（肺癌．2021;61:130-137）

CASE REPORT

Spontaneous Regression of Malignant Pleural Mesothelioma, Possibly Caused by CD8$^+$ Tumor-infiltrating Lymphocytes

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ABSTRACT —— ***Background.*** Malignant pleural mesothelioma is a disease with a poor prognosis and limited therapeutic options. With only a few reports available, spontaneous regression of malignancy is very rare, and the mechanism underlying spontaneous regression of malignancy is unknown. ***Case.*** A 68-year-old man was referred to our hospital because of dyspnea on exertion, anorexia, and weight loss. Chest computed tomography (CT) revealed multiple tumors in the pleura and interlobar pleura at the right side along with right pleural effusion. We performed a percutaneous needle biopsy of a pleural mass and thoracentesis. We finally diagnosed the patient with epithelioid malignant pleural mesothelioma, pathological T3N0M0 stage III. However, the patient's performance status was 3, so we concluded that chemotherapy would be difficult and suggested best supportive care instead. One year later, he was referred to our hospital again. Chest CT indicated that multiple tumor shadows on the pleura and interlobar pleura had been reduced and decreased despite no medical treatment, such as radiotherapy, chemotherapy, or immunotherapy. Thus, spontaneous regression of malignant pleural mesothelioma was considered to have occurred. ***Conclusion.*** Spontaneous regression of malignant pleural mesothelioma is very rare. CD8$^+$ T cells are known to play a critical role in antitumor immunity. In our case, we performed immunohistochemical anti-CD8 antibody staining, and many tumor-infiltrating lymphocytes were stained. There was a significantly greater increase of CD8$^+$ than CD4$^+$ tumor-infiltrating lymphocytes. This significant increase in CD8$^+$ tumor-infiltrating lymphocytes further supported the possibility that the spontaneous regression of the malignant pleural mesothelioma had been caused by the antitumor effect of CD8$^+$ tumor-infiltrating lymphocytes. These findings suggest that the spontaneous regression of malignant pleural mesothelioma may have been caused by CD8$^+$ tumor-infiltrating lymphocytes. The presence of CD8$^+$ tumor-infiltrating lymphocytes may correlate with spontaneous regression and an improved clinical outcome.

(*JJLC.* 2021;61:130-137)

KEY WORDS —— Malignant pleural mesothelioma, Tumor-infiltrating lymphocytes, Spontaneous regression

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a disease with a poor prognosis and limited therapeutic options. MPM is associated with a history of exposure to asbestos. A total of 103,000 deaths in Japan are predicted to be caused by MPM over the next 40 years. MPM carries an extremely poor prognosis, and the median survival of patients with MPM is 9 to 12 months from the diagnosis. The incidence of MPM is increasing worldwide because of the history of exposure to asbestos in industrialized countries up to the 1970s. Only 10-15% of patients have resectable disease at presentation. MPM is naturally resistant to chemotherapy; even with standard cisplatin (CDDP) plus pemetrexed (PEM) therapy, the median survival is only 12.1 months. According to the MERIT

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Received September 6, 2020; accepted January 8, 2021.

