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wild-type and SCID mice, and a dose of 100 mg/kg body weight was used to examine the systemic reaction to the s.c. administered agent in SCID mice.

Chronological Alteration of BAL Cell Counts after Administration of BLM and BCG Vaccine

With i.t. injection of 0.1 mg/mouse BLM, the total cell and macrophage numbers in BAL fluid were increased dramatically starting from day 3 in both wild-type and SCID mice. The transition, however, was significantly different between the 2 types of mice. That is to say, they were continuously and linearly increased until day 28 in the case of wild-type mice, whereas in the case of SCID mice, they had returned to significantly lower levels by day 28, although the transition until day 14 was similar to that of wild-type mice (Figure 1*A*, *B*). The change in lymphocyte number in BAL fluid was similar to that in total cell and macrophage numbers, with the exception that the lymphocyte number of SCID mice at days 7 and 14 tended to be lower than that of wild-type mice, completely returning to the level of untreated mice at day 28 (Figure 1*C*). On the other hand, the neutrophil number in BAL fluid was increased at days 3, 7, and 14, and completely returned to the level of untreated mice in both types, but the numbers in SCID mice were generally lower than in wild-type mice (Figure 1*D*).

As the results with i.t. injection of BLM suggested that the interactions among these cells might be different between wild-type and SCID mice, similar experiments with i.t. injection of BCG, known to cause pulmonary inflammation that is triggered by initial recruitment of neutrophils [28], were performed to verify the difference. It was observed that the 2 types of mice again displayed different transitions of these cell numbers in BAL fluid with the i.t administration of BCG at a dose of 0.5 mg/mouse. This time, the total cell and macrophage numbers were increased after the treatment with a peak at day 3, followed by lower but still high levels until day 28 in the wild-type mice, whereas the numbers were gradually decreased from days 7 to 28, with a clear trend of returning to the level of untreated mice in the SCID mice (Figure 2*A* and *B*). The lymphocyte number in BAL fluid differed greatly between the 2 types of mice. Contrary to the significant and continuous increase according to the time course in wild-type mice, lymphocytes in SCID mice completely returned to the untreated level as early as day 14, with a similar peak at day 3 (Figure 2*C*). The change in neutrophil number was similar to that in lymphocyte number in SCID mice. The neutrophil number of wild-type mice, however, demonstrated a complex transition: it jumped to a peak much the same as the total cell, macrophage, and lymphocyte numbers, returned to an untreated level as early as day 7, elevated again at day 14, and once again decreased at day 28 (Figure 2*D*). In contrast to i.t. injection, s.c. administration of sublethal dose (50 mg/kg) of BML did not cause drastic alteration of cell numbers in BAL in both wild-type and SCID mice during the period of 28 days (Figure 3).

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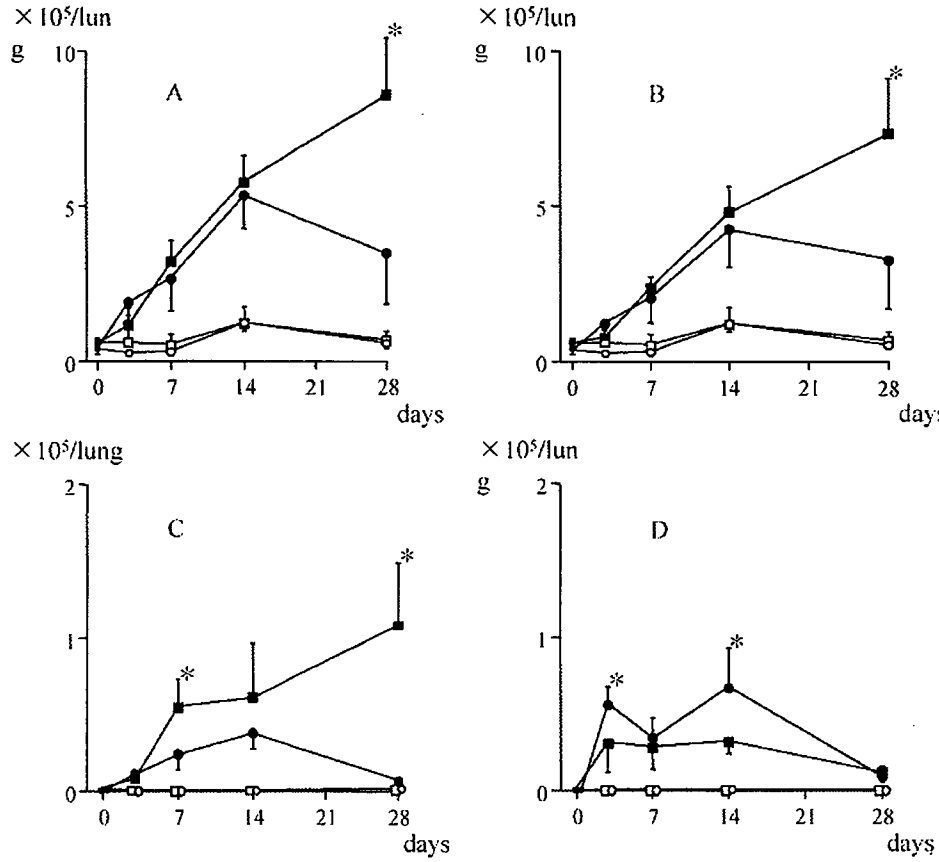

FIGURE 1 Chronological alteration of total cells (*A*), macrophages (*B*), lymphocytes (*C*), and neutrophils (*D*) in BAL fluids after i.t. injection of BLM (0.1 mg/mouse) or saline. Although the number of total cells, macrophages, and lymphocytes were dramatically increased similarly in wild-type and SCID mice, they were again decreased at day 28 in SCID mice, in contrast to the continuous increase in wild-type mice. Neutrophils were transiently increased in number after BLM injection at days 3 to 14 in both mouse types. Injection of saline did not cause significant cell count shift in either type. Closed and open squares represent data of wild-type CB17 mice with i.t. injection of BLM and saline, respectively. Closed and open circles represent data of SCID mice with i.t. injection of BLM and saline, respectively. Dots and bars represent means and standard deviations ($n = 5$ for each point). Asterisks indicate statistically significant differences between SCID and wild-type mice treated with BLM ($P < .05$; Mann-Whitney's U test).

Alteration of Pathological Findings of the Lung by i.t. and s.c. Administration of BLM

Histological examinations of the lung also revealed pulmonary inflammation mainly in the interstitium at 28 days after i.t. BLM administration. Although the severities of pathological alterations were generally similar between the wild-type and SCID mice, as demonstrated by elastica van Gieson stain, lesions in wild-type mice had more abundant lymphocyte infiltration than those in SCID mice ($n = 13$ for SCID, $n = 11$ for wild-type; Figure 4). In contrast, s.c. BLM administration caused focal interstitial changes limited to the subpleural regions in both wild-type and SCID mice ($n = 6$; Figure 5).

