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Inflammatory myopathy associated with PD-1 inhibitors

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ABSTRACT

Objective: To characterize the inflammatory myopathy associated with programmed cell death 1 inhibitors (PD-1 myopathy).

Methods: We studied 19 Japanese patients with PD-1 myopathy (13 men and 6 women, mean age 70 years), who were referred to Keio University. As control groups, we used 68 patients with anti-signal recognition particle antibodies, 51 patients with anti-aminoacyl transfer RNA synthetase antibodies and 460 healthy subjects.

Results: In regard to muscle-disease severity, 10 patients showed a mild form of disease and 9 patients showed a severe form. Non-small cell lung cancer was the most common underlying cancer. PD-1 inhibitor consisted of 11 nivolumab and 8 pembrolizumab. PD-1 myopathy occurred 29 days on average after the first administration of PD-1 inhibitor. The initial manifestation of muscle weakness was ptosis in 10 patients, 15 patients had ptosis, 13 diplopia, 8 facial muscle weakness, 10 bulbar symptoms, 13 limb weakness, 14 neck weakness, 4 cardiac involvement, 6 respiratory involvement and 16 myalgia. Ocular, facial, cardiac and respiratory involvement and myalgia were more frequently observed than controls. Serum creatine kinase was increased to 5247 IU/L on average. Autoantibodies related to inflammatory myopathy were negative, while anti-striational antibodies were found in 13 (68%) patients. HLA-C*12:02 alleles were more frequently detected than healthy controls. Muscle pathology was characterized by multifocal necrotic myofibers with endomysial inflammation and expression of MHC class I. Immunosuppressive therapy with corticosteroids was generally effective for muscle weakness.

Conclusions: Based on our clinical, histological and immunological findings, PD-1 myopathy is a discrete subset of inflammatory myopathy.

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1. Introduction

Programmed cell death 1 (PD-1), first identified by Dr. Honjo's laboratory in 1992 [1], is highly expressed on T cells from patients with tumors and causes tumor-related immune suppression. PD-1 inhibitors including nivolumab and pembrolizumab have shown remarkable benefit in the treatment of a range of cancer types [2]. As cancer treatment with these drugs has become more common, the safety management of immune-related adverse events (irAEs) due to cancer immunotherapy has become more important [3–5]. There is an active debate whether cancer treatment should be conducted in cooperation with organ specialists in order to achieve the best management of irAEs, such as dermatological, gastrointestinal, endocrine, hepatic, and pulmonary toxicities. Most of these irAEs are low-grade and can be managed successfully with supportive care. In contrast, neuromuscular irAEs are sometimes serious and require prompt diagnosis.

The specific characteristics of neuromuscular irAEs have not been elucidated because the frequency is generally low, ranging from 1% to 2% of cancer patients undergoing therapy with PD-1 inhibitors [6]. However, certain general traits of neuromuscular irAEs have been recognized [7–12]. The diseases affect the central nervous system (CNS), peripheral nerves, neuromuscular junction, and muscles. Disease onset and progression may be rapid with a critical clinical course. The clinical presentation may be different from that of patients unrelated to drugs. In addition, paraneoplastic syndrome, a remote effect of malignancy on neuromuscular organs, is always considered as a differential diagnosis.

Myositis is one of the representative neuromuscular irAEs. A variety of studies have demonstrated that myositis as an irAE (irAE myositis) is often accompanied by ocular muscle symptoms, which physicians have often termed “myasthenia-like” or “pseudo-myasthenic” symptoms [13–15]. Physicians often recognize the unique clinical manifestations of irAE myositis and may hesitate to diagnose these patients as pre-existing myositis alone. To date, no comparison with well-recognized subsets of inflammatory myopathy has been done. Recently, the morphological findings in skeletal muscle of patients with irAE myositis have been clearly described [16]. However, immunological features, including human leucocyte antigen (HLA) association and autoantibodies profiles, have not been fully elucidated. The present study aimed to comprehensively characterize the inflammatory myopathy associated with PD-1 inhibitors (PD-1 myopathy).

2. Materials and methods

2.1. Patients

We examined 19 Japanese patients with PD-1 myopathy, who were referred to Keio University for autoantibody detection between July 2016 and June 2018. Patients were included if all of the following entry criteria were fulfilled: (i) the patient received monotherapy with either nivolumab or pembrolizumab, (ii) the patient could provide blood samples, accompanied by full clinical information, (iii) the patient showed increase levels of serum creatine kinase (CK), (iv) the patient exhibited objective muscle weakness supported by needle EMG, muscle MRI and muscle biopsy, and (v) the patient signed an informed consent agreement. Four of these cases were previously reported [17]. Two other cases were also previously reported as case reports [18,19].

As the control groups for inflammatory myopathy, we selected 68 patients with anti-signal recognition particle-positive (SRP+) and 51 patients with anti-aminoacyl transfer RNA synthetase-positive (ARS+). These 119 patients were included in the Integrated Diagnosis Project for Inflammatory Myopathies, for which they were recruited between October 2010 and December 2014 [20,21]. This study was approved by the Institutional Review Boards of Keio University, the National Center of Neurology and Psychiatry, and Tokai University.

2.2. Clinical features

Each patient's clinical information was provided by his or her referring physician, who completed detailed charts describing the clinical course, the results of neurological examinations and laboratory findings. We also obtained follow-up information. Disease severity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5 (https://ctep.cancer.gov/protocolDevelopment). Briefly, grade 1 corresponded to asymptomatic CK elevation or myalgia alone, grade 2 to mild muscle weakness with minimal limiting of daily living abilities, grade 3 to moderate muscle weakness and a requirement for hospitalization, and grade 4 to severe muscle weakness and a life-threatening condition. Activities of daily living were assessed using the patient's performance status according to the Eastern Cooperative Oncology Group [22]. The tumor response to PD-1 inhibitors was evaluated based on the new response evaluation criteria in solid tumors (revised RECIST) [23].

