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Anti-interferon gamma-inducible protein 16 antibodies: Identification of a novel autoantigen in idiopathic interstitial pneumonia and its clinical characteristics based on a multicenter cohort study

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ABSTRACT

Autoantibodies are detected in idiopathic interstitial pneumonias (IIPs) without a clear connective tissue disease diagnosis, and their clinical significance is unclear. This study aimed to identify a novel autoantibody in IIPs. We screened 295 IIP patients using a ^{35}S-methionine labeled protein immunoprecipitation assay. Candidate autoantigens were identified via protein array and confirmed by immunoprecipitation. Six sera from 295 IIP patients immunoprecipitated common tetrameric proteins (100 kDa). The protein array identified interferon gamma-inducible protein 16 (IFI16) as the candidate autoantigen. Patients with anti-IFI16 antibodies received immunosuppressants less frequently. Five-year survival rates were 50 %, 69 %, and 63 % ($P = 0.60$), and acute exacerbation-free rates were 50 %, 96 %, and 84 % ($P = 0.15$) for patients with anti-IFI16, anti-aminoacyl tRNA antibodies, and others. Anti-IFI16 is a novel autoantibody in IIPs. Patients with this antibody often receive less immunosuppressive therapy and could have a poor prognosis. Further research is needed to refine patient stratification and management.

Abbreviations: IIPs, Idiopathic interstitial pneumonias; IP, immunoprecipitation; IFI16, interferon gamma-inducible protein 16; ILD, interstitial lung disease; CTD, connective tissue disease; IPF, idiopathic pulmonary fibrosis; CTD-ILD, CTD-associated interstitial lung disease; IPAF, interstitial pneumonia with autoimmune features; ERS, European Respiratory Society; ATS, American Thoracic Society; ARS, anti-aminoacyl tRNA; HRCT, high-resolution computed tomography; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia; OP, organizing pneumonia; PPFE, pleuroparenchymal fibroelastosis; LTOT, long-term oxygen therapy; cDNA, complementary DNA; IgG, immunoglobulin; mRNA, messenger RNA; ANAs, antinuclear antibodies; ELISA, enzyme-linked immunosorbent assay; ABC, avidin-biotin-peroxidase complex; PBS, phosphate-buffered saline; MxA, myxovirus resistance protein A; HRP, horseradish peroxidase; FVC, forced vital capacity; KL-6, Krebs von der Lungen-6; anti-dsDNA, percentage of predicted diffusing capacity for carbon monoxide (%DLcoanti-double stranded DNA; STING, stimulator of interferon genes; IRF, interferon regulatory factor; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

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1. Introduction

Interstitial lung disease (ILD) is a heterogeneous group of diffuse parenchymal lung disorders characterized by radiographic findings that may result in pulmonary fibrosis [1]. ILD has various causes, including connective tissue disease (CTD), drugs, and environmental exposure. Cases with unknown etiology were classified as having idiopathic interstitial pneumonias (IIPs). IIPs are subdivided into nine groups based on clinical, radiological, and pathological findings [2]. Among the groups of IIPs, idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung disease that usually advances gradually, and some patients with IPF experience rapid deterioration or acute exacerbation, which can be fatal [3]. Recently, antifibrotic agents such as pirfenidone or nintedanib have been used to treat IPF, but their prognosis remains poor [4].

In contrast, CTD-associated interstitial lung disease (CTD-ILD) generally has a more favorable clinical course than IPF [5]. There are some CTD-ILD cases where lung lesions precede the systemic symptoms of CTD or cases where systemic symptoms are poor and only lung lesions are conspicuous [6–8]. These ILD types, which exhibit clinical or serological features of CTD but fail to meet the established diagnostic criteria for CTD, are reported to have a good prognosis [9]. Such patients have been classified as those with interstitial pneumonia with autoimmune features (IPAF) following the 2015 publication of a research guideline that coined the term and proposed the standardized classification criteria of the European Respiratory Society (ERS)/American Thoracic Society (ATS) [10]. The responsiveness and prognosis of CTD-ILD differ from those of IPF, and the concept of IPAF arises from the overlap between CTD-ILD and IIPs. Therefore, the classification of the IPAF group in daily practice could have significant clinical implications.

