAg. Table 1 shows the schedules, dosages, and routes of administration of SSAg treatment in each of the 14 patients. Patients were monitored for adverse effects in hospital for 24 h after treatment and then followed at 3- to 6-day intervals for recurrence of pleural fluid by physical examination, ultrasound, and chest radiography.

Evaluation of Response

Pleural effusions were assessed on serial chest radiographs. For pleural effusions, complete response (CR) was defined as the absence of any reaccumulation of pleural fluid confirmed by chest radiography and sonography at 30 days. A partial response (PR) was defined as reaccumulation of pleural fluid that did not induce symptoms or require repeat thoracentesis at 30 days.

Table 1—*SSAg Treatment in Patients With MPE From NSCLC

Patient No./Age, yr/Sex	NSCLC Histology	SSAg Regimen
1/82/male	Adenocarcinoma	Initial treatment: SSAg, 250 pg IP every week for 3 wk SSAg, 500 pg IP every week for 3 wk SSAg, 100 pg IV every day for 30 d Repeat treatment at 6 mo: SSAg 100 pg IV every day for 30 d
2/67/male	Adenocarcinoma	SSAg, 250 pg IP every week for 4 wk SSAg, 50 pg IV every day for 21 d
3/66/male	Squamous cell carcinoma	SSAg, 250 pg IP every week for 4 wk
4/61/male	Adenocarcinoma	SSAg, 250 pg IP every week for 4 wk SSAg, 500 pg IV every day for 14 d
5/47/male	Adenocarcinoma	SSAg, 200 pg IP every week for 3 wk
6/73/male	Squamous cell carcinoma	SSAg, 250 pg IP every 3 to 4 d five times
7/68/male	Adenocarcinoma	SSAg, 250 pg IP every 3 to 4 d four times
8/69/male	Adenocarcinoma	SSAg, 250 pg IP every week for 3 wk
9/56/male	Adenocarcinoma	SSAg, 250 pg IP every 3 to 4 d five times SSAg, 100 pg IV every day for 21 d
10/65/male	Squamous cell carcinoma	SSAg, 250 pg IP every 3 to 4 d three times SSAg, 100 pg IV every day for 14 d
11/79/male	Adenocarcinoma	SSAg, 100 pg IP every week for 2 wk
12/71/male	Squamous cell carcinoma	SSAg, 250 pg IP every week for 5 wk
13/46/male	Adenocarcinoma	SSAg, 250 pg IP once SSAg, 100 pg IV every day for 3 d
14/47/male	Adenocarcinoma	SSAg, 250 pg IP every 3 to 4 d three times

*IP = intrapleural.

Short-term responses were recorded at 30 days, and long-term responses were recorded at 90 days after the completion of SSAg treatment. Failure was defined as reaccumulation of fluid that caused dyspnea and required an additional thoracentesis. Lung tumors present on chest radiographs were measured and graded according to the World Health Organization guidelines. In addition, the patients were monitored continuously for adverse effects, which were graded according to the National Cancer Institute common toxicity criteria. KPS was recorded for all patients before and 30 days after the completion of SSAg treatment without prior knowledge of the pretreatment KPS scores of the talc-treated group.

Patients Treated with Talc Poudrage

Between 1993 and June 1, 1998, 18 consecutive, unselected patients with symptomatic MPE from NSCLC (stage IIIb) referred to the Interventional Pulmonary Service of the University of California at San Diego Medical Center underwent pleurodesis by thoracoscopic insufflation of sterile, asbestos-free US Pharmacopoeia-approved talc powder. The diagnosis was established by positive pleural fluid cytology results on thoracentesis, or evidence of NSCLC on pleural biopsy prior to referral. Follow-up to the date of death was obtained on 17 patients, and survival duration was measured from the date of first treatment with talc poudrage via thoracotomy to the date of death. KPS scores were recorded before treatment without knowledge of the pretreatment KPS scores of the SSAg-treated group. A study of this patient population was reported previously,[13] and is extended herein as a basis of comparison with the SSAg-treated patients.

Hematologic Studies

Peripheral blood and pleural fluid were sampled before and 6 to 24 h after SSAg treatment in 10 patients for determination of total and differential cell counts.