2.3. Autoantibodies detection

We performed screening of autoantibodies related to inflammatory myopathy using RNA immunoprecipitation [24]. Briefly, 10 μl aliquots of serum were mixed with 2 mg of protein A-Sepharose CL-4B (Pharmacia Biotech AB) in 500 μl of immunoprecipitation buffer and incubated for 2 h. After washing three times with immunoprecipitation buffer, antigen-bound Sepharose beads were mixed with 100 μl of HeLa cell extract (6×10^6 cell equivalents per sample) for 2 h, and then 30 μl of 3 M sodium acetate, 30 μl of 10% sodium dodecyl sulfate, and 300 μl of phenol:chloroform:isoamyl alcohol (50:50:1, containing 0.1% 8-hydroxyquinoline) were added to extract the bound RNA. After ethanol precipitation, the RNA was resolved using a 7-M urea-8% polyacrylamide gel, and the gel was silver-stained.

Anti-striational antibodies were first described as serum immunoglobulins reacting with cross-striations of skeletal muscle in patients with myasthenia gravis [25]. Main autoantigens of anti-striational antibodies were titin and voltage-gated potassium channel, Kv1.4. To screen for anti-striational antibodies, we performed cytometric cell-based assays using human embryonic kidney cells transfected with titin and Kv1.4. Briefly, 5.0×10^6 transfected cells were incubated on ice for 1 h with the patients' serum (1:100). Then the cells were washed and incubated with phycoerythin-labeled secondary anti-human IgG (1:1000) antibodies (Thermo Fisher Scientific, Tokyo) for 30 min. Finally, the cells were applied to a flow cytometer and analyzed with Kaluza software (Gallios, Beckman Coulter, Brea, CA). Autoantibodies were determined by differences in phycoerythin fluorescence between the transfected and control cells.

2.4. HLA genotyping

Genomic DNA was extracted from peripheral blood using standard methods. The patients were genotyped by the next generation sequencing based HLA DNA typing method [26] for six loci (HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1). Four-hundred sixty and 114 healthy Japanese subjects were genotyped by the PCR-SSO-Luminex method (Wakunaga Japan or One Lambda CA) for four loci (HLA-A, -B, -C and -DRB1) and two loci (HLA-DQB1, DPB1), respectively.

2.5. Statistical analyses

Analyses were performed using statistical analysis software (IBM/SPSS version 20, Armonk, NY) or R software (version R-3.5.1). Comparisons of relative frequencies were tested for significance using the chi-square test for 2×2 tables and Fisher's exact test. Continuous variables were compared using the Mann-Whitney U test. Values of $p < 0.05$ were considered significant.

Table 1
Demographic and clinical features of 19 patients with PD-1 myopathy.

No/age /gender	CTCAE grade	Cancers	Drugs × cycles	Onset (d)[a]	Initial muscle weakness	Creatine kinase (IU/L)	Anti -titin	Anti -Kv1.4	Anti-AChR	Impaired NMJ Transmission
1/25/M	2	Hodgkin	N × 2	21	Limb weakness	732	–	+	–	N/A
2/46/F	2	NSCLC	N × 2	27	Ptosis	895	+	+	–	+
3/53/M	2	NSCLC	N × 2	33	Ptosis	6418	+	–	–	+
4/63/M	2	Bladder ca.	P × 1	35	Ptosis	3385	–	–	–	N/A
5/73/M	2	NSCLC	P × 1	30	Double vision	7311	+	–	–	–
6/73/M	2	NSCLC	P × 2	25	Limb weakness	1643	–	–	–	–
7/73/M	2	Colon ca.	N × 3	37	Ptosis	2090	+	–	–	N/A
8/79/F	2	RCC	N × 2	24	Ptosis	5350	+	+	–	+
9/83/M	2	NSCLC	P × 2	28	Ptosis	4361	–	–	–	+
10/83/M	2	NSCLC	N × 2	32	Neck weakness	3350	–	–	–	N/A
11/63/F	3	Pharyngeal ca.	N × 3	30	Neck weakness	3021	–	–	–	+
12/75/M	3	NSCLC	N × 2	28	Neck weakness	12,119	+	+	–	+
13/78/M	3	RCC	N × 2	29	Ptosis	10,331	–	–	–	+
14/78/M	3	NSCLC	P × 2	38	Ptosis	6230	–	+	–	–
15/84/F	3	Urothelial ca.	P × 2	24	Ptosis	828	+	–	–	+
16/68/M	4	NSCLC	N × 2	29	Dysphagia	9892	+	+	–	N/A
17/74/F	4	Colon ca.	N × 2	37	Limb weakness	5331	–	+	–	–
18/77/F	4	NSCLC	P × 1	48	Ptosis	6888	+	+	+	+
19/80/M	4	Melanoma	P × 1	19	Dyspnea	9536	+	+	+	–

Patients 5, 9, 12, 16, 17 and 19 were previously reported [17–19].
AChR = acetylcholine receptor, ca. = cancer or carcinoma; CTCAE = Common Terminology Criteria for Adverse Events; N = nivolumab; N/A = not available; NMJ = neuromuscular junction; NSCLC = non-small cell lung cancer; P = pembrolizumab; PD-1 = programmed cell death 1; RCC = renal cell carcinoma.
[a] Onset day after the initial PD-1 inhibitors treatment.

3. Results

3.1. Demographic and background features

The demographic and background features of the 19 patients with PD-1 myopathy are summarized in Table 1. These patients included 13 men and six women with a mean age of 70 years (range, 25–84 years). Disease severity at disease onset was assessed as CTCAE grade 2 in 10 patients (the mild form) and as CTCAE grade 3–4 in 9 patients (the severe form). Patients with asymptomatic CK elevation or myalgia alone (CTCAE grade 1) were not included. Nivolumab was administered in 11 patients, whereas pembrolizumab was used in eight patients. Treatment with PD-1 inhibitors was performed in one cycle (n = 4), two cycles (n = 13), or three cycles (n = 2). The interval between the first PD-1 inhibitor treatment and the onset of PD-1 myopathy ranged from 19 to 48 days; the average was 29 days.

3.2. Clinical features

Ptosis was frequently noticed as the initial muscle weakness in 10 patients. Other initial muscle weaknesses included limb weakness (n = 3), neck weakness (n = 3), diplopia (n = 1), dysphagia (n = 1), and dyspnea (n = 1).