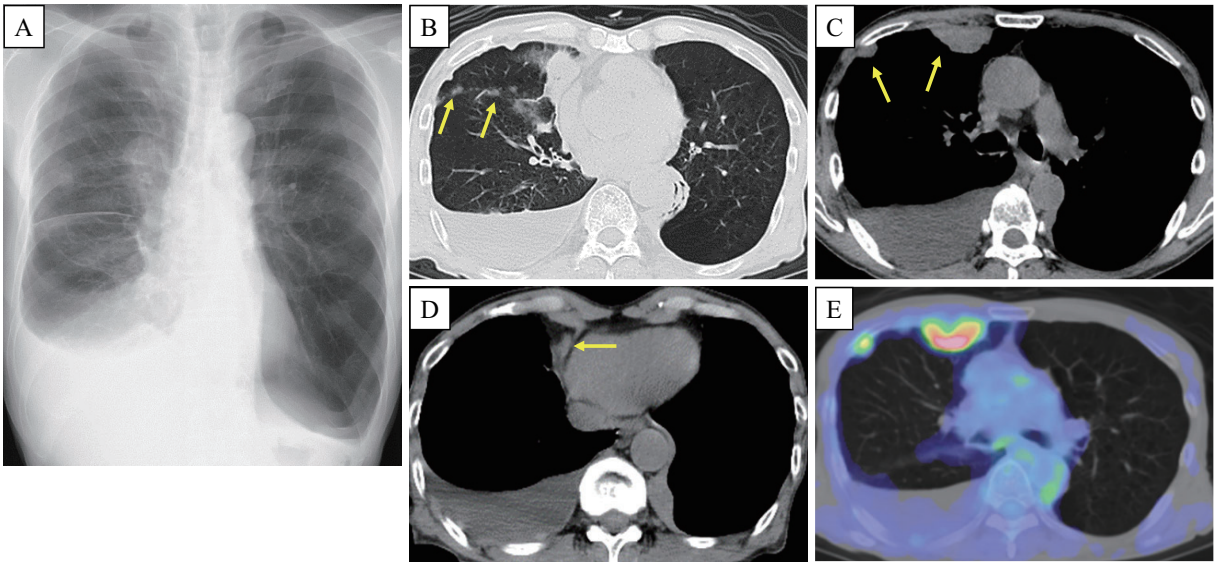

Figure 1. Radiological findings of malignant pleural mesothelioma. **A:** Chest X-ray. X-ray showing multiple nodular shadows and pleural effusion on the right side. **B:** Chest CT of the lung window. CT reveals multiple tumor shadows (yellow arrows) on the interlobar pleura on the right side. **C:** Chest CT of the mediastinal window. CT shows multiple pleural mass shadows (yellow arrows) and right pleural effusion. **D:** Chest CT of the mediastinal window. The nodule had infiltrated into the mediastinal fat tissue (yellow arrow). **E:** PET-CT reveals the accumulation of FDG in the right pleural mass.

study conducted in Japan, nivolumab has meaningful clinical efficacy on previously treated MPM. Nivolumab was approved as a second-line treatment in August 2018 in Japan.[1] Evidence is only available for the first-line treatment of CDDP plus PEM and second-line treatment with nivolumab monotherapy; thus, treatment options are very limited.

Spontaneous regression of malignancy was first defined as the partial or complete disappearance of the tumor without any treatment or in the presence of therapy that is considered inadequate to exert a significant influence on neoplastic disease. Spontaneous regression of malignancy is very rare, with a frequency of approximately 1 in 60,000 to 100,000 cases.[2] Only six cases of spontaneous regression of MPM have been reported.

We experienced a very rare case of spontaneous regression of MPM. This is the first report to suggest that spontaneous regression of MPM may have been caused by $CD8^+$ tumor-infiltrating lymphocytes (TILs).

CASE

In October 2017, a 68-year-old man was referred to our hospital because of dyspnea on exertion, anorexia, and weight loss. He had a history of smoking 20 cigarettes per day for the past 50 years. He worked in a precision machinery factory with no history of asbestos inhalation.

His vital signs at presentation were as follows: body temperature, 36.8℃; blood pressure, 163/98 mmHg; pulse rate, 104 beats/min; and peripheral oxygen saturation (SpO_2) on room air, 93%. A physical examination revealed decreased respiratory sounds in the right lung. Chest X-ray showed multiple nodules and pleural effusion on the right side (Figure 1A). Chest computed tomography (CT) revealed multiple tumors at the pleura and interlobar pleura on the right side and right pleural effusion (Figure 1B, 1C). Some of the nodules had infiltrated into the mediastinal fat tissue (Figure 1D). No abnormalities were found in the lung parenchyma, and no enlargement of the mediastinal lymph nodes was observed. A blood test indicated elevated levels of tumor markers, such as cytokeratin fragment (5.6 ng/ml) and soluble mesothelin-related peptides (2.1 nmol/*l*) (Table 1). Because we suspected malignancy, such as lung cancer or MPM, we performed a percutaneous needle biopsy of a pleural mass and thoracentesis. The pleural effusion was macrophage-predominant and exudative, and elevated hyaluronic acid levels were detected in the pleural effusion (22.5×10^4 ng/ml) (Table 2).