The classification criteria for IPAF include clinical, serologic, and morphological domains. Clinically, lung lesions sometimes precede CTD-associated symptoms, and symptoms other than lung lesions may not be present at the onset of IIPs. These cases could have been diagnosed as IIPs, potentially missing the opportunity for immunosuppressive therapy. In the morphological domain, surgical lung biopsies are highly invasive, and not all patients undergo biopsy. Considering these factors, the serologic domain consisting of autoantibodies is crucial when classifying IPAF cases. In particular, anti-aminoacyl tRNA (ARS) antibodies are frequently found in IPAF. Anti-ARS antibody-positive ILD has recently been considered a distinct disease entity, anti-synthetase syndrome, owing to its common clinical and pathophysiological features and preferable response to immunosuppressive therapy with a relatively good prognosis [11–13]. Therefore, detecting some autoantibodies in patients with IIPs can help avoid missed opportunities for immunosuppressive therapy.

We routinely examined autoantibodies in sera with various CTDs or IIPs using immunoprecipitation (IP) assays. We discovered that some sera from patients with IIPs contained autoantibodies that immunoprecipitated common tetrameric bands at a molecular weight of approximately 80–100 kDa. These were not previously reported as autoantibodies associated with ILD. These patients did not meet the criteria for CTDs. This novel autoantibody may be associated with lung disease. Therefore, in this study, we explored the corresponding autoantigens of this novel autoantibody and analyzed their clinical significance.

2. Material and methods

2.1. Serum samples

This multicenter retrospective observational study obtained serum samples from consecutive patients with IIPs who visited or were referred to Kyoto University Hospital or collaborating medical centers between 2014 and 2020. Other serum samples were collected from Tosei General Hospital ($n = 50$), Osaka Medical and Pharmaceutical University Hospital ($n = 12$), Kobe City Medical Center West Hospital ($n = 10$), and Kobe City Medical Center General Hospital ($n = 8$). IIPs were defined as interstitial pneumonia of unknown cause in which a patient did not fulfill the classification criteria for any specific CTDs. All diagnoses of IPF and other IIPs were made in accordance with the 2018 international guidelines for IPF and the 2013 international statement for IIPs [2,14,15]. We excluded patients diagnosed with CTDs later during their clinical course. Serum samples were collected at the first visit, and informed consent was obtained from all patients following the Declaration of Helsinki. This study was approved by the Medical Ethics Committee of Kyoto University Graduate School of Medicine (approval number; R2784).

2.2. Clinical evaluation

We retrospectively reviewed the clinical findings of each patient at the time of IIP diagnosis, including age, sex, smoking status, pulmonary function, high-resolution computed tomography (HRCT) pattern, and clinical features listed in the criteria for the clinical domain of IPAF. The frequency of malignancy was examined within 3 years of IIP diagnosis. We also collected serological and morphological features listed in the criteria for IPAF. Additionally, we examined the clinical course after diagnosis. All clinical data for each patient with IIPs were collected from medical records, and HRCT patterns were reviewed by respiratory physicians and categorized as usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), organizing pneumonia (OP), NSIP with OP overlap, lymphocytic interstitial pneumonia, pleuroparenchymal fibroelastosis (PPFE), or unclassifiable [10,16,17]. We categorized IIP cases into patients with novel autoantibodies, those with anti-ARS antibodies (frequently found in IPAF), and those with neither novel autoantibodies nor anti-ARS antibodies. We then compared the clinical characteristics and courses, such as overall survival, acute exacerbation-free survival, and long-term oxygen therapy (LTOT)-free survival. The observation period was defined as the period between IIP diagnosis and the last follow-up within 5 years. Survival time was defined as the time from the first IIP diagnosis to death or last follow-up. Acute exacerbation was diagnosed by the attending physician based on worsening dyspnea, new ground-glass opacities evident on HRCT, and after excluding an obvious cause of acutely impaired respiratory function such as infection, pneumothorax, cancer, pulmonary embolism, or congestive cardiac failure [18].