Statistical Evaluation

The programs in the S-plus statistical software package, professional edition 6 for windows (Insightful Corporation; Seattle, WA) were used for data analysis. Patient survival duration was measured from the first day of SSAg and talc poudrage treatment. Kaplan-Meier survival curves were derived with the survival analysis program developed at the Mayo Clinic and incorporated in the S-plus package. Estimates of survival probabilities and median survival time were obtained from the SSAg-treated and talc-treated groups. Confidence intervals (CIs) were based on the log-hazard scale. A log-rank test was performed comparing the survival duration of the SSAg treatment group with the talc-treated group. The Kaplan-Meier analysis was used to compute the median time to progression of measurable tumors and pleural effusions. A nonparametric method, the Wilcoxon rank-sum statistic, was used to compare KPS scores between two groups. The Cox proportional hazards model was used to evaluate whether the initial volume of pleural effusion was related to survival in both SSAg- and talc poudrage-treated groups. Peripheral blood and pleural fluid determinations of total and differential cell counts before and after SSAg treatment were pooled from 10 patients and were analyzed using a *t* test.

Results

Patient Characteristics

Fourteen unselected, consecutive patients with MPE from NSCLC were treated with SSAg; all were men with a median age of 67.5 years (range, 46 to 82 years). Eight patients had COPD or coronary artery disease. Of the NSCLCs, 10 were adenocarcinomas and 4 were squamous cell carcinomas. Six of 14 patients received prior radiation or chemotherapy. In the remaining eight patients, MPE was the first sign of malignancy. In 11 patients, the MPE was associated with a radiographically evident tumor mass. Serum chemistries and liver function test results were normal in all patients before treatment. At initial presentation, all patients were dyspneic and six patients had submassive hemoptysis. Pleural effusions were left sided in 11 patients, and were associated with ascites and pericardial effusion in 2 patients and 1 patient, respectively. The median initial volume of the pleural effusions was 600 mL (range, 350 to 1,100 mL), and the median pretreatment KPS score was 40.0 (range, 30 to 60) [Table 2].

In general, the SSAg- and talc poudrage-treated groups had similar demographics and clinical characteristics, tumor histology, and staging by TMN subset (Table 2). A similar percentage in each group presented *de novo* without prior chemotherapy or radiation ($p = 1.5$), and the median pretreatment KPS scores in both groups were not statistically different ($p = 0.74$). In addition to their pleural effusion, 12 of 14 patients in the SSAg-treated group and 17 of 18 patients in the talc-treated group had radiographically detectable lung tumors or tumor-related lesions. Although the talc-treated group had a larger median initial pleural effusion volume removed than the SSAg-treated group ($p = 0.001$), all patients in both groups had symptomatic pleural effusions (Table 2).

Toxicity

Adverse effects associated with SSAg treatment are shown in Table 3. In general, SSAg was well tolerated. The most common adverse event was fever ranging from 37.4 to 39.8°C (grade 2), which was unrelated to SSAg dosage. Peak fever was 38°C in five patients and 39.8°C in six patients, and lasted for 24 to 36 h. In two patients, fever persisted for > 36 h and was relieved by indomethacin suppository. Minimal ipsilateral chest pain occurred in three patients and abated spontaneously. There was no evidence of respiratory distress, congestive heart failure, or significant changes in hepatic or renal function during or after treatment. No stage 3 or 4 toxicity was observed in any case.

Responses of the MPE and Tumor to SSAg

Responses of MPE and tumors to SSAg treatment are provided in Table 4. With respect to MPE, 11 patients had a CR and 3 patients had a

Table 2—*Characteristics of Patients Prior to Treatment With SSAg and Talc Poudrage