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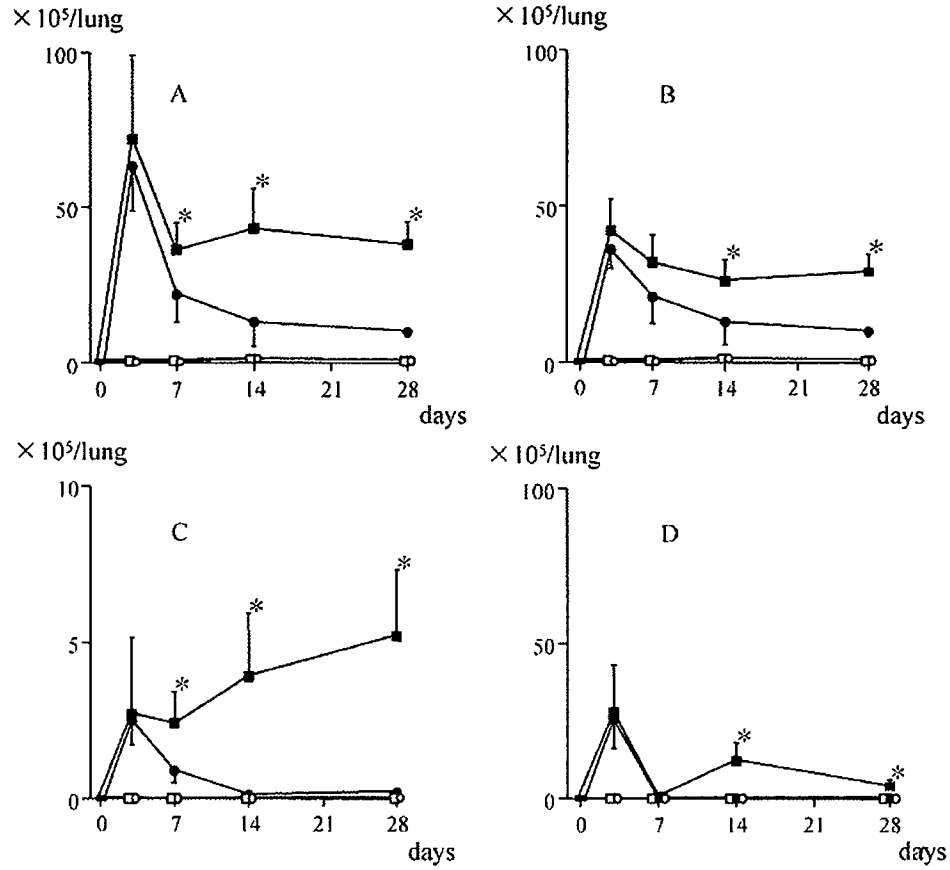

FIGURE 2 Chronological alteration of total cells (*A*), macrophages (*B*), lymphocytes (*C*), and neutrophils (*D*) in BAL fluids after i.t. injection of BCG vaccine (0.5 mg/mouse) or saline. When injected with BCG, the total cells and macrophages were increased in number, with their peak at day 3. Although they were decreased from days 7 to 28, they still remained at higher levels than in the mice injected with saline. The decrease during this period was more prominent in SCID than in wild-type mice. Lymphocytes after BCG vaccine injection continued increasing in number up to day 28 in wild-type mice, whereas in SCID mice they had returned to a normal level at day 14. Neutrophils were transiently increased after BCG vaccine injection at days 3 and 14 in wild-type mice, and only at day 3 in SCID mice. Closed and open squares represent data of wild-type CB17 mice i.t. injected with BCG vaccine and saline, respectively. Closed and open circles represent data of SCID mice i.t. injected with BCG vaccine and saline, respectively. Dots and bars represent means and standard deviations ($n = 5$ for each point). Asterisks indicate statistically significant differences between SCID and wild-type mice treated with BLM ($P < .05$; Mann-Whitney's U test).

Alteration of Hydroxyproline Contents in the Lung by i.t. and s.c Administration of BLM

Hydroxyproline content in the lung elevated significantly at day 28 after i.t. BLM injection, when compared to that after saline injection, in both SCID and wild-type mice. Both before and after BLM administration, there was no significant difference in hydroxyproline content between wild-type and SCID mice ($n = 6$; Figure 6*A*). In contrast, s.c. administration of the

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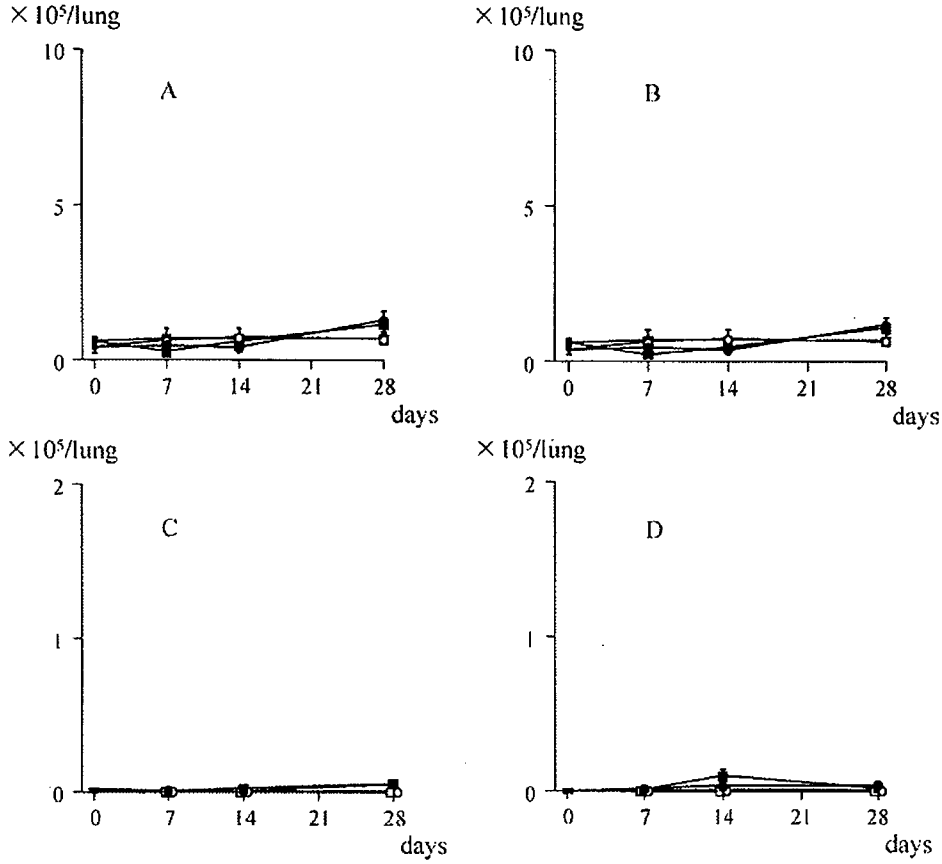

FIGURE 3 Chronological alteration of total cells (*A*), macrophages (*B*), lymphocytes (*C*), and neutrophils (*D*) in BAL fluids after s.c. administration of BLM (50 mg/kg) or saline. Administration of BLM and saline did not cause significant cell count shift in either mice type. Closed and open squares represent data of wild-type CB17 mice with s.c administraion of BLM and saline, respectively. Closed and open circles represent data of SCID mice with s.c. administration of BLM and saline, respectively. Dots and bars represent means and standard deviations ($n = 7$ for each point).

agent did not alter hydroxyproline content in the lung at day 28 after implantation of osmotic minipumps, in both wild-type and SCID mice ($n = 6$; Figure 6*B*).

Changes in Organs Other Than Lungs

At day 11 after the start of continuous s.c. administration of BLM at a lethal dose (100 mg/kg), histological examination of brain, heart, liver, kidneys, stomach, and small and large intestines failed to demonstrate any abnormalities in SCID mice, and there was no difference between SCID mice treated with BLM and saline ($n = 5$; data not shown). Esophagi of SCID mice treated with s.c. BLM, however, showed a remarkable degree of hyperplasia of cells with large nuclei, accompanied by severe keratosis in the