The cytology revealed many multinucleated cells that

Table 1. Blood Test Findings in November 2017

Hematology		Blood chemistry	
WBC	11,810/μl	TP	7.4 g/dl
Neu	76.0%	Alb	3.9 g/dl
Lym	15.2%	T-bil	0.62 mg/dl
Eos	0.6%	AST	24 U/*l*
Baso	0.4%	ALT	19 U/*l*
Mono	7.8%	LDH	180 U/*l*
Hb	14.3 g/dl	BUN	6.1 mg/dl
Plt	12.6×10^4/μl	Cre	0.58 mg/dl
Serology		Na	139 mEq/*l*
CRP	0.17 mg/dl	K	3.64 mEq/*l*
		Cl	103 mEq/*l*
		CEA	2.82 ng/ml
		CYFRA	5.6 ng/ml
		ProGRP	62.7 pg/ml
		SMRP	2.1 nmol/*l*

WBC: white blood cell; Neu: neutrophil; Lym: lymphocyte; Eos: eosinophil; Baso: basophil; Mono: monocyte; Hb: hemoglobin; Plt: platelet; CRP: C-reactive protein; TP: total protein; Alb: albumin; T-bil: total bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; BUN: blood urea nitrogen; Cre: creatinine; Na: sodium; K: potassium; Cl: chlorine; CEA: carcinoembryonic antigen; CYFRA: cytokeratin fragment; ProGRP: progastrin-releasing peptide; SMRP: soluble mesothelin-related peptides.

Table 2. Pleural Effusion Findings in November 2017

Specific gravity	1.035
TP	5.1 g/dl
Glu	48 mg/dl
Cell count	2,844/μl
Histiocyte	49.0%
Lym	29.0%
Seg	2.0%
Atypical cells	19.0%
Mesothelial cells	1.0%
LDH	1,094 U/*l*
ADA	33.2 U/*l*
CYFRA	825.0 ng/ml
Hyaluronic acid	225,000 ng/ml

TP: total protein; Glu: glucose; Lym: lymphocyte; Seg: segmented neutrophil; LDH: lactate dehydrogenase; ADA: adenosine deaminase; CYFRA: cytokeratin fragment.

had formed spherical and papillary cell clumps. Cells with large nuclei had mutual inclusion, and cells with hump-like cytoplasmic protrusions were observed (Figure 2A, 2B). In the cell block, cell nests composed of atypical cells with a high nuclear/cytoplasm ratio and large nuclei were scattered, and abnormal cell division was observed. Epithelial membrane antigen staining was strongly positive. A histological examination of the pleural mass revealed that atypical epithelial-like cells had formed a glandular duct, and there was invasive proliferation in the hyaline-thickened pleural tissue (Figure 2C, 2D). According to the immunohistochemical analysis, mesothelioma markers, including calretinin and podoplanin, were positive, but adenocarcinoma markers, such as carcinoembryonic antigen, thyroid transcription factor-1, and napsin A, were negative. Thus, we diagnosed the patient with epithelioid MPM.

We performed ^{18}F-fluorodeoxyglucose-positron emission tomography-computed tomography (^{18}F-FDG/PET-CT), which revealed the accumulation of FDG in the mass at the right pleura and in the mediastinal fat tissue (Figure 1E). Contrast-enhanced magnetic resonance imaging of the brain did not identify any abnormalities, such as brain metastasis. We finally diagnosed the patient with epithelioid MPM, pathological T3N0M0 stage III (seventh edition of the TNM classification for MPM). However, his performance status (PS) was 3 (he used a wheelchair to move and required oxygen during exertion). Therefore, we concluded that chemotherapy would be difficult and thus suggested best supportive care (BSC). The patient agreed with BSC, and we referred him to a palliative care center in November 2017.