Characteristics	SSAg (n = 14)	Talc (n = 18)*	Significance
Primary lung cancer histology, No.			
Adenocarcinoma	10	17	Not significant
Squamous cell carcinoma	4	1	
Stage by TMN subset, %			
T4 N0–3: IIIb	100	100	Not significant
Median age (range), yr	67.5 (47–82)	68.5 (51–80)	Not significant
No prior chemotherapy/radiation, %	57	56	Not significant
COPD/atherosclerotic heart disease, %	57	44	Not significant
Pretreatment KPS, %			
70–90	0	28	
10–60	100	72	All not significant
Median KPS	40	50	
Median KPS (10–60)	40	30	
Symptomatic effusion, %	100	100	Not significant
Median initial pleural fluid, mL	600	1,600	p = 0.001
Volume removed, mL	350–1,400†	750–4,500	
Chest radiographic findings, No. of lesions (%)			
Parenchymal/hilar tumor	11 (79)	11 (61)	
Bronchial obstruction/atelectasis	4 (29)	9 (43)	All not significant
Nodular/interstitial infiltrates	1 (7)	2 (10)	

*All 18 patients were evaluable for histology, tumor stage, age, pretreatment KPS, symptomatic effusions. Seventeen of 18 patients were evaluable for prior chemotherapy/radiation, ASHD, or COPD, pleural effusion volume and chest radiographic lesions.

†The pleural volumes removed and quantitated during the first thoracentesis or thoracopy in the SSAg- and talc-treated groups respectively. The volume removed in the SSAg-treated group represented 50 to 75% to the total effusion volume estimated by postthoracentesis ultrasound and chest radiographs.

PR. Twelve of 14 patients did not have recurrent effusion for > 90 days after their last SSAg treatment, with a median time to recurrence of 5 months (range, 3 to 23 months). Pleural fluid samples obtained 6 to 24 h after SSAg treatment from two patients demonstrated tumor cell degeneration that was not evident in pretreatment samples. One month after SSAg treatment, the median pretreatment KPS score of 40 (range, 10 to 60) improved to a median KPS score of 70 (range, 40 to 90) [p = 0.005] in association with resolution of the effusions. Patient 1 and patient 3 were retreated with SSAg for recurrence of MPE. In patient 1, a recurrent left pleural effusion 6 months after the first SSAg treatment was retreated with intrapleural SSAg every 3 to 4 days for four doses, after which the effusion resolved and has not returned. Patient 1 was disease free for 27 months after starting a second course of treatment, and is alive 36 months from the first SSAg treatment (Table 4). Patient 3 had a recurrent pleural and pericardial effusion 15 months after the first treatment, and was retreated twice with intrapleural and intrapericardial SSAg. However, the patient refused additional treatment; therefore, the effect of this limited retreatment could not be evaluated. Patient 4 had a recurrent pleural effusion 4 months after starting SSAg and was not retreated. Recurrent effusion was noted in patient 2 and patient 6 at the time of death, 11 months and 8 months, respectively, after starting SSAg treatment. Notably, patient 11 (pretreatment KPS score of 10) with a persistent hydropneumothorax failed intrapleural chemotherapy 2 months earlier, and had an indwelling chest tube in place draining > 600 mL/24 h. Following the second intrapleural SSAg injection, air leakage and catheter drainage ceased and the chest tube was successfully removed.

Table 3—*Toxicity of SSAg Treatment*

Adverse Event	Severity of Adverse Event, Grade (%)*		
	0	1	2
Fever	8 (57)	5 (36)	6 (43)
Chills	10 (71)	2 (14)	None
Pain	11 (79)	3 (21)	None
Dyspnea	14 (100)	None	None
Leukopenia	14 (100)	None	None

*There was no grade 3 or 4 toxicity.

Pretreatment tumor masses were measurable in 12 patients at the start of SSAg treatment. One patient showed a CR lasting 27 months after the last SSAg treatment, and 11 patients exhibited a median time to progression of their tumor mass of 4 months (range, 2.5 to 14 months) [Table 4]. In general, progression of tumor mass (median, 4 months) was noted before recurrence of the pleural effusion (median, 5 months).