The clinical characteristics of PD-1 myopathy are summarized in Table 2.

Ocular muscle involvement was frequently observed in PD-1 myopathy patients. It was noted that five patients had limited involvement of ocular muscles. Ocular symptoms included both ptosis and diplopia in 12 patients, ptosis alone in three and diplopia alone in one. Ptosis was found ipsilateral in three patients and bilateral in 12 patients. Regarding bulbar symptoms, all 10 patients experienced dysphagia, but only five patients noticed difficulty in speaking. Severe dysphagia requiring nasogastric tubes was seen in five patients. A videofluoroscopic examination of swallowing showed severe dysphagia mainly in the pharyngeal stage (Supplementary Video 1).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.jaut.2019.03.005.

Limb muscle weakness was observed in 13 patients. Among the 14 patients with neck muscle weakness, six had marked weakness presenting with continuous dropped head. Cardiac muscle involvement was observed in four patients. A definite histological diagnosis of myocarditis was obtained in two patients. Respiratory muscles were affected in six patients.

Myalgia was frequently observed in 16 patients and localized especially in the neck, back, or legs.

3.3. Comparison among inflammatory myopathy subsets

To investigate PD-1 myopathy as an individual subset of inflammatory myopathy, we compared clinical and laboratory features between PD-1 myopathy and SRP + or ARS + myopathies (Table 2). PD-1 myopathy was associated with older age at disease onset and male predominance. Patients with SRP+ and ARS + myopathy lacked ocular symptoms. The frequencies of facial muscle weakness and cardiac and respiratory muscle involvement were significantly higher in patients with PD-1 myopathy than in those with SRP + or ARS + myopathy. Bulbar symptoms and neck weakness were seen in patients with PD-1 and SRP + myopathies with a similar frequency. Myalgia was more common in PD-1 myopathy than in SRP + or ARS + myopathy.

3.4. Laboratory findings

An elevation of serum CK was observed several days before the onset of PD-1 myopathy. The peak serum CK in 19 patients with PD-1 myopathy was 5247 IU/L on average. Three patients had CK activity less than 1000 IU/L; however, their values were increased 5-fold compared to before disease onset. CK levels were significantly higher in the nine patients with the severe form than in the 10 patients with the mild form (7131 IU/L versus 3354 IU/L, p = 0.03).

Needle EMG electromyography was performed in 11 patients. Myopathic motor unit potentials with positive sharp waves and/or fibrillation potentials were recorded in eight patients.

Representative muscle MR images are shown in Fig. 1. Nine patients underwent muscle MRI. Fat-suppression T2-weighted or gadolinium enhancement T1-weighted MR images showed high intensity signals with enhancement in six patients. Focal or diffuse abnormal signals were detected in the corresponding muscles including muscles of the neck, trunk and extremities.

Table 2
Comparison among three subsets of inflammatory myopathies.

	PD-1 myopathy (n = 19)	SRP + myopathy (n = 68)	ARS + myopathy (n = 51)	P-value PD-1 vs. SRP+	P-value PD-1 vs. ARS+
Age at disease onset (average)	70	55	60	0.0008	0.02
Male (%)	13 (68)	28 (41)	20 (39)	0.04	0.03
Predisposing factors (%)					
Statin	4 (21)	3 (4)	1 (2)	0.02	0.006
Cancer	19 (100)	4 (6)	6 (12)	< 0.0001	< 0.0001
Rheumatic disease	1 (5)	8 (12)	8 (16)	0.7	0.4
Muscle weakness (%)					
Diplopia	13 (68)	0 (0)	0 (0)	< 0.0001	< 0.0001
Ptosis	15 (79)	0 (0)	0 (0)	< 0.0001	< 0.0001
Facial muscle involvement	8 (42)	3 (4)	2 (4)	< 0.0001	0.0002
Bulbar symptoms	10 (53)	46 (68)	15 (29)	0.2	0.07
Limbs weakness	13 (68)	68 (100)	51 (100)	< 0.0001	< 0.0001
Severe limbs weakness	7 (37)	43 (63)	14 (27)	0.04	0.6
Neck weakness	14 (74)	48 (71)	17 (33)	0.8	0.003
Cardiac involvement	4 (21)	1 (1)	1 (2)	0.007	0.03
Respiratory muscle involvement	6 (32)	8 (12)	6 (12)	0.04	0.05
Muscle atrophy	2 (11)	46 (68)	15 (29)	< 0.0001	0.0003
Decreased deep tendon reflex	5 (26)	31 (46)	8 (16)	0.3	0.3
Myalgia	16 (84)	27 (40)	15 (29)	0.002	0.0001
Blood examination					
Creatine kinase (average, IU/L)	5247	6589	4288	0.17	0.37
Elevated C-reactive protein (%)	3 (16)	13 (19)	31 (61)	0.7	0.0008
Antinuclear antibody positivity (%)	1 (5)	10 (15)	6 (12)	0.5	0.7

ARS+ = anti-aminoacyl transfer RNA synthetase-positive; PD-1 = programmed cell death 1; SRP+ = anti-signal recognition particle-positive.

3.5. Serological profiles

RNA immunoprecipitation can detect many autoantibodies based on electrophoresis patterns. Autoantigens include SRP, ARS, U1–U5 ribonucleoprotein, ribosome, Sm, Ku, Th/To, SSA and SSB. However, RNA immunoprecipitation revealed no autoantigens in our 19 patients with PD-1 myopathy (Supplementary Fig. 1). In-house and commercially available enzyme-linked immunoassays also indicated no autoantibodies. Additionally, autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase, Mi-2, transcriptional intermediary factor 1γ and melanoma differentiation-associated gene 5 were negative.

With regard to anti-striational antibodies, we investigated autoantibodies against titin and Kv1.4 using cytometric cell-based assays.