One year later in November 2018, he was referred to our hospital because of dyspnea on exertion again. We performed a blood test, chest X-ray, chest CT, and pulmonary function test. Surprisingly, chest X-ray showed that the right pleural effusion had disappeared, and the nodular shadow had shrunk. Chest CT indicated that the multiple tumor shadows on the pleura and interlobar pleura had been reduced despite no medical treatment, such as radiotherapy, chemotherapy, or immunotherapy. The only changes the patient had made were to cease smoking and eat regularly. We were shocked by the clinical course of spontaneous regression of MPM. His PS improved from 3 to 1, he gained 5 kg, and his resting SpO_2 increased from 93% to 96%. He became able to move without a wheelchair and did not require oxygen inhalation during exertion. Upon further investigation, the pleural effusion and multiple tumor shadows were found to have worsened until December 2017, but by February 2018, both entities had improved without

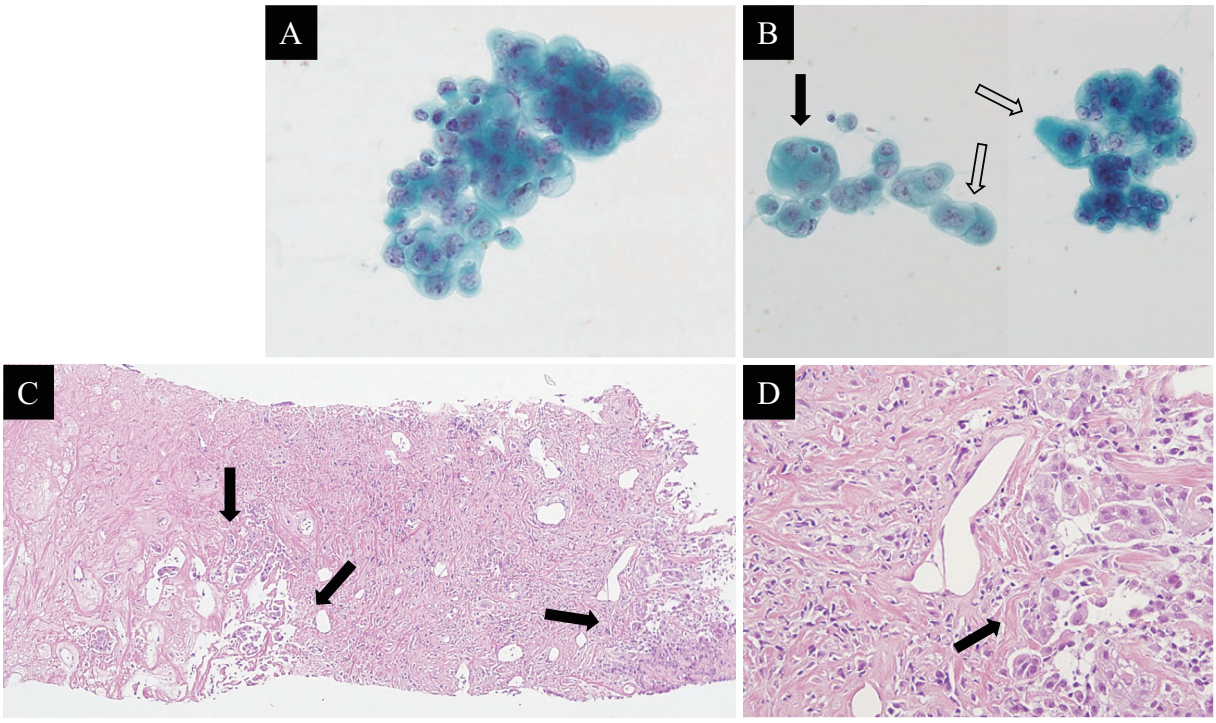

Figure 2. Pathological findings of malignant pleural mesothelioma. **A:** Pleural effusion cytology. Many multinucleated cells form spherical and papillary cell clumps. **B:** Cells with large nuclei showed mutual inclusion (black arrow), and cells with a hump-like cytoplasmic protrusion (white arrows) were observed. **C, D:** Histological findings of the pleural mass revealed that atypical epithelial-like cells formed a glandular duct and invasive proliferation (black arrows) occurred in the hyaline-thickened pleural tissue.

any treatment. From February 2018 to November 2018, the MPM continued to regress without regrowth (Figure 3). Pulmonary function tests revealed obstructive ventilation disorders and a maximal expiratory flow-volume curve described as concave downward. Compared to 1 year earlier, his forced vital capacity had improved from 1.79 to 2.23 *l* (71.0% of predicted), and his forced expiratory volume in 1 second had improved from 0.77 to 1.03 *l*. We diagnosed him with chronic obstructive pulmonary disease, and he started inhalation therapy.