Table 4—*Results of SSAg Treatment

Patient No.	Pleural Effusion: Response/Duration, mo	Lung Tumor: Response/Duration, mo	Survival Duration, mo
1	CR/6 (initial treatment) CR/27 (recurrent pleural effusion retreated at 6 mo, see Table 1)	CR/29	36
2	CR/11	NC/9	11.4
3	CR/15	NC/14	16.6
4	CR/7	NC/4	8.6
5	CR/5	NC/3	5.9
6	CR/8	NC/6	9.2
7	CR/4	NC/2.5	5.3
8	CR/4	NC/5	7.9
9	CR/7	NC/4	7.7
10	CR/5	NC/3	5.4
11	PR/5	No hilar or parenchymal tumor	6.7
12	PR/1	NC/1	2
13	PR/3	No hilar or parenchymal tumor	LTF
14	CR/5	NC/2	LTF
Summary	CR/11; PR/3; median time to progression, 5.0 mo (range, 3 to 23 mo)	CR/1; NC/11; median time to progression, 4.0 mo (range, 2.5 to 14 mo)	Median: 7.9 mo (range, 2 to 36 mo)

*LTF = not available for follow-up; NC = no change.

Survival

The median survival for all 14 patients in the SSAg-treated group was 7.9 months (range, 2 to 32 months; 95% CI, 5.9 to 11.4), compared to the median survival of 2.5 months (range, 0.1 to 57 months; 95% CI, 1.3 to 3.4 months) for 18 unselected, consecutive patients with MPE from NSCLC treated with talc poudrage ($p = 0.044$) [Fig 1]. Thirteen of 18 patients from the latter group with a pretreatment KPS range of 10 to 60 (median 30) and distribution comparable to the 14 patients in the SSAg-treated group (KPS range, 10 to 60; median 40; $p = 0.5$), had a median survival of 2.0 months (95% CI, 0.4 to 2.9), which was significantly different from 7.9 months for the SSAg-treated group ($p = 0.0023$) [Fig 2]. Patients in the SSAg-treated group survived three to four times longer than those

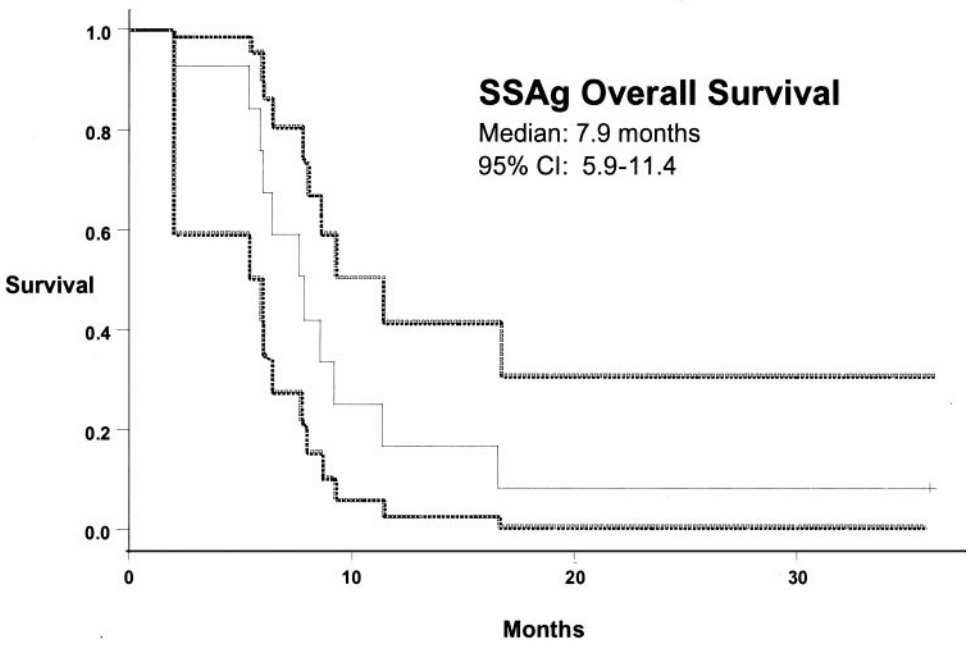

FIGURE 1. Kaplan-Meier survival curve of 14 patients who received SSAg intrapleurally for treatment of MPE from NSCLC showing a median survival of 7.9 months (range, 2 to 32 months; 95% CI, 5.9 to 11.4). Solid line represents survival of SSAg-treated patients, and dotted lines indicate 95% CIs.