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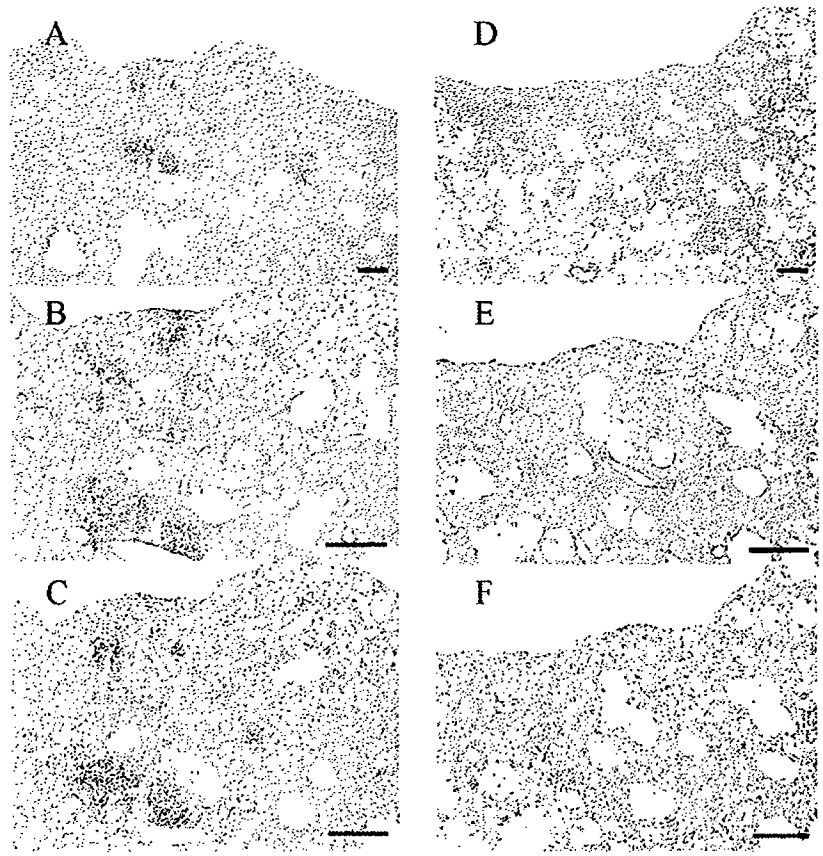

FIGURE 4 Pulmonary lesions in wild-type (*A*, *B*, and *C*) and SCID (*D*, *E*, and *F*) mice at day 28 by i.t. injection of BLM (0.1 mg/body). Histological examination revealed thickening of alveolar septa, proliferating fibroblasts, and infiltration of histiocytes and lymphocytes in both animals. The degree of severity and distribution of the lesions were generally similar between the 2 mouse types when demonstrated by low power magnification (*A* and *D*, original magnification ×10, H&E stain). Elastica van Gieson stain (*B* and *E*, original magnification ×20) also disclosed similarity in collagen fiber deposition between the 2 types. Closer observation with higher magnification (*C* and *F*, original magnification ×20, H&E stain), however, uncovered more prominent lymphocyte infiltration in wild-type than in SCID mice. The bars represent 100 μm.

epithelium. In contrast, SCID mice treated with saline and wild-type mice treated with s.c. BLM did not show such alteration (Figure 7). Bone marrow cell counts in SCID mice treated with s.c. BLM were significantly elevated when compared with those with saline, and no myelosuppression with s.c. BLM administration was noted (Table 3). According to the observation of mice during breeding, SCID mice treated with s.c. BLM ate and drank far less than the ones treated with saline, although there was no evidence of diarrhea in either group (data not quantified). As a consequence, mice treated with s.c. BLM lost body weight in a 10-day period, whereas those treated with saline gained weight probably because of their natural growth (Table 3).

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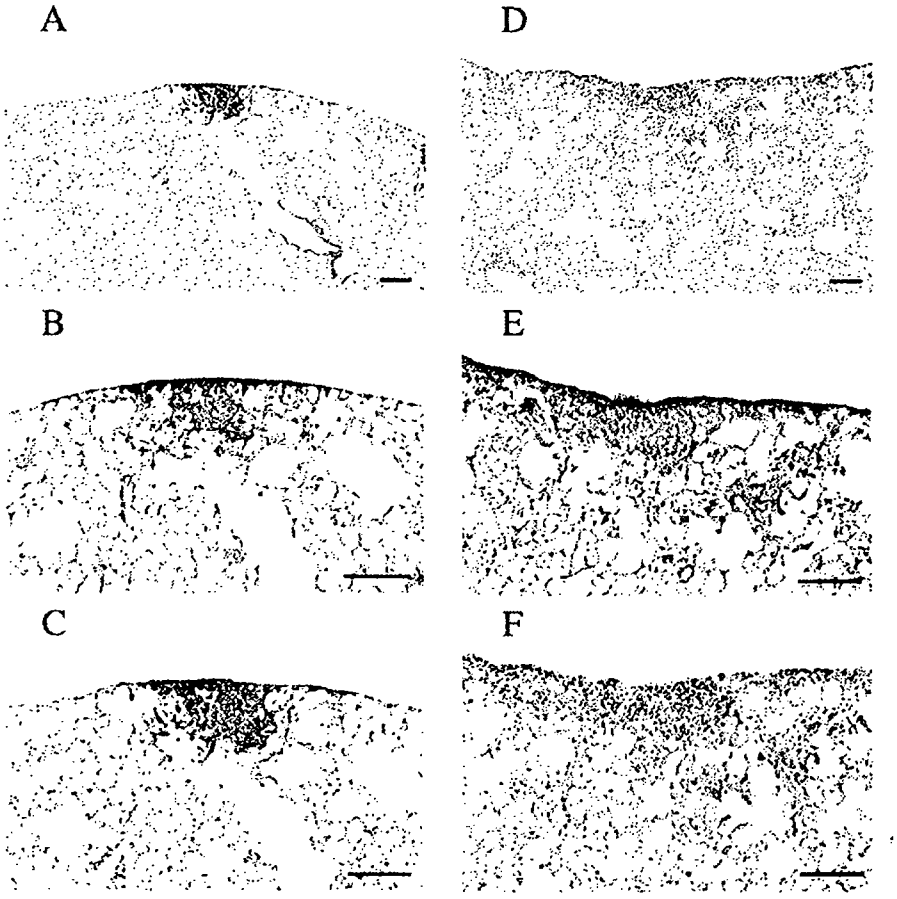

FIGURE 5 Pulmonary lesions in wilsd-type (*A*, *B*, and *C*) and SCID (*D*, *E*, and *F*) mice at day 28 by s.c. administration of BLM (50 mg/kg). Histological examination revealed focal fibrosis limited to subpleural regions in both wild-type and SCID mice. The degree of severity and distribution of the lesions were generally similar between the 2 mouse types with low- and higher-power magnifications (*A* and *D*, original magnification ×10; *C* and *F*, original magnification ×20, H&E stain). Elastica van Gieson stain (*B* and *E*, with original magnification ×20) also disclosed similarity in collagen fiber deposition between the 2 types. The bars represent 100 μm.

DISCUSSION

Intratracheal instillation of sublethal dose BLM induced diffuse pulmonary infiltration and fibrosis, whereas its systemic administration caused focal subpleural scarring in wild-type mice. This was concordant with the well-established data of the experimental model of BLM-induced pulmonary fibrosis [5]. This situation in pulmonary lesions was similar in case of SCID mice. BAL and hydroxyproline content analyses with sublethal dose BLM also did not cause significant alterations in inflammatory cell numbers and pulmonary fibrosis in both wild-type and SCID mice. The significant differences between the outcomes of i.t. and s.c. injection routes may be due to the organ specificity of BLM-induced damage [29] and the fact that the

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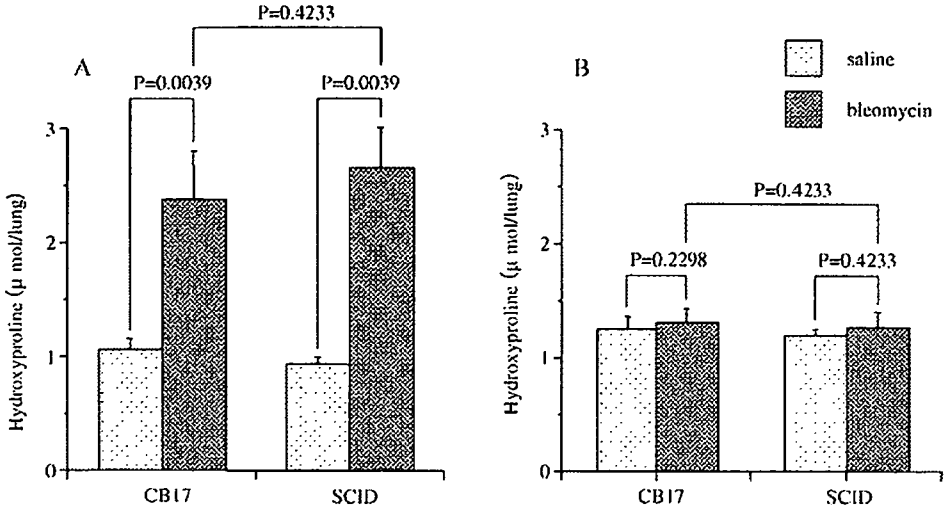