We have been observing him in the outpatient clinic for about three years since the diagnosis. Chest CT in September 2020 showed that the MPM had continued to regress, and there was no recurrence.

We performed CD4, CD8, and PD-L1 staining. Hematoxylin and eosin staining revealed atypical epithelial-like cells that formed glandular duct and invasive proliferation and lymphocytes that infiltrated around the tumor (Figure 4A). Most lymphocytes were not stained on anti-CD4 antibody staining (Figure 4B), but many were stained on anti-CD8 antibody staining (Figure 4C). The infiltration of $CD8^+$ T lymphocytes around the tumor was significantly higher than that of $CD4^+$ T lymphocytes. The expression of programmed cell death-ligand 1 (PD-L1) was 1-5% (Figure 4D).

DISCUSSION

MPM is a disease with a poor prognosis. The current chemotherapeutic regimens are limited, and even with the use of chemotherapy, treatment has little effect on the prognosis. In our case, MPM showed partial disappearance with no treatment and was thus considered a case of spontaneous regression. Only six reports have described the spontaneous regression of MPM (Table 3).[3-8] Those five of the six previous cases and our case displayed an epithelioid histological type, in one case, histological type was unknown. In three of the six previous cases and our case, spontaneous regression was recognized relatively early within 3 months of diagnosis. However, in four of the six previous cases, MPM recurrence was later observed. In these reports, the mecha-