2.3. Immunoprecipitation assay

The IP assay was conducted using HeLa cell extracts as previously described [19]. For polypeptide studies, $3*10^6$ HeLa cells in 100 mL of methionine-free minimal essential medium were labeled with 18.5 MBq ^{35}S-methionine (Perkin Elmer, Waltham, MA, USA) and incubated at 37 °C for 18 h. Furthermore, ^{35}S-methionine-labeled HeLa cells were sonicated using a Misonix Microson (Misonix, Farmingdale, NY, USA) after four washes in IP buffer (10 mM Tris-HCL, 500 mM NaCl, 0.1 % Nonidet P-40, pH 8.0) and resuspension in 4 mL of IP buffer. The soluble supernatant was recovered by centrifugation (10,000 $\times g$ for 15 min). Notably, 10 μL of serum were mixed with 3 mg of protein A CL-4B Sepharose beads (GE Healthcare, Uppsala, Sweden) in IP buffer on a rotator for 2 h at room temperature. The IgG-coated Sepharose beads were washed four times and mixed with ^{35}S-methionine-labeled HeLa cell extracts for 2 h at 4 °C. After washing in 500 μL of IP buffer four times and 500 μL of distilled water once, the Sepharose beads were resuspended in sodium dodecyl sulfate sample buffer. The polypeptides were then fractionated using 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The radiolabeled polypeptide components were analyzed by autoradiography using a Fuji Bio-Imaging Analyzer System-5000 (Fuji Photo-Film, Tokyo, Japan).

2.4. Serum autoantibody screening using protein array

Serum autoantigen screening was conducted using the HuPEX® Comprehensive Protein Array (ProteoBridge, Tokyo, Japan). The protein array was kept under wet conditions during the entire handling process, which enabled the displayed antigens to maintain their three-dimensional structure [20]. Proteins were synthesized in vitro from 13,350 clones of the HuPEX® complementary DNA (cDNA) library using a Gateway cloning system (Thermo Fisher Scientific, San Jose, CA, USA), as previously described [21]. Protein synthesis was conducted using a wheat germ cell-free translation system (FASMAC, Kanagawa, Japan). The synthesized proteins harboring FLAG-GST-tag were diluted with a solution containing 12.5 % (*v*/v) glycerol and spotted in duplicate onto glutathione-coated glass plates using a multidispensing system (BIOTEC, Tokyo, Japan). After spotting, the plates were incubated at room temperature for 30 min and washed with Tris-buffered saline containing 0.1 % Tween 20. The plates were incubated in a blocking buffer and stored at −80 °C until the serum reaction. Serum was diluted at a ratio of 3:1000, added to the arrays, and allowed to react for 1 h at room temperature. Subsequently, the arrays were washed, and goat anti-human immunoglobulin G (IgG) (H + L) Alexa Fluor 647 conjugate (Thermo Fisher Scientific) diluted at 1:1000 was added to the arrays and allowed to react for 1 h at room temperature. Finally, the arrays were washed and air-dried, and fluorescent images were acquired using a fluorescence imager (Amersham Typhoon; Cytiva, Marlborough, MA, USA). During protein preparation, negative control spots were prepared using distilled water instead of messenger RNA (mRNA). Positive control spots were prepared using the mRNA encoding human IgG for protein synthesis. Autoantibody quantification was performed based on fluorescence values obtained from serum reactions with protein spots. The levels of each antibody were calculated as follows:

$$\text{Index value} = \frac{\text{F autoantigen} - \text{F negative control}}{\text{F positive control} - \text{F negative control}} \times 100$$

F autoantigen: fluorescent intensity of each autoantigen duplicate spot.

F negative control: fluorescence intensity of negative control spot.

F positive control: fluorescence intensity of positive control spot.

The positive threshold of the index value was defined as >4.0, based on the fact that it can be recognized as a specific signal rather than background noise and the mean value for the healthy serum plus three standard deviations.