FIGURE 6 Hydroxyproline content in the lung. Lungs were removed after the mice were sacrificed, and were evaluated for hydroxyproline content. Lungs of wild-type and SCID mice contained similar amounts of hydroxyproline at day 28 of i.t. saline injection. They had significantly increased amounts of hydroxyproline at day 28 of i.t. administration of a sublethal dose (0.1 mg/body) of BLM. There was no significant difference between wild-type and SCID mice, suggesting that i.t. BLM administration caused severe fibrosis of the lungs of comparable degree between the two types of mouse (*A*). In contrast, there was no significant difference in hydroxyproline content between wild-type and SCID mice at day 28 of s.c administration of a sublethal dose (50 mg/kg) of the agent (*B*). The columns and bars represent means and standard deviations ($n = 6$).

distribution of the BLM-inactivating enzyme, bleomycin hydrolase, is lacking in the lung and skin [30].

Due to deficiency in DNA DSB repair in SCID mice, the cells of the animals are hypersensitive to BLM that causes DNA DSB [24]. At the same time, the SCID phenotype includes lymphocyte dysfunction due to the lack of V(D)J recombination capability [16, 21–23], and this may influences the

TABLE 3 Body weight and bone marrow cell counts in the mice treated with s.c. BLM

	Before	After*1	*p* value*2
Bone marrow cell ($\times 10^7$)			
BLM (100 mg/kg)	NT*4	1.17 ± 0.45 ($n = 10$)	—
Saline	NT	0.65 ± 0.10 ($n = 6$)	—
p value*3		0.004	
Body weight (g)			
BLM (100 mg/kg)	24.8 ± 0.7 ($n = 12$)	17.9 ± 0.5 ($n = 12$)	0.0018
Saline	24.8 ± 1.2 ($n = 6$)	27.5 ± 1.4 ($n = 6$)	0.0231
p value*3	0.8419	0.0003	

*1: at 14 days after the start of continuous s.c. administration.
*2: comparison between before and after with Wilcoxon's rank sum test (paired).
*3: comparison between BLM and saline with Mann-Whitney's *U* test.
*4: not tested.

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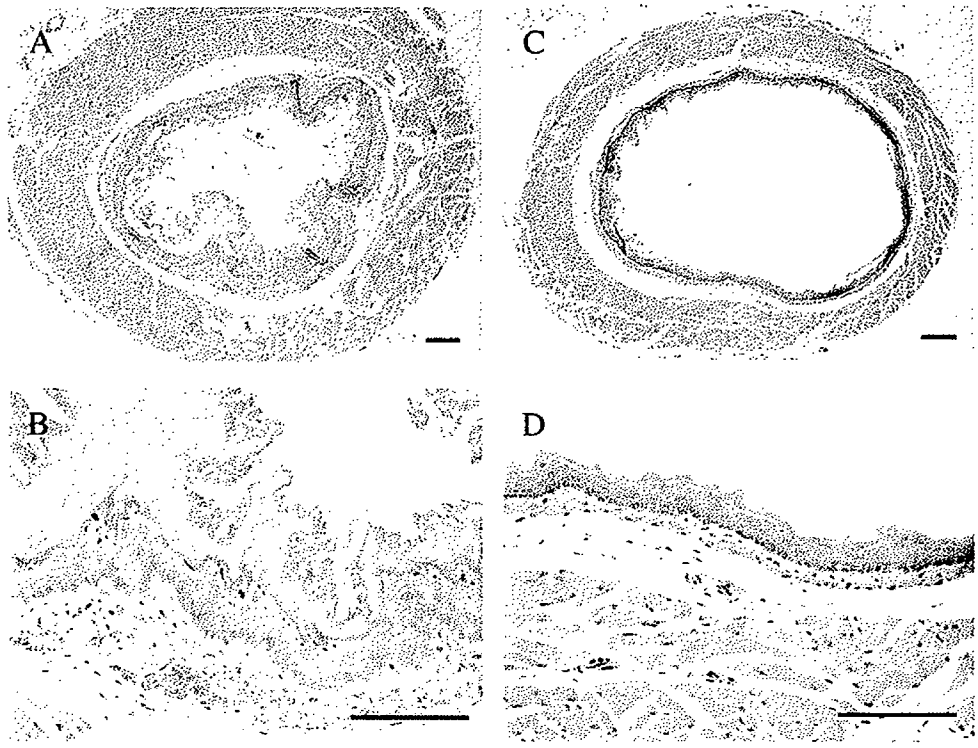

FIGURE 7 Esophageal lesion by s.c. administration of BLM. BLM of 100 mg/kg was continuously administered for 10 days by the osmotic minipumps implanted in the flank subcutis. Severe esophagitis characterized by thick epithelium, significant undulation, keratinization, congested submucosal vessels, and hyperplasia of cells with large-sized nuclei was evident in SCID mice (*A* and *B*), whereas no signs of esophagitis developed in wild-type mice with the same dose of the agent (data not shown) or in SCID mice treated with saline (*C* and *D*). Esophagitis seemed to be the most likely cause of death in SCID mice treated with systemic delivery of BLM. Original magnification was ×10 for *A* and *C*, and ×40 for *B* and *D* (H&E stain). The bars represent 100 μm.

development of pulmonary lesions by the agent, considering the lymphocyte roles in this process. These 2 factors may interfere with each other in the pathogenesis of pulmonary lesions by BLM in SCID mice.

As expected, SCID mice had a much higher mortality than wild-type mice with the systemic administration of BLM. Bone marrow suppression, however, was not evident in SCID mice, confirming the fact that the agent has only a mild effect on bone marrow [31]. Pathological examinations revealed no abnormality in organs except for the esophagus, in which severe esophagitis with remarkable hyperkeratosis and severe dysplasia developed. Although the precise cause of death in SCID mice was not identified, this severe esophageal damage might have had some impact on body weight loss preceding death.

In contrast to systemic administration, mortality with i.t. injection of BLM to SCID and wild-type mice was similar. That is to say, i.t. BLM-induced pulmonary damage was comparable in terms of functional damage severity.

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The chronology of the alteration of BAL cells, however, was significantly different between the 2 mouse types. Lymphocytes in BAL fluid of SCID mice increased in number until 14 days after BLM administration, the same as in wild-type mice, but then declined by day 28 to the level of the saline-injected control animals, whereas they continued to increase in number at day 28 in wild-type mice. This phenomenon is in fact quite reasonable, as SCID mice rendered impaired lymphocyte function with leaky lymphocytes [16]. Accordingly, the total cells and alveolar macrophages in BAL fluid at day 28 were significantly less in number in SCID mice than ones in wild-type mice.

To verify this phenomenon, a strong stimulant of cellular immunity, BCG, was injected to mice by i.t. route. Again, the lymphocyte number in BAL fluid of SCID mice was significantly less than that of wild-type mice when evaluated at days 7, 14, and 28. In addition, total cells, alveolar macrophages and neutrophils in BAL fluid of SCID mice were significantly less than those of wild-type mice at days 14 and 28. These observations confirmed the existence of an impaired lymphocyte response in the process of pulmonary inflammation in SCID mice, as well as the diversity in pulmonary damage between SCID and wild-type mice despite of the similar mortality. Pathological examinations of the lungs after i.t. injection of BLM demonstrated that interstitial pneumonitis developed in both types of mice, but that the pneumonitis in SCID mice was characterized by less lymphocyte infiltration than that in wild-type mice. This was in agreement with the findings regarding the aforementioned differentiated cell numbers in BAL fluid. Significantly elevated hydroxyproline contents by i.t. injection of BLM were observed similarly in both mouse types. All these findings suggested that the severity of pulmonary fibrosis from i.t. BLM injection in SCID mice was comparable to that in wild type, regardless of the lesser degree of lymphocyte involvement in the process in SCID mice.