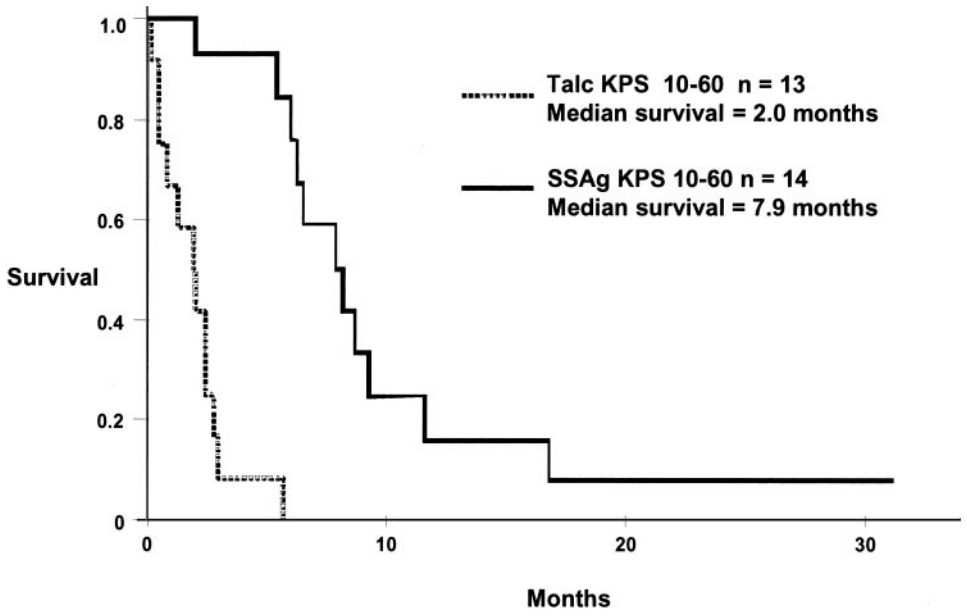

FIGURE 2. Kaplan-Meier survival curve comparing 14 patients who received SSAg intrapleurally with 13 patients who received talc poudrage for treatment of MPE from NSCLC who had similar pretreatment KPS scores (range, 10 to 60; medians, 40 and 30, respectively) and distribution. The patients who received SSAg had a significantly increased median survival of 7.9 months, compared to 2.0 months for the patients who received talc pleurodesis ($p = 0.0023$).

treated with talc poudrage (Fig 1, 2). Twelve of 14 patients in the SSAg-treated group survived > 4 months, 9 patients survived > 6 months, 4 patients survived > 9 months, and 1 patient is still alive 36 months after starting therapy. In contrast, only 1 of 13 patients in the talc-treated group survived > 4 months, and none survived > 6 months (Fig 2). Twelve-month survival for the SSAg-treated group was 14%, vs 0% for the talc poudrage-treated group (Fig 2). Survival in both SSAg- and talc-treated groups could not be predicted from pretreatment pleural fluid volume ($p = 0.26$ and $p = 1.0$, respectively).

Route of Administration of SSAg

Eight patients received intrapleural SSAg only, and six patients received intrapleural SSAg together with daily IV SSAg (Table 1). Despite receiving significantly more SSAg, the group receiving intrapleural and IV therapy showed no significant difference in survival compared to the group receiving only intrapleural treatment ($p = 0.3$) [Tables 1, 4].

Hematologic Changes in the Blood and Pleural Fluid

Peripheral WBC and neutrophil counts increased significantly 6 to 24 h after treatments in all patients (both $p < 0.05$) [Table 5]. Total nucleated cells, neutrophil, and lymphocyte counts in pleural effusions increased significantly 6 to 24 h after treatment (all $p < 0.05$) [Table 4]. While lymphocytes did not change significantly in peripheral blood following SSAg treatment, there was a significant increase in lymphocyte count in the pleural fluid after SSAg treatment.