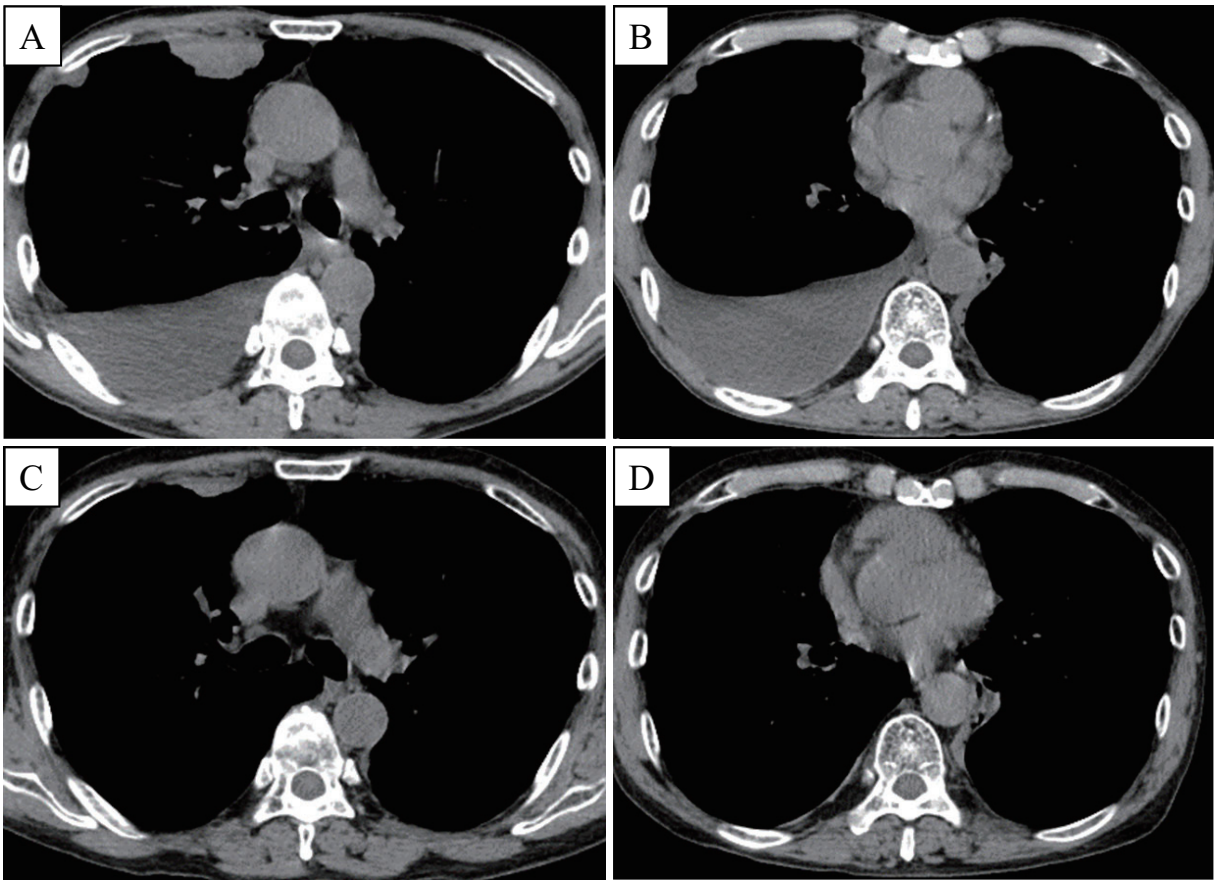

Figure 3. Changes in radiological findings. **A, B:** Chest CT in November 2017 with a diagnosis of malignant pleural mesothelioma. CT reveals multiple tumor shadows at the pleura and interlobar pleura on the right side along with right pleural effusion. **C, D:** Chest CT in November 2018. The pleural effusion had disappeared, and the pleural mass lesion showed regression.

nism underlying the spontaneous regression was described as having a potential immunological basis. Robinson et al. reported that lymphoid infiltration was observed in areas where spontaneous regression occurred but not in areas where progression was observed.[4] Pilling et al. reported that in a case of spontaneous regression of MPM, a moderate host inflammatory response with small round lymphocytes was easily visible around the tumor cells.[5]

Immune infiltration into tumors includes the infiltration of natural killer (NK) cells, B and T lymphocytes, mast cells, neutrophils, macrophages, and dendritic cells (DCs). NK cells, cytotoxic T cells, mature DCs, and T helper cells are known to have antitumor properties. TILs consist of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells. TILs recognize tumor-specific antigens, thus playing an important role in the immune defense against cancer. Among TILs, $CD8^+$ TILs are cytotoxic and are thought to be involved in the antitumor immune response in the cancer-immunity cycle.[9] Several clinical studies have revealed that an increased density of $CD8^+$ TILs is associated with a better prognosis in a variety of malignant tumors, including MPM.[10] In patients with MPM treated with induction chemotherapy followed by extrapleural pneumonectomy, $CD8^+$ TILs remained an independent prognostic factor associated with a better progression-free survival.[11] In another study, a high density of $CD8^+$ TILs was a significantly better prognostic factor for the overall survival of patients with extrapleural pneumonectomy ($P<0.05$).[12] Previous studies have reported that the presence of intratumoral $CD8^+$ T cells was correlated with improved clinical outcomes of patients with MPM.[11,12] High levels of $CD8^+$ TILs are reportedly associated with a better prognosis than intermediate and low numbers in non-small-cell lung cancer. The prognosis was considered to be better when the infiltration of