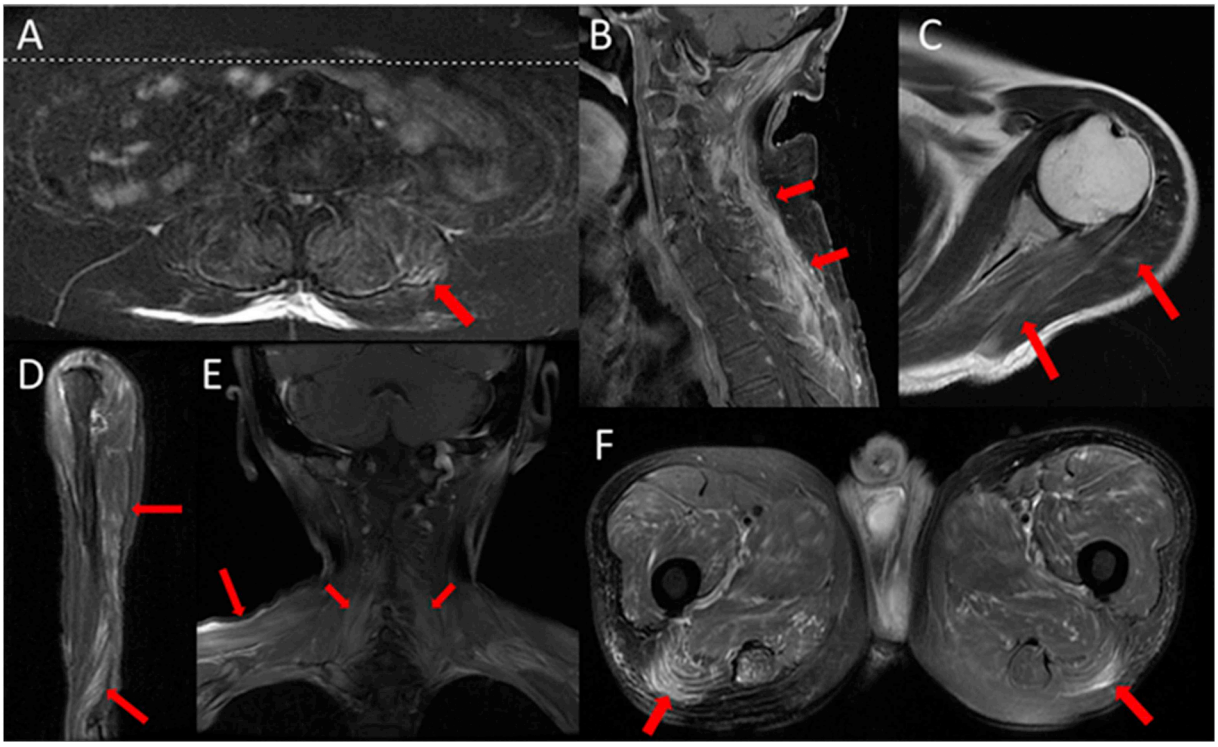

Fig. 1. Muscle MRI findings of PD-1 myopathy. Representative MRI obtained from patient 8 (A), patient 10 (B), patient 11 (C–E) and patient 13 (F) show abnormal high intensity signals in the corresponding muscles. (A) Axial fat-suppression T2-weighted image of the paraspinal muscles. (B) Sagittal gadolinium enhancement fat-suppression T1-weighted image of the posterior cervical muscles. (C) Axial T2-weighted image of the shoulder girdle muscles including the supraspinatus, infraspinatus and deltoid muscles. (D) Coronal fat-suppression T2-weighted image of the triceps brachii. (E) Coronal gadolinium enhancement fat-suppression T1-weighted image of the spinal muscles and trapezius. (F) Axial fat-suppression T2-weighted image of the hamstring muscles.

Table 3
Frequencies of HLA class I and II alleles.

n (%)	PD-1 myopathy (n = 30)	Healthy controls (n = 920)	P-value
A*02:06	3 (10)	82 (9)	0.7
A*24:02	16 (53)	358 (39)	0.1
A*26:01	4 (13)	72 (8)	0.3
B*15:01	3 (10)	70 (8)	0.7
B*35:01	5 (17)	60 (7)	0.05
B*52:01	8 (27)	116 (13)	0.05
C*01:02	5 (17)	141 (15)	0.8
C*03:03	7 (23)	109 (12)	0.08
C*07:02	5 (17)	137 (15)	0.8
C*12:02	9 (30)	117 (13)	0.01[a]
DRB1*01:01	3 (10)	58 (6)	0.4
DRB1*04:05	4 (13)	103 (11)	0.8
DRB1*09:01	4 (13)	128 (14)	1
DRB1*15:01	3 (10)	62 (7)	0.5
DRB1*15:02	6 (20)	114 (12)	0.3

HLA = human leucocyte antigen; PD-1 = programmed cell death 1.
[a] 95% confidence interval: 1.16–6.89.

Seropositivity for anti-striational antibodies was regarded as detection of one of these autoantibodies. We found anti-titin antibodies in 10 patients and anti-Kv1.4 antibodies in nine patients. Among 19 patients with PD-1 myopathy, 13 (68%) had at least one anti-striational antibody. In contrast, only two patients were positive for anti-acetylcholine receptor antibodies (350 nM and 37 nM) (Table 1).

3.6. HLA genotyping

HLA genotyping was performed in 15 patients with PD-1 myopathy. The results for HLA class I and II alleles at nine loci were obtained using the next-generation gene sequence (Supplementary Table 1). We proceeded to analyze HLA associations, as summarized in Table 3. Among HLA class I alleles, HLA-C*12:02 alleles were frequently detected in patients with PD-1 myopathy compared to 460 healthy controls (30% vs. 13%). The HLA class I haplotype HLA-A*24:02-B*52:01-C*12:02 was found in seven (47%) of 15 patients with PD-1 myopathy.