A body of evidence has already illustrated the important roles of T cell–mediated immune reactions in the development of BLM-induced pulmonary damage; suppression of T-cell function by anti-CD4, anti-CD8 antibodies [6] and anti-CD3 antibody [32] diminished BLM-induced pulmonary fibrosis as measured by hydroxyproline content in the lung, and suppression of suppressor T cells by means of cyclophosphamide augmented pulmonary fibrosis in a BLM-resistant mouse strain [33]. Observations in athymic nude mice, inherently defective in T-cell functions, however, seem controversial. One study demonstrated significantly decreased BLM-induced pulmonary fibrosis as measured by histological findings, hydroxyproline content, and net collagen synthesis in the lung [34], whereas another failed to demonstrate differences in pulmonary fibrosis between athymic nude and its wild-type mice as measured by histological examinations [35]. Although the reasons for these conflicting results are not fully explained, ultimately, the difference in injection route

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of BLM, that is, the i.t. route in the former and i.p route in the latter, possibly had a not insignificant role in these contrasting outcomes. In any events, as far as the i.t. route is concerned, T-cell dysfunction in mice leads to attenuation of pulmonary cell–mediated immune reaction, as was also observed in the BAL findings of the current study. On the other hand, other studies [36, 37] demonstrated BLM (i.t. route)–induced pulmonary fibrosis development in SCID mice with a comparable degree of severity to wild-type mice, similarly to the present study. They also suggested a direct effect of BLM on macrophages, based on the fact that IL-12 and TNF-α mRNA were sustained at a high level in the affected lungs, and speculated that pulmonary fibrosis in SCID animal was not the result of leaky T-cell development [36]. A study concerning topically injected BLM-induced skin lesions also disclosed comparable dermal fibrosis between SCID and wild-type mice [38]. Accordingly, those studies concluded that lymphocyte functions were not necessary in the development of fibrosis in these organs. Although they did not compare the kinetics of inflammatory-cell recruitments in the affected organs, our research clearly showed remarkable lymphocyte infiltration in BAL fluid in the process of BLM-induced inflammation in wild-type mice, as well as significantly higher sensitivity of SCID mice to BLM.

SCID mice have somatic cells including pulmonary epithelial and pulmonary vascular endothelial cells that are hypersensitive to BLM, and impaired lymphocyte function. These 2 factors may cancel each other out, thereby establishing pulmonary fibrosis with a similar degree of severity to wild-type mice. In other words, the pulmonary lesions in SCID and wild-type mice represent 2 types of interstitial lung disease models: one with severe direct tissue damage and mild inflammatory process, and the other with mild direct tissue damage and severe inflammatory process. Although the present study is preliminary in that it does not elucidate the kinetics of potentially involved cytokines and chemokines, it proposes 2 types of lung injury models by means of a single causative agent.

In conclusion, the i.t. injection of BLM to SCID and wild-type mice established a novel experimental model for pulmonary fibrosis. In clinical situations, administrations of BLM and radiotherapy are exclusively for cancer-bearing patients who, in many cases, are relatively immune deficient due to their tumor burden and pretreatments with cytotoxic agents and/or radiation. Therefore, the present experimental lung injury model promises to be of significant value for practical lung injury by BLM and radiation exposure.

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INTERNAL MEDICINE

CASE REPORT

Pulmonary Toxicity by a Cytotoxic Agent, S-1

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Abstract

A 72-year-old man with tongue carcinoma complained of dyspnea on exertion 18 days after starting treatment with S-1. Chest radiograph and CT scan suggested diffuse interstitial lesions with ground glass opacity on both lungs. Bronchoalveolar lavage and transbronchial lung biopsy revealed moderate lymphocyte infiltration with granuloma. Drug lymphocyte stimulation test was positive against tegafur, one of the components of S-1. These findings were consistent with S-1-induced lung injury. Both his symptoms and the radiographic findings were resolved dramatically after high-dose corticosteroid therapy. Clinicians should be aware that S-1 has the potential to cause lung injury when it is included in chemotherapy.

Key words: lung injury, corticosteroid, drug lymphocyte stimulation test (DLST), S-1

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Introduction

Pulmonary toxicity by cytotoxic agents, in theory, falls into three categories according to the mechanisms involved. The first one relates to direct tissue damage by agents, the second one is due to abnormal inflammatory cytokine induction or suppression by the agent's original function or via the effect of cytotoxicity, and the final one is by allergic reaction to agents (1, 2). Now, a majority of the pulmonary toxicity cases caused by cytotoxic agents are considered a consequence of a combination of the three mechanisms. For example, pulmonary toxicity by bleomycin is initiated by pulmonary vascular endothelial cell damage, followed by imbalance of cytokines and adhesion molecules, resulting in severe damage to the alveolar epithelium and interstitium (3).

S-1 is a recently approved oral anti-neoplastic agent composed of three components, tegafur, 5-C-2, 4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo). Tegafur is a prodrug of 5-FU that has been a key drug for gastrointestinal cancers (4), and the other two components are combined to enhance the cytotoxicity and suppress toxicity. In addition to the clinical relevance of 5-FU, there is an increasing body of evidence to suggest the clinical usefulness of S-1 (5-7). The major adverse effects of S-1 are hematological and gastrointestinal, but pulmonary toxicity is not well described. Here, we report a case with S-1-induced lung injury, in which the causative component of the drug was identified by lymphocyte stimulation test (DLST), and treatment was performed successfully with corticosteroid therapy.

Case Report

A 72-year-old man with advanced tongue carcinoma (T4N2M0) was scheduled to undergo hemi-glossectomy. S-1 (80 mg/day) was started prior to surgery as induction chemotherapy by an oral and maxillofacial surgeon. Eighteen days after initiation, he experienced dyspnea on exertion and fever (39.0℃), accompanied by severe hypoxemia (PaO_2 of 54.8 torr and $PaCO_2$ of 24.8 torr). Then he was admitted to the respiratory department of our hospital. He was a current smoker (two packs daily for 50 years) and his past clinical history included mild renal dysfunction from chronic glomerulonephritis, alcoholic liver disease, and gastroesophageal reflux disease. He had been given a proton pump inhibitor (rabeprazole sodium), in addition to S-1.

When he was admitted the respiratory department, physical examination revealed no abnormality except for bilateral fine crackles and pyrexia. Laboratory data included leuko-

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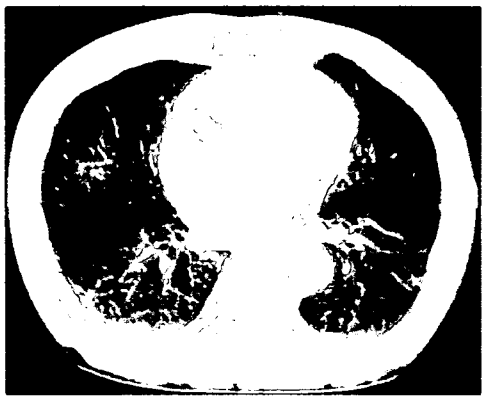
(A)



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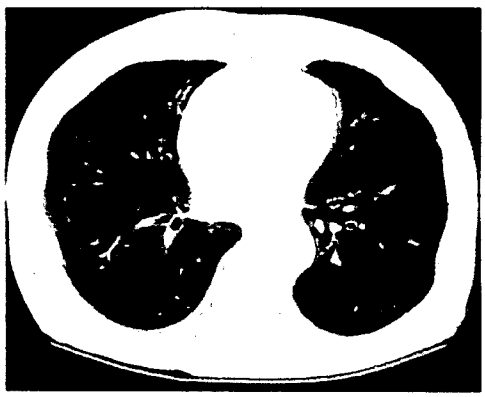

Figure 1. (A) HRCT on admission showed diffuse a reticular shadow and ground glass opacity predominantly at subpleural lesions. (B) The lesions were dramatically resolved 7 days after corticosteroid treatment.

cyte counts of 3.9×10^9/L with eosinophilia (12%) and monocytosis (25%), elevated lactate dehydrogenase of 279 U/L, alkaline phosphatase of 495 U/L, IgG of 1,945 mg/dl, IgE of 811 mg/dl, blood urea nitrogen of 27 mg/dl, serum creatinine of 1.45 mg/dl, CRP of 3.4 mg/ml and KL-6 of 750 U/ml. Chest radiograph showed reticular shadows on both lung fields. High-resolution CT scan (HRCT) revealed diffuse interstitial lesions with alveolar septal thickening and ground glass opacity (GGO), predominantly at subpleural lesions without findings of lung volume loss and honeycombing (Fig. 1A).