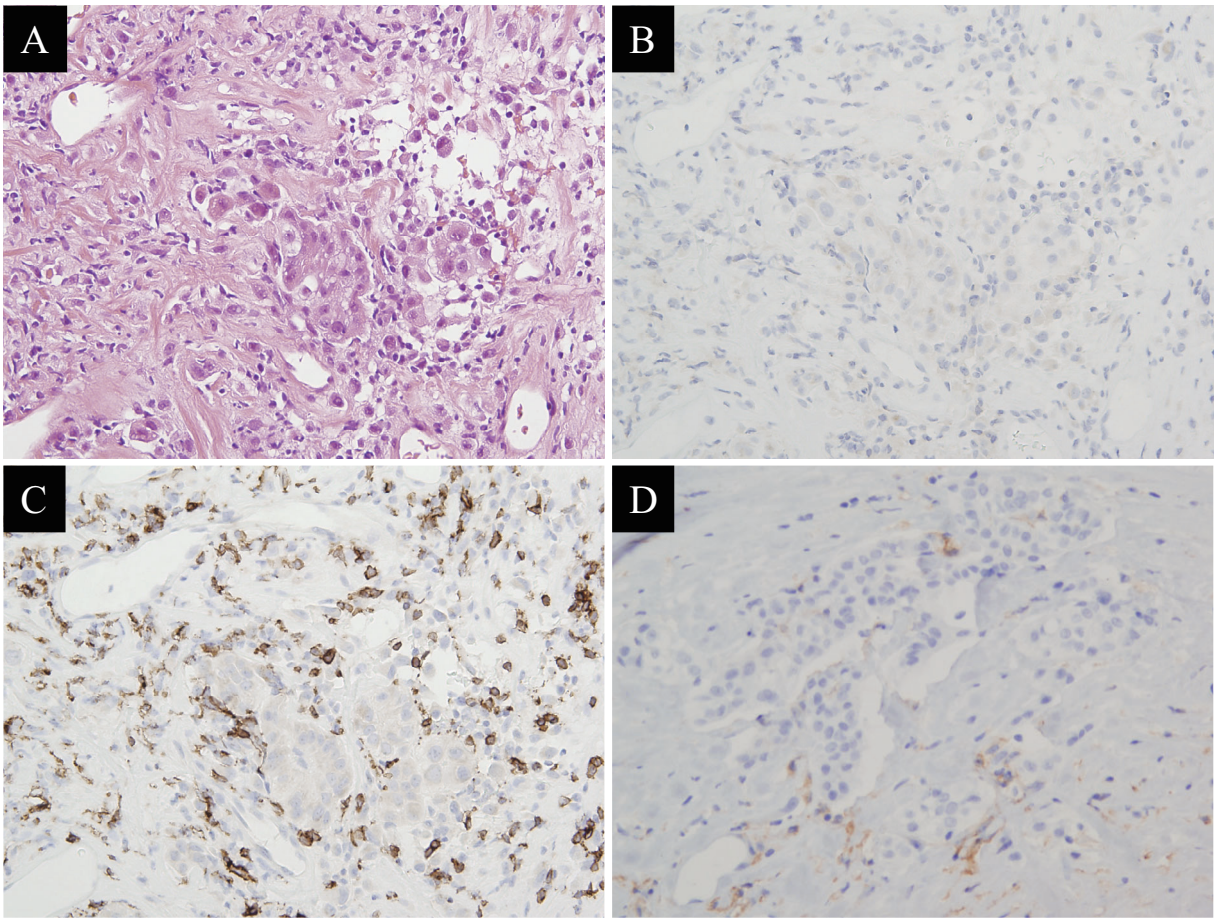

Figure 4. Immunohistochemical antibody staining. **A:** Hematoxylin and eosin staining. Atypical epithelial-like cells formed a glandular duct and invasive proliferation is shown with lymphocytes infiltrating around the tumor. **B:** Anti-CD4 antibody staining. Most lymphocytes were not stained. **C:** Anti-CD8 antibody staining. Most lymphocytes were stained. The infiltration of $CD8^+$ T lymphocytes was significantly increased. **D:** PD-L1 staining. The expression of PD-L1 was 1-5%.

$CD8^+$ T cells was high in the stroma and around the tumor.[13] A previous report described the relationship between spontaneous regression and $CD8^+$ TILs in lung cancer. In that case, $CD4^+$ T cells and $CD8^+$ T cells had infiltrated around the tumor, and spontaneous regression of the lung cancer occurred temporarily, but recurrence later developed. A biopsy performed before the spontaneous regression and again after enlargement showed that the $CD8^+$ TILs decreased as the tumor progressed but increased with spontaneous regression.[14] A significant increase in $CD4^+$ TILs and a decrease in $CD8^+$ TILs in the tumor stroma occurred concomitantly with enlargement of the lesion. Thus, $CD8^+$ TILs, but not $CD4^+$ TILs, might play a crucial role in spontaneous regression. $CD8^+$ TILs may play a pivotal role in the antitumor immune response against these tumors and result in better local control and a prolonged survival. Therefore, $CD8^+$ TILs might be involved in both good outcomes as well as spontaneous regression. The significant increase in $CD8^+$ TILs further strengthens the possibility that the spontaneous regression of MPM was caused by the antitumor effect of $CD8^+$ TILs. In fact, even in our case, the $CD8^+$ TIL numbers were significantly increased compared to those of $CD4^+$ TILs. It is thus possible that $CD8^+$ TILs caused spontaneous regression of MPM.