3.7. Histological evaluation

Muscle histology was evaluated in seven patients. Multifocal confluent areas comprising 10–15 muscle fibers undergoing degeneration including necrosis with some features of regeneration were found on hematoxylin-eosin staining (Fig. 2A). Aberrant expressions of major histocompatibility complex class I antigen were observed on the cell surface mainly in the areas of degenerating muscle fibers (Fig. 2B). Scattered foci of inflammatory cell infiltration were found predominantly in the endomysium. At the foci of the infiltrating cells, $CD4^+$ and $CD8^+$ T-cells were seen to roughly the same degree. $CD20^+$ B-cells and $CD68^+$ macrophages were also detected. Perifascicular atrophy, rimmed vacuoles and vasculitic change were not observed. Lesions of necrotic or regenerated of muscle fibers in PD-1 myopathy were different from those in SRP + myopathy (scattered distribution) or ARS + myopathy (perifascicular distribution) (Supplementary Fig. 2).

Cardiac muscle biopsy was performed in two patients. Histological analysis showed lymphocyte infiltration of the myocardium with much greater infiltration of $CD8^+$ T-cell lymphocytes than of $CD4^+$ T-cells.

3.8. Tests for neuromuscular junction

Since patients with PD-1 myopathy presented ocular symptoms, physicians often initially considered a diagnosis of myasthenia gravis. Fluctuation of muscle weakness was reported in seven patients. Among 14 patients available for neuromuscular junction tests, nine patients showed the impairment of neuromuscular transmission (Table 1). An edrophonium test was performed in 10 patients. Although the physicians regarded four patients as showing positive responses to cholinesterase inhibitors, the improvement was not as remarkable as is typically observed in myasthenia gravis. The other six patients showed no improvement. An ice pack test revealed improvement of ptosis in three of six patients. Repetitive nerve stimulation revealed an abnormal decrement in only three of 14 patients. Single fiber EMG revealed increased jitter in the two patients who had no decrement.

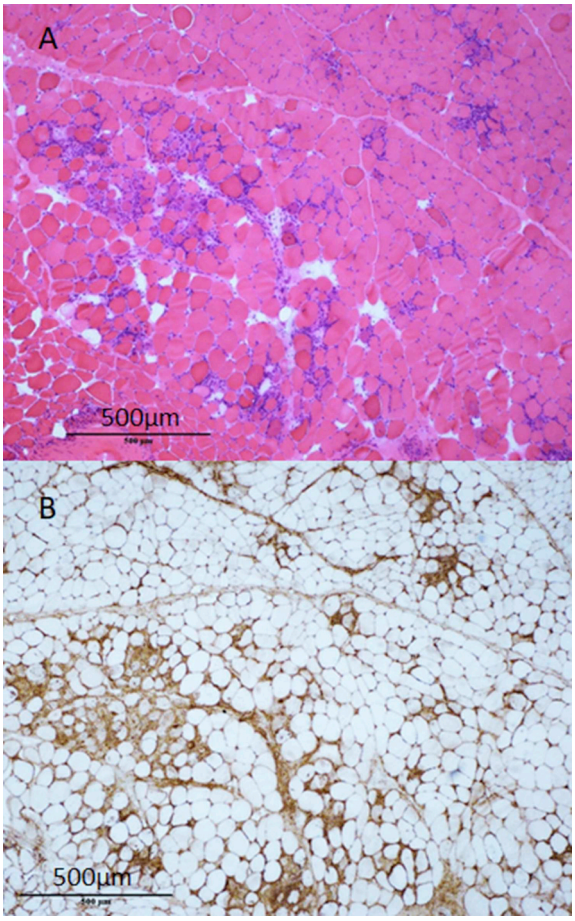

Fig. 2. Muscle histology. (A) Myophagocytosis and necrotizing muscle fibers are multifocally confluent. Hematoxylin and eosin stain. (B) The expression of major histocompatibility complex class I antigen is highlighted on the cell surface mainly in the area of degenerating muscle fibers. Scale bar: 500 μm.

3.9. Treatment

Treatment and follow-up data of PD-1 myopathy are given in Table 4. The following course of PD-1 inhibitor treatment was discontinued in all patients. Seventeen patients, excluding two with the mild form, received immunosuppressive therapy with corticosteroids. Intravenous methyl-prednisolone pulse therapy was initially performed in nine patients, followed by oral or intravenous administration of prednisolone. The other eight patients were initially treated with the oral administration of prednisolone. Five patients with the severe form required additional treatment including intravenous immunoglobulin (n = 4), plasma exchange (n = 4), and tacrolimus (n = 1). Respiratory support was necessary in four patients.

3.10. Serial changes of serum CK

We assessed the serial changes of serum CK levels in 17 patients (Fig. 3). Serum CK levels were dramatically decreased soon after the

Table 4
Treatment and follow-up data.

No	Immunotherapy	PS	Tumor response	Other irAEs	Follow-up duration (m)	Neurological outcome[a], tumor status
1	IVMP, PSL (10 mg)	2	PR	–	10	Remission at 20 wks, tumor growth
2	None	2	PD	–	3	Remission at 2 wks, died of cancer
3	IVMP, PSL (30 mg)	2	PD	+	6	Improvement over 7 wks
4	PSL (30 mg)	2	PR	+	5	Remission at 8 wks
5	IVMP, PSL (20 mg)	2	PR	+	12	Remission at 14 wks, died of cancer
6	PSL (70 mg)	2	PR	+	10	Remission at 2 wks
7	PSL (30 mg)	2	PR	+	6	Remission at 8 wks
8	None	2	SD	–	17	Remission at 4 wks
9	PSL (20 mg)	2	PR	+	13	Remission at 6 wks, restart of pembrolizumab, tumor growth
10	PSL (10 mg)	2	PD	–	5	Remission at 5 wks, died of cancer
11	PSL (40 mg)	3	SD	+	10	Improvement over 8 wks, tumor growth
12	PSL (70 mg), IVIg	3	SD	–	6	Improvement over 18 wks
13	IVMP, PSL (50 mg)	3	SD	–	11	Remission at 10 wks
14	IVMP, PSL (80 mg)	3	PD	+	3	Improvement over 13 wks, died of cancer
15	IVMP, PSL (30 mg)	4	PR	–	6	Improvement over 12 wks, died of cancer
16	IVMP, PSL (60 mg), PP, IVIg, MV	5	SD	–	2	No improvement, died of myocarditis
17	IVMP, PSL (50 mg), PP, IVIg, MV, tacrolimus	4	SD	+	8	Improvement over 12 wks, confined to a where chair, tumor growth, died of cancer
18	PSL (40 mg), PP, MV	4	PR	+	14	Recovered over 29 wks, fracture of femoral hip
19	IVMP, PSL (100 mg), PP, IVIg, MV	4	SD	+	6	Wean off ventilation, bedridden, transferred to a nursing home

irAE = immune-related adverse events; IVIg = intravenous immunoglobulin; IVMP = intravenous methyl-prednisolone pulse therapy; MV = mechanical ventilation; PD = progressive disease; PP = plasmapheresis; PR = partial response; PS = performance status; PSL = prednisolone (maximum daily dose); SD = stable disease.
[a] Duration after the immunosuppressive therapy.