A pulmonary function test showed a marked decrease in diffusing capacity of carbon monoxide (32.5% of predicted). Microbiological examination was unremarkable including negative antigen of *Cytomegalovirus, Legionella pneumophilia*, negative PCR data for *Mycobacterium tuberculosis*, *Pneumocystis jiroveci*, and negative antibodies to *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Bronchoalveolar lavage (BAL) from the right middle lobe showed moderately increased cell counts (3.0×10^5/ml) with lymphocyte predominance (alveolar macrophages 17%, lymphocytes 79%, eosinophils 2%, and neutrophils 2%) and a normal T lymphocyte ratio of CD4/CD8 as 2.48. Transbronchial lung biopsy (TBLB) from the anterior segment of the right lower lobe demonstrated moderate lymphocyte infiltration with granulomas (Fig. 2). DLST was employed for the possible drugs that he was given. The three components of S-1 were examined independently. The stimulation index (SI) cut-off was set at 200%, and each drug was examined at multiple concentrations. At higher concentrations, S-1 and tegafur showed an apparently positive response (the maximum SI of 460 and 619, respectively), whereas for rabeprazole sodium, CDHP yielded negative results. DLST for Oxo showed a positive result with mild elevation of SI (246%) only at one dilution point (Fig. 3). Taken together, a diagnosis of drug-induced lung injury was established. By the time of the diagnosis, S-1 and rabeprazole had been discontinued, although hypoxia and fever were still present.

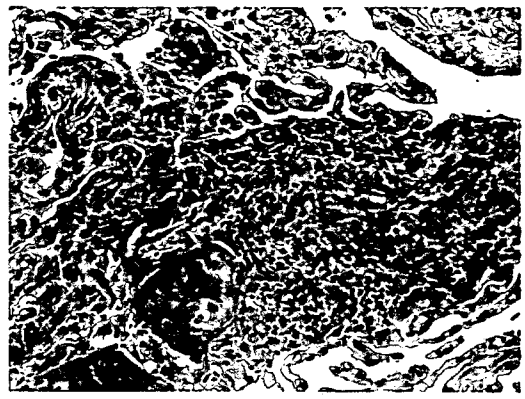

Figure 2. Specimen of transbronchial lung biopsy showed moderate lymphocyte infiltration in the pulmonary interstitium with fibro-edematous thickening and mild vasculitis. Granuloma formation (arrow) was also seen (HE, original magnification: × 10).

High-dose corticosteroid (500 mg of methylprednisolone) was initiated intravenously for three consecutive days followed by oral prednisone (1 mg/kg/day). Seven days after treatment, both his symptoms and radiographic findings were dramatically resolved (Fig. 1B). Also, alveolar-arterial oxygen gradient ($A\text{-}aDO_2$) was significantly decreased from 64.2 to 40.8 torr, and predicted diffusing capacity was increased from 32.5 to 60.3% after treatment (Fig. 4).

Discussion

Most antineoplastic drugs have the potential to induce pulmonary toxicity, involving lung parenchyma, airways, pleura, and pulmonary circulation (8). The mainstay of treatment of drug-induced pneumonia is to identify and remove the causative agent as soon as possible. However, diagnosis is often difficult due to the patient's confounding factors, i.e. pulmonary co-morbidities and other modalities such as concomitant chemotherapy and radiation therapy (9).

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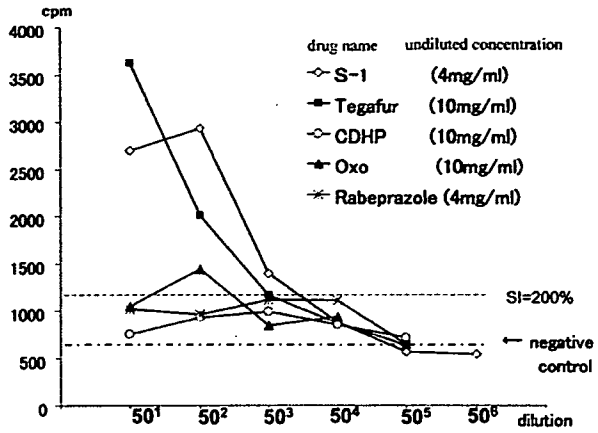

Figure 3. Results of DLST with drugs administered to the patient. The three components of S-1 were also examined separately. Each agent, diversely diluted from 50 ~50^6 fold of the original concentration (indicated in the figure), was added to culture medium containing the patient's peripheral lymphocytes. Note that the tests with tegafur and S-1 provided positive results at multiple dilution points. The figure also indicates the level of negative control and threshold for judging as positive.

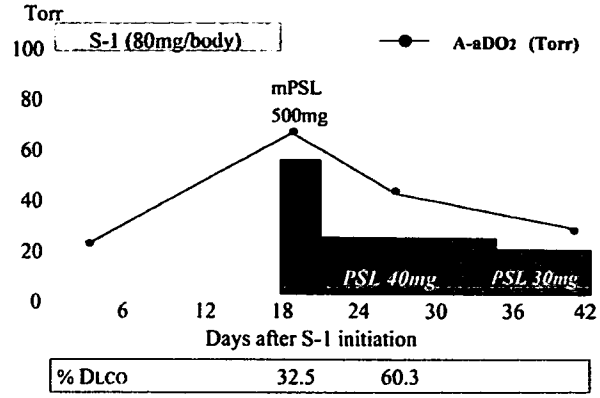

Figure 4. Clinical course. Interstitial pneumonia induced by S-1 dramatically responded to corticosteroid therapy.

Interstitial lung disease is the most common form of drug-induced lung injury. Based on TBLB findings, the present case was proven to have interstitial pneumonia, which indeed responded to corticosteroid treatment. DLST was also helpful for establishing the diagnosis. Although the result of DLST with Oxo was positive with mild elevation of SI at one dilution point, this was not reproducible with other dilution points. As the test was performed with multiple dilution points for each agent, and the three components of S-1 were examined independently, the pneumonitis of the present patient was considered to be drug-induced, most likely by tegafur. Kurakawa et al reported the first case of S-1 associated lung injury diagnosed by DLST, without specifying a causative component (10).

The interpretation of DLST for antineoplastic drug requires a certain caution. Some anti-metabolites directly affect the ^{3}H-thymidine uptake of lymphocytes by inhibiting DNA de novo synthesis, which could result in a false negative response. However, tegafur is able to elicit enhanced T-cell proliferation even in non-sensitized individuals. In fact, 1 out of 10, and 4 out of 20 healthy volunteers showed positive response against UFT (11), and S-1 (12), respectively, when the SI cut-off was set at 200%. It would be important to note, however, that the SI in these healthy volunteers was not so very high, except for one subject with SI of 460% against S-1 (12), that is, it ranged from 124 to 204% against UFT, and from 90 to 247% against S-1. This means that attention should be paid to the evaluation of DLST data with anti-metabolites, and diagnosis should be made in combination with other circumstantial evidence. The present case was most likely affected with hypersensitivity to tegafur, with other clinical findings including eosinophilia and an elevated IgE level.