DISCUSSION

We found that intrapleural administration of SSAg to 14 unselected, consecutive patients with MPE

Table 5—*Peripheral Blood Leukocyte Counts and Pleural Fluid Nucleated Cell Counts in Patients Treated With SSAg**

Variables	WBC/μL	Neutrophils/μL	Lymphocytes/μL
Peripheral blood (n = 10)			
Pretreatment	5,200 ± 0.398	3,285 ± 2.50	1,850 ± 0.144
Posttreatment‡	8,533 ± 1.534†	6,455 ± 1.535†	1,916 ± 0.587
Pleural fluid (n = 10)			
Pretreatment	761 ± 0.150	553 ± 0.150	201 ± 0.134
Posttreatment‡	1,178 ± 0.381†	661 ± 0.185†	541 ± 0.167†

*Data are presented as mean ± SD.
†Significant at $p < 0.05$.
‡Peripheral blood and pleural effusion cell counts 6 to 24 h after initial treatment.

from NSCLC resulted in resolution of the MPE within 1 month after concluding treatment with persistence for > 90 days in 12 of 14 patients. In several cases, resolution lasted for as long as 6 months, 8 months, 12 months, and 15 months, with a median time to recurrence of 5 months. The response rate (100%) for resolution of the MPE exceeded that for talc, bleomycin, doxycycline, and indwelling catheter drainage, which are commonly used for local palliation of MPE from NSCLC. None of the latter treatments have been shown to improve survival.[3–6,14]

While MPEs resolved with SSAg, it appeared that a substantial number of the patients also survived longer than would be expected than if the SSAg only induced palliation.[2,9–13] The median survival of 7.9 months in the NSCLC patients with MPE included three patients who survived > 350 days. At the time of this report, one patient is still alive 36 months after starting treatment.

The median survival of 7.9 months for all 14 consecutive, unselected SSAg-treated patients stood in comparison to a median survival of 2.5 months for all 18 consecutive, unselected patients with MPE from NSCLC treated with talc poudrage at the University of California, San Diego from 1993 to 1998.[13] Thirteen of these 18 patients with a pretreatment KPS range of 10 to 60, median of 30, and distribution statistically similar to that of the entire SSAg-treated group had a median survival of 2.0 months. Additional groups of 61 patients and 35 patients with MPE from NSCLC treated with indwelling catheter drainage and doxycycline pleurodesis in multicenter trials led by investigators at MD Anderson Cancer Center from 1994 to 1996 had median survivals of 2.0 months[9] and 3.0 months,[10] respectively. A meta-analysis of 156 patients with MPE from lung cancer showed a median survival of 3.0 months.[1,9–13] Compared to current populations treated with the best available palliative measures (talc, doxycycline, and indwelling catheter drainage), SSAg-treated patients had a 2.4-fold to fourfold greater survival.

The median survival of 7.9 months for the 14 SSAg-treated patients was surprising in view of the low median pretreatment KPS score of 40 or ECOG score of 3 (disabled, bedridden > 50% waking hours) for this group. Nine patients with KPS scores ≤ 40 had a median survival of 8.6 months. Platinum-based chemotherapy is generally not recommended for patients with KPS scores ≤ 70 (ECOG score ≥ 2), since it induces a greater level of toxicity compared to those with KPS scores ≥ 70 (ECOG score 0 to 1). Recent chemotherapeutic regimens used in stage IIIb patients (with pleural effusion) selected for ECOG score 0 to 1, KPS score ≥ 70, have shown an improved median survival of approximately 8 months comparable to the survival reported herein for SSAg-treated patients with a median KPS of 40 (ECOG score of 3).[34,35] As a single agent, SSAg appears to be capable of inducing an MPE response rate exceeding talc with less morbidity and a survival duration in a group with poor performance status (KPS score of 40) comparable to cisplatinum-based chemotherapy in patients with better performance (KPS score ≥ 70). Thus, SSAg treatment may be useful in stage IIIb patients with MPE, KPS score of 40, or ECOG score of 3 who are ineligible for chemotherapy. Notably, pretreatment KPS scores < 70 (range, 30 to 60) in eight patients improved to ≥ 70 (ECOG score of 2) after a single course of SSAg treatment, suggesting that patients considered ineligible for chemotherapy might become eligible after SSAg therapy

Notwithstanding the limitations of comparing populations in different countries with different medical systems, the SSAg- and talc poudrage-treated groups had certain similarities. Both groups were comprised of unselected and consecutive patients with MPE from NSCLC. Median age, TMN subset grouping, and pretreatment KPS scores were not statistically different. In both groups with stage IIIb lung cancer due to adenocarcinoma, symptomatic pleural effusions were present at the time of first treatment, and there was a comparable degree of parenchymal tumor or tumor-related lung lesions. Fifty-seven percent of SSAg-treated and 56% of talc-treated patients had not received prior cancer chemotherapy and/or radiation treatment, consistent with the experience of Schrump and Nyugen[7] and Maghfoor and colleagues,[8] who noted that 46 to 64% of NSCLC patients present with MPE as the first sign of malignancy. The median survival for MPE from NSCLC is compatible with findings in previous reports[36,37] of similar patients in China showing no better survival rates than in Western populations. Given the similarity of the two populations, a comparison of survival rates for talc and SSAg was considered to be reasonable.