immunosuppressive therapy. Serum CK levels returned to the normal ranges within 20 days on average (range 5–54 days). There were no differences in the duration of CK normalization between patients with the mild and severe forms (16 days versus 25 days on average).

3.11. Responses to treatment

The patients' activities of daily living were severely impaired by PD-1 myopathy. The performance status at the worst condition was graded as performance status 2 in 10 patients with the mild form. In contrast, nine patients with the severe form showed performance status 3–5 and required long-term hospitalization.

Immunosuppressive therapy was generally effective for treating muscle weakness. The symptoms of patients with the mild form started to respond to corticosteroids within several days. In contrast, the patients with the severe form showed a gradual improvement in muscle strength over several months. With regard to the order of recovery, ptosis initially responded to the immunosuppressive therapy, followed by limb and neck weakness, and finally bulbar symptoms. The dose of prednisolone was gradually tapered in accordance with the improvement in muscle weakness.

Tumor responses to PD-1 inhibitors were favorable. Follow-up evaluation of cancers showed a partial response in eight (42%) patients who only underwent one to three cycles of PD-1 inhibitors. Seven patients had stable disease, but the other four patients showed a progressive disease course.

3.12. Additional irAEs

Fifteen additional irAEs of other organs were found in 11 (58%) of the 19 patients with PD-1 myopathy. They included hepatotoxicity (n = 8), pneumonitis (n = 2), skin toxicity (n = 2), arthritis (n = 2), and thyroid gland disorders (n = 1).

3.13. Final outcome

We followed the patients for a mean duration of 12 months (range, 2–17 months). The neurological outcomes indicated that 10 patients,

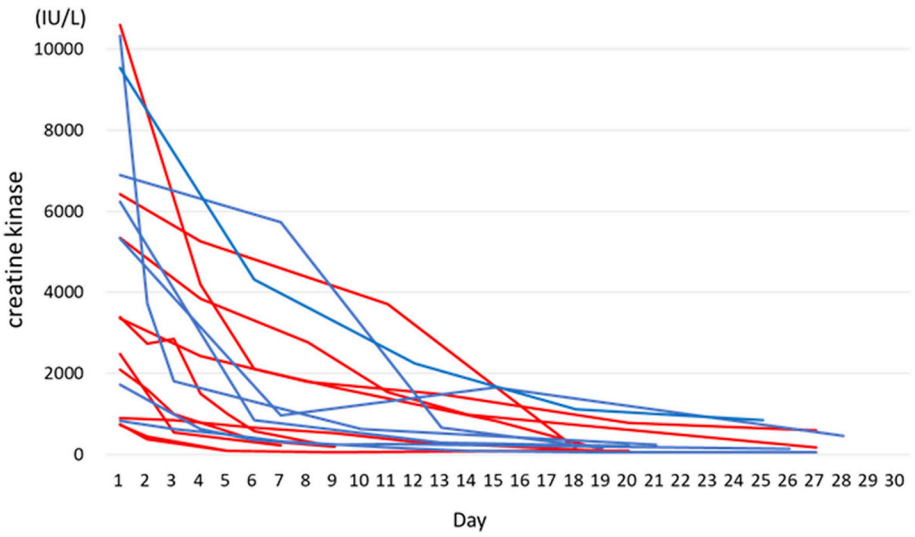

Fig. 3. Serial changes of serum creatine kinase. Serum creatine kinase levels were decreased soon after the initiation of corticosteroids. Day 1 corresponds to the first day of the corticosteroids. Red lines show the serial changes of serum creatine kinase levels in patients with the mild form, while blue lines show those in patients with the severe form.

most of them with the mild form, achieved a remission of muscle weakness. These remissions ranged from two to 20 weeks after the start of immunosuppressive therapy. Among these 10 patients, one patient received the minimal dose of prednisolone (5 mg/day). In spite of the discontinuation of immunosuppressive therapy, the other nine patients did not experience relapses.

On the other hand, nine patients did not achieve a remission of PD-1 myopathy. Clinical symptoms gradually improved over eight to 29 weeks in seven of these patients. At the final follow-up, these patients received oral prednisolone of 12 mg/day on average because of residual muscle weakness. As for the remaining two patients, one was weaned from the mechanical ventilation, but was still bedridden. The other died of myocarditis despite intensive therapy.

Six patients with PD-1 myopathy died of cancer. Among them, two patients initially showed a partial response, but their cancers subsequently deteriorated. Only one patient with the mild form started pembrolizumab again, because of its marked effectiveness against his NSCLC.

4. Discussion

4.1. Presentation

The clinical characteristics of PD-1 myopathy can be summarized as follows: (i) the disease severity was mild for 10 patients and severe for nine; (ii) PD-1 myopathy occurred 29 days on average after the first treatment; (iii) ocular, facial, cardiac, and respiratory muscle involvement as well as myalgia were frequently observed compared to the control groups; (iv) immunosuppressive therapy was effective with the prompt normalization of CK.