Pulmonary toxicity of S-1 has rarely been reported. Other cytotoxic agents, 5-FU (active form of S-1) and UFT, which also includes tegafur, however, have been reported to cause mortality by lung injury (13, 14). As the use of S-1 becomes more common, the incidence of S-1 related pulmonary toxicity may also increase. All clinicians should be aware that S-1 has the potential to cause lung injury.

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Short Communication

Randomised phase II trial of irinotecan plus cisplatin *vs* irinotecan, cisplatin plus etoposide repeated every 3 weeks in patients with extensive-disease small-cell lung cancer

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Patients with previously untreated extensive-disease small-cell lung cancer were treated with irinotecan 60 mg m^{-2} on days 1 and 8 and cisplatin 60 mg m^{-2} on day 1 with ($n = 55$) or without ($n = 54$) etoposide 50 mg m^{-2} on days 1–3 with granulocyte colony-stimulating factor support repeated every 3 weeks for four cycles. The triplet regimen was too toxic to be considered for further studies.

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Keywords: small-cell lung cancer; chemotherapy; irinotecan; etoposide; three drug combination

Small-cell lung cancer (SCLC), which accounts for approximately 14% of all malignant pulmonary tumours, is an aggressive malignancy with a propensity for rapid growth and early widespread metastases (Jackman and Johnson, 2005). A combination of cisplatin and etoposide (PE) has been the standard treatment, with response rates ranging from 60 to 90% and median survival times (MSTs) from 8 to 11 months in patients with extensive disease (ED)-SCLC (Fukuoka *et al*, 1991; Roth *et al*, 1992). A combination of irinotecan and cisplatin (IP) showed a significant survival benefit over the PE regimen (MST: 12.8 *vs* 9.4 months, $P = 0.002$) in a Japanese phase III trial for ED-SCLC (Noda *et al*, 2002), although another phase III trial comparing these regimens failed to show such a benefit (Hanna *et al*, 2006). Thus, irinotecan, cisplatin and etoposide are the current key agents in the treatment of SCLC. A phase II trial of the three agents, IPE combination, in patients with ED-SCLC showed a promising antitumour activity with a response rate of 77%, complete response (CR) rate of 17% and MST of 12.9 months (Sekine *et al*, 2003).

We have developed these IP and IPE regimens in a 4-week schedule where irinotecan was given on days 1, 8 and 15. The dose of irinotecan on day 15, however, was frequently omitted because of toxicity in both regimens (Noda *et al*, 2002; Sekine *et al*, 2003). The objectives of this study were to evaluate the toxicities and antitumour effects of IP and IPE regimens in the 3-week schedule in patients with ED-SCLC and to select the right arm for subsequent phase III trials.

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PATIENTS AND METHODS

Patient selection

Patients were enrolled in this study if they met the following criteria: (1) a histological or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) ED, defined as having distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) predicted life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function as documented by a white blood cell (WBC) count $\geqslant 4.0 \times 10^3\,\mu l^{-1}$, neutrophil count $\geqslant 2.0 \times 10^3\,\mu l^{-1}$, haemoglobin $\geqslant 9.5\,g\,dl^{-1}$, platelet count $\geqslant 100 \times 10^3\,\mu l^{-1}$, total serum bilirubin $\leqslant 1.5\,mg\,dl^{-1}$, hepatic transaminases $\leqslant 100\,IU\,l^{-1}$, serum creatinine $\leqslant 1.2\,mg\,dl^{-1}$, creatinine clearance $\geqslant 60\,ml\,min^{-1}$, and $PaO_2 \geqslant 60$ torr; and (9) providing written informed consent.

Patients were not eligible for the study if they had any of the following: (1) uncontrollable pleural, pericardial effusion or ascites; (2) symptomatic brain metastasis; (3) active infection; (4) contraindications for the use of irinotecan, including diarrhoea, ileus, interstitial pneumonitis and lung fibrosis; (5) synchronous active malignancies; (6) serious concomitant medical

illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or breast feeding.

Treatment schedule

In the IP arm, cisplatin, 60 mg m^{-2}, was administered intravenously over 60 min on day 1 and irinotecan, 60 mg m^{-2}, was administered intravenously over 90 min on days 1 and 8. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not administered in this arm. In the IPE arm, cisplatin and irinotecan were administered at the same dose and schedule as the IP arm. In addition, etoposide, 50 mg m^{-2}, was administered intravenously over 60 min on days 1–3. Filgrastim 50 μg m^{-2} or lenograstim 2 μg kg^{-1} was subcutaneously injected prophylactically from day 5 to the day when the WBC count exceeded 10.0×10^3 μl^{-1}. Hydration (2500 ml) and a $5HT_3$ antagonist were given on day 1, followed by an additional infusion if indicated in both arms. These treatments were repeated every 3 weeks for a total of four cycles.

Toxicity assessment, treatment modification and response evaluation

Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0.

Doses of anticancer agents in the following cycles were modified according to toxicity in the same manner in both arms. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000).

Study design, data management and statistical considerations

This study was designed as a multi-institutional, prospective randomised phase II trial. This study was registered on 6 September 2005 in the University hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan (http://www.umin.ac.jp/ctr/index.htm), which is acceptable to the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/faq.pdf). The protocol and consent form were approved by the Institutional Review Board of each institution. Patient registration and randomisation were conducted at the Registration Center. No stratification for randomisation was performed in this study. The sample size was calculated according to the selection design for pilot studies based on survival (Liu *et al*, 1993). Assuming that (1) the survival curve was exponential for survivals; (2) the MST of the worse arm was 12 months and that of the better arm was 12 months × 1.4; (3) the correct selection probability was 90%; and (4) additional follow-up in years after the end of accrual was 1 year, the estimated required number of patients was 51 for each arm. Accordingly, 55 patients for each arm and their accrual period of 24 months were planned for this study.

The dose intensity of each drug was calculated for each patient using the following formula as previously described:

$$\text{The dose intensity (mg m}^{-2}\text{ week}^{-1}\text{)} = \frac{\text{Total milligrams of a drug in all cycles per body surface area}}{\text{Total days of therapy}/7}$$

where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 21 days for both arms (Hryniuk and Goodyear, 1990).

Differences in the reason for termination of the treatment and the frequencies of grade 3–4 toxicities were assessed by χ tests. Survival was measured as the date of randomisation to the date of death from any cause or the date of the most recent follow-up for overall survival and to the date of disease progression or the date of death for progression-free survival (PFS). The survival of the arms was estimated by the Kaplan–Meier method and compared in an exploratory manner with log-rank tests (Armitage *et al*, 2002).

RESULTS

Patient characteristics

From March 2003 to May 2005, 55 patients were randomised to IP and 55 patients to IPE. One patient in the IP arm was excluded because the patient was ineligible and did not receive the study treatment. The remaining 109 patients were included in the analyses of toxicity, tumour response and patient survival. There were no differences between the two arms in any demographic characteristics listed (Table 1).

Treatment delivery

Treatment was well tolerated with respect to the number of cycles delivered in both arms (Table 2). Among reasons for termination of the treatment, disease progression was noted in nine (17%)

Table 1 Patient characteristics

	IP ($n=54$)	IPE ($n=55$)
Sex		
Female	11	8
Male	43	47
Age (years)		
Median (range)	63 (42–70)	62 (48–70)
PS		
0	11	12
1	42	41
2	1	2
Weight loss		
0–4%	38	43
5–9%	10	10
⩾10%	6	2

Table 2 Treatment delivery

	IP ($n=54$) No. (%)	IPE ($n=55$) No. (%)
Number of cycles delivered		
6[a]	—	1 (2)
4	41 (76)	36 (65)
3	6 (11)	6 (11)
2	3 (6)	6 (11)
1	4 (7)	6 (11)
Reasons for termination of the treatment[†]		
Completion	40 (74)	35 (64)
Disease progression	9 (17)	2 (4)
Toxicity	3 (6)	13 (24)
Patient refusal	2 (4)	4 (7)
Others	0 (0)	1 (2)
Total number of cycles delivered	192 (100)	186 (100)
Total number of omission on day 8	35 (18)	37 (17)
Total number of cycles with dose reduction	28 (15)	31 (17)

[†]$P=0.013$ by χ^2 test. [a]Protocol violation.