2.5. Expression of interferon gamma-inducible protein 16 and identification of autoantigen

Overexpression of the interferon gamma-inducible protein 16 (IFI16) protein was achieved with plasmids encoding IFI16 cDNA (constructed by Vector Builder, ID: VB230426-1671xyx) using a TnT Quick Coupled Transcription/Translation system (Promega, Madison, WI, USA), following the manufacturer's protocol. In contrast to IP using HeLa cells containing various proteins, this method allowed us to obtain pure IFI16 protein. We then examined the reactivity of patient sera with a novel autoantibody against in vitro translated IFI16 using an IP assay.

2.6. Antinuclear antibody assay and measurement for autoantibody

Antinuclear antibodies (ANAs) were detected by indirect immunofluorescence using Hep-2 cell slides (MBL, Nagoya, Japan) as the substrate, combined with fluorescein isothiocyanate-conjugated anti-human IgG. A titer of ≥1:40 was considered positive for ANA. If the serum tested positive at an initial 1:40 dilution, it was serially titrated to 1:2560. Antibodies listed in the serologic domain of the classification for IPAF were tested using enzyme-linked immunosorbent assay (ELISA), chemiluminescent enzyme immunoassay, immunoblotting, latex turbidimetric immunoassay, RNA-IP assay, or protein-IP assay (Supplementary Table 1).

2.7. Pathological analysis

A lung biopsy was conducted on a patient with an anti-IFI16 antibody. The specimens were stained with hematoxylin and eosin. The pathological pattern was diagnosed following the 2013 ATS/ERS consensus classification of IIPs [2,14]. We also obtained lung specimens from patients with IPF and those with pneumothorax as control specimens. All specimens were collected from patients who provided informed consent. Immunohistochemical analysis of the IFI16 expression was performed on paraffin-embedded tissue sections using a conventional avidin-biotin-peroxidase complex (ABC) method [22]. Incubation and washing procedures were performed at 121 °C for 5 min. After deparaffinization and antigen retrieval, endogenous peroxidase activity was blocked with 0.3 % hydrogen peroxide in methyl alcohol for 30 min. Subsequently, an anti-IFI16 antibody (clone D8B5T, Cell Signaling) at a dilution of 1:200 as primary antibody was applied overnight at 4 °C. They were then incubated with a biotinylated secondary antibody diluted to 1:300 in phosphate-buffered saline (PBS) for 40 min, followed by washing in PBS (six times, 5 min). The ABC (ABC-Elite; Vector Laboratories, Burlingame, CA, USA) was diluted at 1:100 in bovine serum albumin for 50 min. After washing in PBS (six times, 5 min), the coloring reaction was performed with 3,3′-Diaminobenzidine, and the nuclei were counterstained with hematoxylin. The expression of myxovirus resistance protein A (MxA) was evaluated using a tyramide signal amplification-based method with an Opal 7-Color Automation immunohistochemistry Kit following the manufacturer's modified protocols (Akoya Biosciences, Marlborough, MA, USA). An anti-MxA antibody (MABF938; Merck KGaA, Darmstadt, Germany) at a dilution of 1:300 was incubated with the specimen as the primary antibody. Following incubation with the primary antibody, sections were washed and incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (ImmPRESS® HRP Universal PLUS Polymer Kit, Vector Laboratories, CA, USA). After washing the slides, fluorophore-conjugated tyramide was added, and sample slides were imaged using Mantra™ quantitative pathology workstation with inForm® image analysis software (PerkinElmer, Massachusetts, USA).

2.8. Statistical analysis

Categorical variables are presented as counts and percentages, whereas continuous variables are presented as medians with interquartile ranges. Fisher's exact and Kruskal-Wallis tests were used for categorical variables, and the Wilcoxon signed-rank test was used for continuous variables. The *P* values for multiple comparisons were adjusted using the Bonferroni correction. Survival curves were generated using the Kaplan-Meier method, and the log-rank test was used to compare survival distributions. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) for the R software (R Foundation for Statistical Computing, Vienna, Austria, version 4.3.3) [23].