In a post-market survey of Japanese cancer patients undergoing nivolumab monotherapy as of July 2017 (https://www.opdivo.jp/basic-info), the frequency of irAE myositis was estimated to be 0.25%. In contrast, a US database showed the frequency of irAE myositis to be 0.15% in patients undergoing nivolumab monotherapy [27]. It is likely that Japanese individuals are more prone to develop PD-1 myopathy compared to other ethnic groups. HLA genotyping in the present study may be ascribed to this tendency. Although the haplotype frequency of HLA-A*24:02-B*52:01-C*12:02 was 23% in PD-1 myopathy, this haplotype was reported to be extremely rare in Hispanics, Caucasians, and North Americans. On the other hand, the frequency in Asians was 1% [28], and it was especially common in Japanese, with a frequency of up to 10% (HLA laboratory: http://www.hla.or.jp/haplo).

There is a close relationship between cancer and inflammatory myopathy. This association was well recognized in dermatomyositis, particularly with respect to autoantibodies against transcriptional intermediary factor 1γ and nuclear matrix protein 2 [29]. Recently, anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase antibodies were also shown to be related to malignancy [30]. Since PD-1 inhibitors augment autoimmune reactions, the drugs may trigger to the development of paraneoplastic myopathy. In this regard, we note that our 19 patients did not have a dermatomyositis rash such as Gottron's sign/papule or heliotrope rash. In addition, none had anti-transcriptional intermediary factor 1γ or anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase antibodies, although anti-nuclear matrix protein 2 antibodies were not tested. We considered that PD-1 myopathy did not arise from paraneoplastic syndrome in any of our patients.

Anti-striational antibodies are found especially in patients with thymoma-associated myasthenia gravis, although their pathogenetic roles are not fully elucidated. Recently, some investigators have reported that anti-striational antibodies were detectable in the serum of patients who developed myositis after treatment with PD-1 inhibitors [10,14,31,32]. We also demonstrated that 68% of our patients with PD-1 myopathy possessed anti-striational antibodies. Taken together, we speculate that anti-striational antibodies can be potential diagnostic biomarkers for PD-1 myopathy.

Based on the clinical severity, patients with PD-1 myopathy were divided into those with the mild form and those with the severe form. Some patients with the mild form presented only ocular symptoms such as ptosis and/or diplopia. In this clinical setting, the differentiation of ocular myasthenia is challenging. One of the patients who underwent muscle biopsy had only ocular symptoms but did not have limb muscle weakness. His muscle histology obtained from the biceps brachii muscle showed inflammation with necrosis and regeneration process. We speculate that marked CK elevation reflects necrosis of systemic skeletal muscles even if the patient exhibits only ocular symptoms.

The severe form of PD-1 myopathy was associated with a risk of respiratory and cardiac muscle involvement. It should be noted that myocarditis can cause both severe heart failure and lethal arrhythmias, resulting in fatal outcomes [27]. In addition, bulbar and trunk muscles were also affected in PD-1 myopathy with a frequency similar to that in SRP + myopathy. As compared to limb muscle weakness, dropped head and dysphagia were usually severe, and limited patients' performance status. Furthermore, these symptoms were resistant to immunosuppressive therapy with delayed recovery.

Previous studies reported the necrotic and regenerating process of muscle fibers as a common major pathological finding of PD-1 myopathy, as in immune-mediated necrotizing myopathy [16]. However, in contrast to immune-mediated necrotizing myopathy, necrotic fibers are multifocally clustered; they are isolated or scattered in immune-mediated necrotizing myopathy. Our observation is consistent with the reports suggesting that the pathological features are not identical to those of already-known idiopathic inflammatory myopathies [29,33].

4.2. Management

There are guidelines for the treatment of neuromuscular irAEs [3,4]. For all but the minimum symptoms (CTCAE grade 1), therapy with PD-1 inhibitors should be withheld. In cases with mild symptoms (CTCAE grade 2), treatment with prednisolone 0.5–1 mg/kg is considered. High-dose steroid therapy with oral prednisolone (1–2 mg/kg) or the i.v. equivalent is preferentially used in severe neuromuscular irAEs (CTCAE grade 3–4). In the present study, other immunotherapies such as intravenous immunoglobulin or plasmapheresis were required. Immune-modulating medication is generally effective for PD-1 myopathy as well as the prompt normalization of CK levels. Corticosteroids should be started in cooperation with consultant neurologists as soon as possible. In this regard, cancer outcomes for patients whose irAEs were treated with corticosteroids were not worse overall than the outcomes for patients who did not receive corticosteroids for irAEs [5].

The development of irAEs has been found to be associated with a survival benefit in melanoma. In a retrospective analysis of the use of nivolumab monotherapy in 576 patients, the objective response rate was significantly higher in patients with irAEs compared to those without irAEs [34]. In addition, the development of irAEs was associated with a better survival outcome of nivolumab treatment with advanced or recurrent NSCLC [35]. We demonstrated that the response of PD-1 inhibitors to cancers was observed in 42% of patients, though the overall response rates of nivolumab monotherapy range from 20% to 30% in various cancers, except in Hodgkin's disease, where the response is much better [6].

Making a decision to permanently discontinue PD-1 inhibitors is difficult, since these drugs are potentially the last choice for survival against advanced cancer. Patients and their doctors would hope to continue the treatment with PD-1 inhibitors if the muscle symptoms of PD-1 myopathy are tolerable. In our cohort, PD-1 inhibitors were re-initiated in only one patient. During the follow-up period, seven patients died; six due to cancers and one due to severe myocarditis. An absolute contraindication to restarting PD-1 inhibitors is life-threatening toxicity, particularly cardiac, pulmonary, and neuromuscular toxicity. However, in our experience, PD-1 myopathy itself is not always severe if it is properly treated.