The smaller median initial pleural effusion volume removed in the SSAg-treated patients was partially due to differences in the techniques used to withdraw pleural fluid. In the SSAg-treated group, an indwelling needle was used for thoracentesis with drainage of only part of the initial effusion; whereas in the talc-treated group, all of the initial pleural fluid was removed during thoracoscopy. In both the SSAg- and talc-treated groups, initial pleural effusion volume was not predictive of survival duration. Putnam and coworkers[9,10] observed a median survival of approximately 3 months in 21% of MPE patients with initial pleural fluid volumes of ≤ 1,000 mL.

Therefore, initial pleural effusion volume in patients with MPE from NSCLC is variable, dependent on the drainage technique and does not appear to be predictive of survival.

The only toxicity of SSAg treatment was fever, which never exceeded grade 2. Fever was easily managed with conventional antipyretics. There was no grade 3 or 4 toxicity, and all patients were discharged from the hospital within 24 h after the procedure. Notable was the absence ARDS, as has been observed following talc insufflation or instillation,[38] and hypotension and noncardiogenic pulmonary edema that has been reported after treatment of cancer patients with preparations containing staphylococcal superantigens, enterotoxins A and B.[39–42] In addition to the lack of significant toxicity, SSAg may offer potential advantages over approved palliative agents used for MPE in requiring minimal hospitalization while also avoiding thoracotomy, chest tube insertion, and prolonged in-hospital chest tube drainage.

Following intrapleural SSAg, the pleural fluid showed significant accumulation of lymphocytes in addition to neutrophils. In contrast, the response to acute pleural injury caused by infection or induced by inflammatory or sclerosing agents in rabbits is manifest by a neutrophil influx, which persists in the pleural fluid as long as the injury is maintained. If the effusion persists, lymphocytes predominate; however, if the injury ceases, blood monocytes transiently become more prevalent.[43,44] The acute neutrophilia and lymphocytosis noted in the SSAg-treated patients may be ascribed, in part, to superantigen induction of T-cell lymphotactin and interleukin-8, which are chemotactic for lymphocytes and neutrophils, respectively, at the site of superantigen administration.[45,46]

Superantigens derive their name from the shared property of activating a high proportion of T-cells via binding to the T-cell receptor Vβ region. Each superantigen activates a unique cluster of Vβs on the T-cell receptors of CD4+ and CD8+ T-cells. The drug used in these studies appears to be a SSAg polypeptide with a Vβ profile distinct from that of classical staphylococcal enterotoxin superantigens, and free of toxicity noted with the use of other preparations containing staphylococcal enterotoxins A and B.[39–42]

The resolution of pleural effusions and prolonged survival of patients with NSCLC with SSAg therapy may be ascribed, in part, to a SSAg-induced tumoricidal reaction in the pleura and pleural space. This notion is supported by the tumor killing noted histologically in the pleural fluid obtained 6 to 24 h after the first SSAg treatment (Fig 3). Superantigens are known to induce a population of CD4+ and CD8+ effector T-cells expressing CD44 and CD62low capable of trafficking to tumor sites and killing tumor cells directly or via release of tumoricidal cytokines and chemokines.[26–32,45–47] Intrapleurally administered SSAg may traffic to regional lymphatic lacunae via stoma and foramina in the macula cribiformis, and ultimately drain into the parasternal, costal, bronchial, and mediastinal lymph nodes,[25] where they activate effector and migratory T-cells.[26–32] These same effector T-cells translocate