3. Results

3.1. Study flow and detection of autoantibody in IIPs

Overall, 308 patients with IIPs were enrolled in this study. From the 308 IIP sera collected, we re-examined the medical records. We excluded the sera of patients who were eventually diagnosed with CTDs during the clinical course ($n = 13$). This process identified 295 IIP sera (Fig. 1). A surgical lung biopsy was performed in 33 % of cases in this study. Based on HRCT patterns and/or pathological findings, most cases were diagnosed as unclassifiable IIPs (58 %), followed by IPF (31 %). Notably, several serum samples precipitated common tetrameric bands at

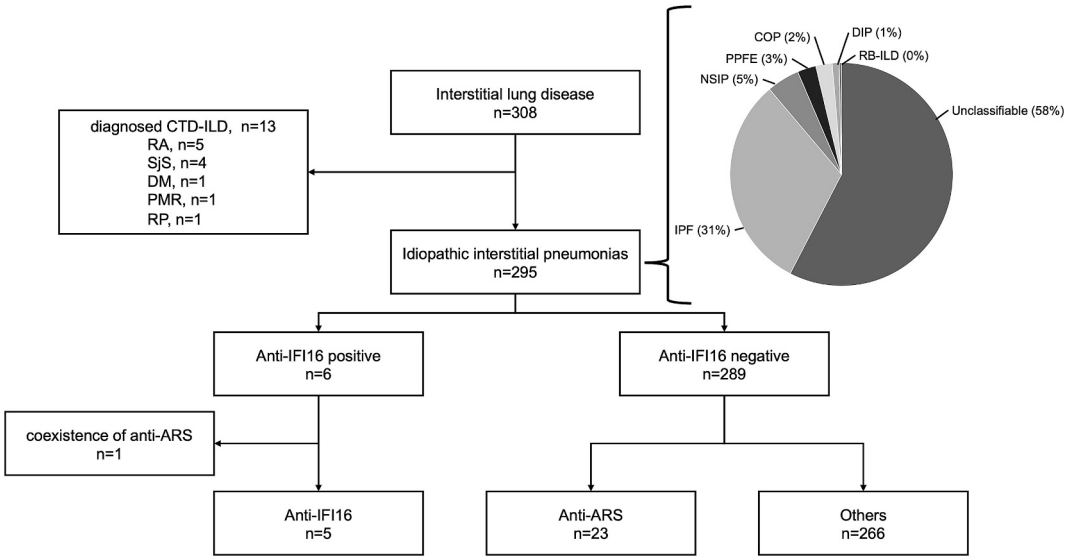

Fig. 1. Enrollment and selection of patients.
This study enrolled 295 patients diagnosed with idiopathic interstitial pneumonia of the 308 patients from five institutions. Thirteen patients were excluded because they were diagnosed with connective tissue disease during the observation period. The most common diagnosis was unclassifiable IIP (58 %), followed by IPF (31 %), NSIP (5 %), PPFE (3 %), COP (2 %), DIP (1 %), and RB-ILD (0 %). Abbreviations: RA, rheumatoid arthritis; SjS, Sjogren syndrome; DM, dermatomyositis; PMR, polymyalgia rheumatica; RP, relapsing polychondritis; IFI16, interferon gamma-inducible protein 16; ARS, aminoacyl-tRNA synthetase; IPAF, interstitial pneumonia with autoimmune features; IPF, idiopathic pulmonary fibrosis; NSIP, non-specific interstitial pneumonia; PPFE, pleuroparenchymal fibroelastosis; COP, cryptogenic organizing pneumonia; DIP, desquamative interstitial pneumonia; RB-ILD, respiratory bronchiolitis-associated interstitial lung disease.