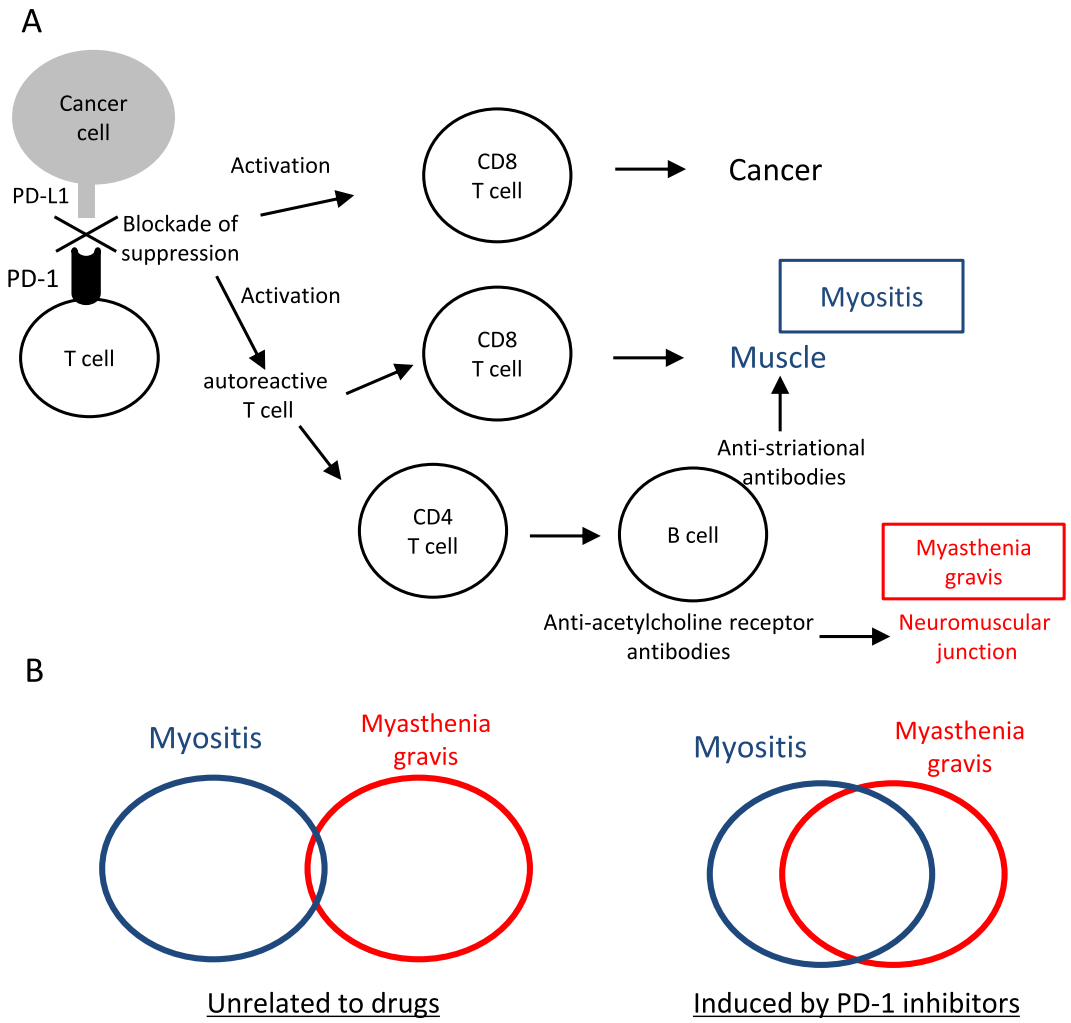

Fig. 4. Hypothesis. (A) Pathogenesis underlying PD-1 myopathy. (B) Relationship between myositis and myasthenia gravis.

4.3. Pathophysiology

We observed an association between PD-1 myopathy and HLA, but this relation was not as strong as that between immune-mediated necrotizing myopathy and HLA [36]. We estimate that tumors are more closely involved in the pathogenesis of PD-1 myopathy than HLA background. Similarly, the HLA association was observed in myasthenia gravis patients without thymoma, but not found in those with thymoma [37]. Cancer cells can escape the immune system by multiple mechanisms, including by secreting soluble suppressive factors and by expressing immune-suppressive molecules [2]. The inhibition of PD-1 may increase the baseline T-cell-specific immune response against the tumor. T-cell immunity, a major target for oncologic immunotherapy, can directly recognize and kill antigen-expressing cells using $CD8^+$ cytotoxic T-cells. The disadvantage of an augmented immune response driven by T-cell activation is that it may activate an autoimmune reaction, and thereby lead to irAEs.

While the exact pathogenesis underlying PD-1 myopathy remains unclear, we speculate that the T-cell-mediated autoimmune mechanism against molecules in muscles may be crucial (Fig. 4A). T-cells that are autoreactive to muscle autoantigens including titin, Kv1.4, and others may be latent in the peripheral blood. Since the blockade of PD-1 signaling causes their activation, the cytotoxicity of autoreactive $CD8^+$ T-cells results in the development of myositis. In addition, the activation of autoreactive $CD4^+$ T-cells leads to the production of anti-striational antibodies. If autoreactive T-cells to acetylcholine receptors are also activated, neuromuscular junction transmission will be impaired. Ocular symptoms in PD-1 myopathy may be ascribed to the impairment of neuromuscular junction.

4.4. Limitations

It is important to disclose the relationship between myositis and myasthenia gravis. Our present patient group was not sufficiently large to address this issue, however, we indicate the potential relation of myositis and myasthenia gravis (Fig. 4B). Although myositis and myasthenia gravis are usually independent disorders, a few myositis patients have also myasthenia gravis [38]. In contrast, PD-1 inhibitor induced myositis and myasthenia gravis may share the same pathophysiology, suggesting an emerging clinical entity (PD-1 myopathy). From the view point of myasthenia as an irAE, CK-level elevations are frequently observed with its concomitant occurrence with myositis [17,39,40]. Our PD-1 myopathy cohort did not include patients with myasthenia gravis alone. To address this question, the development of an international database with complete clinical information and samples is necessary to confirm the entity of PD-1 myopathy. In addition, comprehensive research involving electrophysiological, histological, and immunological studies will be needed to elucidate the pathophysiological mechanisms.

5. Conclusion

Our clinical, histological, and immunological findings indicated that PD-1 myopathy is a discrete subset of inflammatory myopathy.

Conflicting interests

Dr. Suzuki holds a patent on cytometric cell-based assays for anti-striational antibodies. Dr. Suzuki is a consultant/advisory board member on neurologic side effects for Ono Pharmaceutical Company, Bristol-Myers Squibb and MSD. The other authors had no conflicting interest regarding the present study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.03.005.

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