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patients in the IP arm and in two (4%) patients in the IPE arm, whereas toxicity was noted in three (6%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.013$) (Table 2). The dose of irinotecan on day 8 was omitted in 35 (18%) cycles in the IP arm and 37 (17%) cycles in the IPE arm (Table 2). The total dose and dose intensity of cisplatin and etoposide were similar between the IP and IPE arms in the present study (Table 3).

Toxicity

The myelotoxicity was more severe in the IPE arm (Table 4). Grade 3 febrile neutropaenia was noted in 5 (9%) patients in the IP arm and 17 (31%) patients in the IPE arm ($P=0.005$). Packed red blood cells were transfused in 4 (7%) patients in the IP regimen and 14 (26%) patients in the IPE regimen ($P=0.011$). Platelet concentrates were needed in none in the IP regimen and 2 (4%) patients in the IPE regimen ($P=0.16$). Grade 3–4 diarrhoea was observed in 8 (15%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.262$). Grade 3–4 fatigue was more common in the IPE arm with marginal significance (2 *vs* 11%, $P=0.054$). The severity of other non-haematological toxicities did not differ significantly between the arms. No treatment-related death was observed in this study.

Table 3 Total dose and dose intensity

	3-week regimens in this study		4-week regimen[a]
	IP ($n=54$) Median (range)	IPE ($n=55$) Median (range)	IPE ($n=30$) Median (range)
Total dose ($mg\,m^{-2}$)			
Cisplatin	240 (60–240)	240 (60–360)	240 (60–240)
Irinotecan	420 (60–480)	390 (60–720)	563 (60–720)
Etoposide	0	600 (150–900)	600 (150–600)
Dose intensity ($mg\,m^{-2}\,week^{-1}$)			
Cisplatin	19 (14–25)	20 (16–34)	15 (12–15)
Irinotecan	33 (14–40)	35 (15–55)	35 (19–45)
Etoposide	0	48 (34–68)	37 (28–38)

[a]From our previous study (Sekine *et al*, 2003).

Table 4 Grade 3–4 toxicities

	IP ($n=54$)			IPE ($n=55$)		
	Grade 3	4	3+4 (%)	Grade 3	4	3+4 (%)
Leukocytopaenia	9	1	10 (19)	18	11	29 (53)[a]
Neutropaenia	17	11	28 (52)	24	28	52 (95)[a]
Anaemia	18	0	18 (25)	16	9	25 (45)
Thrombocytopaenia	2	0	2 (4)	13	0	13 (13)[b]
Febrile neutropaenia	5	0	5 (9)	17	0	7 (13)
Diarrhoea	8	0	8 (15)	11	2	13 (24)
Vomiting	4	0	4 (7)	3	0	3 (5)
Fatigue	1	0	1 (2)	5	1	6 (11)[b]
Hyponatraemia	9	3	12 (22)	11	2	13 (24)
AST elevation	0	0	0 (0)	3	0	3 (5)
CRN elevation	1	0	1 (2)	0	0	0 (0)

[a]$P<0.001$; [b]$P<0.01$; and [b]$P=0.054$ by χ^2 test.

Response, treatment after recurrence and survival

Four CRs and 37 partial responses (PRs) were obtained in the IP arm, resulting in the overall response rate of 76 with 95% confidence interval (CI) of 65–87%, whereas six CRs and 42 PRs were obtained in the IPE arm, and the overall response rate was 87% with a 95% CI of 79–96% ($P=0.126$). Median PFS was 4.8 months (95% CI, 4.0–5.6) in the IP and 5.4 months (95% CI, 4.8–6.0) in the IPE arm ($P=0.049$) (Figure 1A). After recurrence, 22 (44%) patients in the IP arm and 8 (16%) patients in the IPE arm received etoposide-containing chemotherapy. The MST and 1-year survival rate were 12.4 months (95% CI, 9.7–15.1) and 54.8% (95% CI, 41.4–68.2%) in the IP and 13.7 months (95% CI, 11.9–15.5) and 61.5% (95% CI, 48.6–74.4%) in the IPE arm ($P=0.52$), respectively (Figure 1B).

DISCUSSION

This study showed that the IPE regimen in a 3-week schedule with CSF support produced a promising response rate, PFS and overall survival. Haematological toxicity in the IPE arm, however, was very severe in spite of the G-CSF support with the grade 3 febrile neutropaenia noted in 31% of patients.

In comparison between the 3-week IPE regimen in this study and the 4-week IPE regimen in the previous study, the delivery of cisplatin and etoposide was improved in the 3-week IPE regimen when compared with the 4-week IPE regimen at the cost of the irinotecan total dose. The response rate and MST were 87% and 13.7 months, respectively, in the 3-week IPE regimen and 77% and 12.9 months in the previous 4-week schedule, and toxicity profiles were comparable to each other (Sekine *et al*, 2003).

The MST of 12.4 months in the IP arm in this study was comparable to that of the previous phase III study, with an MST of 12.8 months (Noda *et al*, 2002). Thus, this study showed the reproducible excellent survival outcome of patients with ED-SCLC who were treated with the IP combination. In contrast, a recent American phase III study of the PE regimen *vs* IP regimen failed to show the superiority of the IP regimen to the PE regimen; the MST

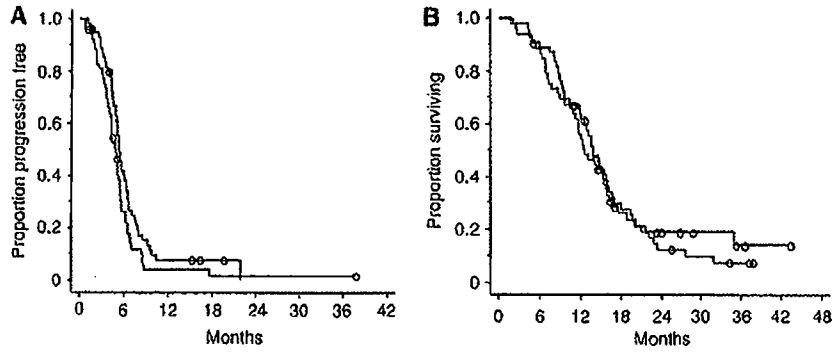

Figure 1 Progression-free survival (**A**) and overall survival (**B**). Thick line indicates the IPE regimen and thin line indicates the IP regimen.

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for the PE regimen was 10.2 months and that for the IP regimen was 9.3 months (Hanna *et al*, 2006). The discrepancy between the Japanese and American trials may be explained by the different cisplatin dose schedules; cisplatin was delivered at a dose of 60 mg m^{-2} on day 1 every 3 or 4 weeks in the Japanese trials, whereas cisplatin was delivered at a dose of 30 mg m^{-2} on days 1 and 8 every 3 weeks in the American one. A platinum agent administered at divided doses was associated with poor survival in patients with ED-SCLC in our previous randomised phase II study (Sekine *et al*, 2003).

The issue of adding further agents to the standard doublet regimen has been investigated in patients with ED-SCLC. The addition of ifosfamide or cyclophosphamide and epirubicin to the cisplatin and etoposide combination produced a slight survival benefit, but at the expense of greater toxicity (Loehrer *et al*, 1995; Pujol *et al*, 2001). Phase III trials of cisplatin and etoposide with or without paclitaxel showed unacceptable toxicity with 6–13% toxic deaths in the paclitaxel-containing arm (Mavroudis *et al*, 2001; Niell *et al*, 2005). The results in these studies and the current study are consistent in the increased toxicity despite the G-CSF support and no definite survival benefit in the three or four drug combinations over the standard doublet in patients with ED-SCLC.

In conclusion, the IPE regimen was marginally more effective than the IP regimen, but was too toxic despite the administration of prophylactic G-CSF.

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