molecular weights of approximately 80–100 kDa in the IP assay, prompting our investigation into this anti-tetramer antibody as a novel autoantibody (Fig. 2a). Among the 295 sera samples of patients with IIPs, anti-tetramer antibodies were detected in six patients. One of the six patients tested positive for anti-tetramer and anti-ARS antibodies. Of the 289 patients without anti-tetramer antibodies, 23 had anti-ARS antibodies, including auto-antibodies to histidyl-tRNA synthetase (anti-Jo-1), anti-glycyl-tRNA synthetase (anti-EJ), anti-asparaginyl-tRNA synthetase (anti-KS), anti-threonyl-tRNA synthetase (anti-PL-7), anti-alanyl-tRNA synthetase (anti-PL-12), and anti-isoleucyl-tRNA synthetase (anti-OJ). Overall, 266 patients tested negative for the anti-tetramer and anti-ARS antibodies. Patient characteristics are summarized in Table 1 and Supplementary Table 2. The median value of the percentage of predicted forced vital capacity (FVC) was 76 %. The rates of each value listed in the clinical and serologic domains of the criteria for IPAF were approximately <10 %, but 29 % of patients were classified as

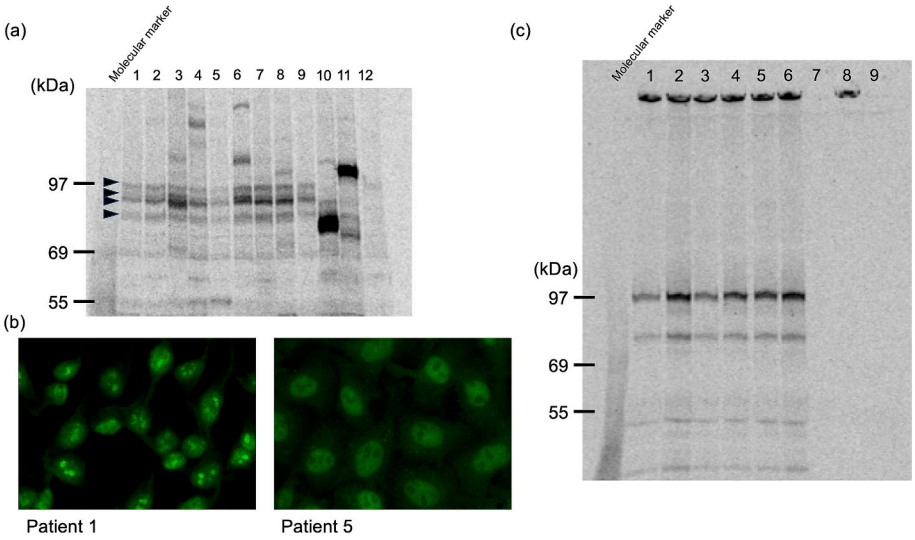

Fig. 2. Immunoprecipitation and indirect immunofluorescence with patient sera.
(a) Using ^{35}S-methionine-labeled HeLa cell extracts, tetrameric bands at a molecular weight of approximately 100 kDa were immunoprecipitated. Lane 1–9, patient sera; Lane 10, anti-PL-7 antibody (approximately 80 kDa)–positive serum; Lane 11, anti-PL-12 antibody (approximately 110 kDa)-positive serum; Lane 12, healthy control. Arrowheads show the tetrameric bands.
(b) An indirect immunofluorescent staining pattern on HEp-2 cell slides produced by a serum positive for anti-tetramer antibody (Patients 1 and 5 in Table 2). Patient 1 showed a nucleolar pattern, and Patient 5 showed a speckled pattern.
(c) Immunoprecipitation analysis of in vitro translated interferon-gamma inducible protein 16 (IFI16). IFI16 protein from a plasmid encoding IFI16 cDNA was expressed in vitro and immunoprecipitated using patients and healthy control. Lanes 1 to 6 correspond to patients 1 to 6 in Table 2, respectively. Sera from healthy control patients (negative control) are shown in lanes 7 to 9.

Table 1
Clinical characteristics of 300 patients from a multicenter cohort study.