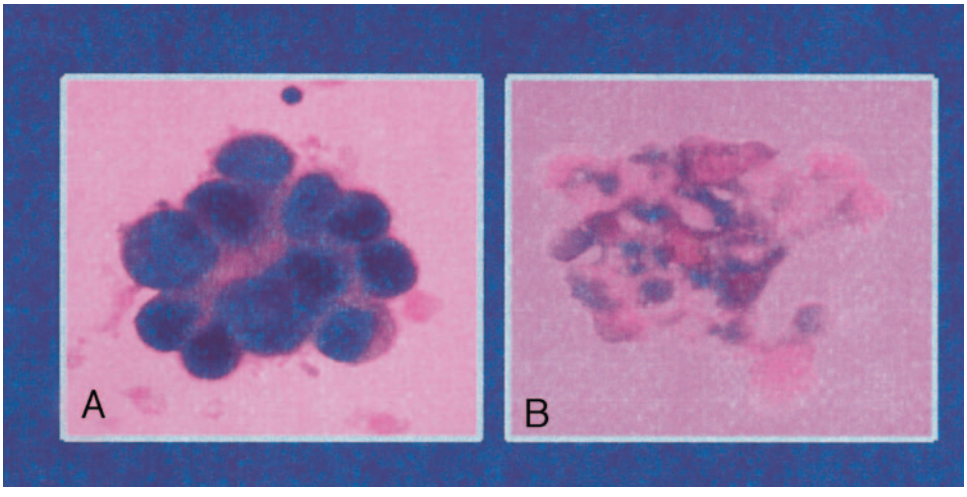

FIGURE 3. Pleural fluid cytology in patient 5 showing clusters of intact, morphologically viable adenocarcinoma cells (*left*, *A*). Tumor cells demonstrate marked vacuolization and cytoplasmic and nuclear degeneration with apoptotic changes and inflammatory cell infiltrate 24 h after treatment (*right*, *B*). Similar findings were noted in the posttreatment pleural fluid of patient 4 (hematoxylin-eosin, original × 400).

into the pleural space,[26,28,32] where their cytotoxic effect is exerted on carcinoma cells with or without surface-bound superantigen.[48,49] Likewise, tumoricidal effector cells generated in the mediastinal lymphatics may limit the growth of parenchymal or hilar tumor to account for the stability of lung tumor masses noted in the SSAg-treated cases for a median of 4 months (Table 4). SSAg-specific antibodies present naturally in the blood of most humans, and considered to be an impediment to superantigen-induced tumor killing when administered IV,[22] may actually contribute to killing of tumor cells displaying surface-bound SSAg[48,49] by complement-mediated tumor lysis and/or antibody-dependent cellular cytotoxicity.

Since the addition of IV SSAg to intrapleural SSAg did not significantly alter MPE resolution or survival and intrapleural SSAg was well tolerated, a regimen consisting of SSAg administered intrapleurally every 2 to 3 days until the MPE resolves shall be adopted for subsequent clinical trials in patients with MPE from NSCLC. Although the exact frequency and duration of response or antineoplastic efficacy cannot be determined from this trial, the lack of toxicity, logistic ease of the therapy, and the relatively low financial cost make this treatment suitable for extension to a larger cohort of patients. Ideally, this report will stimulate additional research that enhances our understanding of the biology and application of this therapy and the mechanism by which SSAg mediates antineoplastic effects.

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Errata

Correction to Text in: Intrapleural Staphylococcal Superantigen Induces Resolution of Malignant Pleural Effusions and a Survival Benefit in Non-Small Cell Lung Cancer

Corrected Text: It has come to our attention that there was a line of incorrect text in "Intrapleural Staphylococcal Superantigen Induces Resolution of Malignant Pleural Effusions and a Survival Benefit in Non-Small Cell Lung Cancer," published in the November 2004 issue of *CHEST* (2004;126(5):1529-1539). In the left column on p. 1533, the sentence beginning "In patient 1…" should read:

> In patient 1, a recurrent left pleural effusion 6 months after the first SSAg treatment was retreated with intravenous SSAg as shown in Table 1, after which the effusion resolved and has not returned.

The information provided in Table 1 has been reviewed and remains accurate.

Intrapleural Staphylococcal Superantigen Induces Resolution of Malignant Pleural Effusions and a Survival Benefit in Non-Small Cell Lung Cancer*

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