A previous study focusing on the relationship between $CD8^+$ TILs and PD-L1 reported that higher $CD8^+$ TIL numbers and a low/negative PD-L1 expression was associated with a favorable prognosis in non-small-cell lung cancer.[15] PD-L1 is a well-known biomarker for immune escape in the tumor microenvironment and is sug-

Table 3. Previous Reports on Spontaneous Regression of Malignant Pleural Mesothelioma and the Current Case

Author	Country Year	Sex Age (years)	Histology	Previous therapy	Spontaneous regression	Recurrence, clinical course	Mechanism of spontaneous regression
Kawanishi R, et al. [3]	Japan 1997	Male 37	Epithelioid	No	3 months after the diagnosis	No recurrence at 1 year	Unknown
Robinson BW, et al. [4]	Australia 2001	Female 54	Epithelioid	No	3 months after the diagnosis	Recurrence in other part, died 20 months later	Possible immunological mechanism
Pilling JE, et al. [5]	UK 2007	Male 58	Epithelioid	No	Regression after the diagnosis	Recurrence after 5 years, performed surgical resection, no recurrence at 7 years	Possible immunological mechanism
Allen RK, et al. [6]	Australia 2007	Female 61	Epithelioid	No	3 months after the diagnosis	No recurrence at 4 years	Possible immunological mechanism
Higashiyama M, et al. [7]	Japan 2009	Male 73	Epithelioid	Surgery, chemotherapy	Relapse after chemotherapy and regression 4 months later	No recurrence at 29 months, but local recurrence	Intake of enzyme parasympathetic therapy
Raphael J, et al. [8]	Canada 2015	Male 76	Unknown	No	Enlargement until 2 years later, but suddenly regression 6 months later	Recurrence after 6 months	Intake of herbal and vegetables
Current case	Japan 2020	Male 68	Epithelioid	No	3 months after the diagnosis	No recurrence at 3 years	Possible immunological mechanism caused by $CD8^+$ TILs

UK: United Kingdom; TILs: tumor-infiltrating lymphocytes.

gested to be associated with a worse prognosis for malignant tumors. Therefore, a low/negative PD-L1 expression was assumed to be associated with a good prognosis.[15] In our case, high $CD8^+$ TIL numbers and a low expression of PD-L1 may have prompted the favorable clinical course.

Several limitations associated with the present study warrant mention. Spontaneous regression of MPM may not be attributed to the role of $CD8^+$ TILs alone. A previous report on MPM showed heterogeneity in the tumor environment, including numbers of $CD8^+$ TILs, $CD4^+$ TILs, and NK cells.[9] This tumor heterogeneity may have allowed tumor cells to escape tumor immunity. Our report mentioned that $CD8^+$ TILs may have been involved in the spontaneous regression of MPM. It is thought that not only $CD8^+$ TILs but also other tumor immunity effects exert antitumor activity, and further research is needed to elucidate the mechanism underlying spontaneous regression, including the involvement of other immune cells, as well as to clarify the method of activation of tumor immunity.

CONCLUSION

We showed that the spontaneous regression of MPM may have been caused by $CD8^+$ TILs. In our case, there was a significantly greater increase in $CD8^+$ TILs than in $CD4^+$ TILs. The presence of $CD8^+$ TILs was correlated with spontaneous regression and an improved clinical outcome. The mechanism underlying the increase in and stimulation of $CD8^+$ TILs has not yet been sufficiently elucidated. Further studies are necessary to clarify this mechanism. Increasing $CD8^+$ TIL numbers and the stimulation of $CD8^+$ TILs may be a potential therapeutic strategy for MPM. In previous studies, four of six cases showed recurrence after spontaneous regression of MPM, so we must continue to carefully observe the present patient at the outpatient clinic.

本論文内容に関連する著者の利益相反：なし

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