	$n = 295$	
Age at the diagnosis of IIPs, years	68	[60–74]
Male sex, n (%)	199	(67)
Smoking history, n (%)	210	(71)
Malignancy, n (%)	43	(15)
Serum KL-6, U/mL	834	[541–1380]
Serum SP-D, ng/mL	207	[117–305]
FVC, % predicted	76	[62–97]
%DLco, % predicted	51	[36–63]
SpO_2, %	96	[95–97]
Satisfaction with clinical domain for IPAF, n (%)	84	(28)
Satisfaction with serologic domain for IPAF, n (%)	113	(38)
Satisfaction with morphologic domain for IPAF, n (%)	109	(37)
Satisfaction with the criteria for IPAF, n (%)	87	(29)
Immunosuppressive therapy, n (%)	100	(34)
Anti-fibrosis agent, n (%)	95	(32)
Long-term oxygen therapy, n (%)	70	(24)
Acute exacerbation, n (%)	38	(13)
Transplantation for lung, n (%)	4	(1)
Mortality, n (%)	95	(32)
Follow-up time, days	1716	[989–1827]

Data are presented as the median [interquartile range] or n (%). Abbreviations: IIPs, idiopathic interstitial pneumonias; KL-6, Krebs von der Lungen-6; SP-D, surfactant protein-D; %FVC, percentage of predicted forced vital capacity; % DLco, percentage of predicted diffusion capacity of carbon monoxide; SpO_2, saturation of percutaneous oxygen.

having IPAF. Immunosuppressive agents, including glucocorticoids and other immunosuppressants, were used in 34 % of patients, and anti-fibrosis agents were used in 32 %. The rate of acute exacerbation was 13 %, and 32 % of patients died during the observation period.

3.2. Confirmation of IFI16 protein as the antigen

Among the six patients with anti-tetramer antibodies, four sera tested negative in the indirect immunofluorescence assay for antinuclear antibody (FANA). In contrast, the others were assessed as nucleolar or speckled patterns using FANA (Fig. 2b). HuPEX® Comprehensive Protein Array analysis was used to identify IFI16 as a common autoantigen among the five anti-tetramer antibody sera (Supplementary Fig. 1). To confirm that the anti-tetramer antibody specifically recognized IFI16, we prepared the human IFI16 protein from its cDNA using an in vitro transcription/translation system. IFI16 labeled with ^{35}S-methionine was immunoprecipitated by each serum sample with an anti-tetramer antibody but was not recognized by sera from healthy controls (Fig. 2c). Therefore, we confirmed that IFI16 is the target antigen recognized by the anti-tetramer antibody in patients with IIPs.

3.3. Clinical features associated with anti-IFI16 antibody

Table 2 and Supplementary Table 3 present the clinical characteristics of the six patients with anti-IFI16 antibodies. The HRCT patterns of the six patients were UIP in two, NSIP/OP in two, PPFE in one, and unclassifiable in one patient, respectively. Two of the six patients were classified as having IPAF based on autoantibody and HRCT patterns. A malignancy was found in only one patient. One patient was treated with glucocorticoids and immunosuppressants; the other was treated with antifibrotic agents. Three patients died during the observation period: one due to malignancy and the others from exacerbation of IIP.

3.4. Clinical features categorized with autoantibodies

Table 3 presents the clinical characteristics of patients with IIP categorized as anti-IFI16 antibody, anti-ARS antibody, or others (neither anti-IFI16 antibody nor anti-ARS antibody). No significant differences were observed in age, sex, history of smoking, or malignancy among the three groups. ILD markers, such as Krebs von der Lungen-6 (KL-6), and pulmonary function indicators, such as %FVC and percentage of predicted diffusion capacity of carbon monoxide (%DLco), did not significantly differ among the three groups. The UIP pattern on HRCT appeared to be common in patients without anti-IFI16 or anti-ARS antibodies, but the difference was not significant. The frequency of immunosuppressive therapy was significantly higher in patients with

Table 2
Clinical features of six patients with anti-IFI16 antibodies.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age at diagnosis, years	52	56	62	70	70	73
Sex	Male	Female	Male	Female	Male	Female
Smoking (pack-years)	Current (1−32)	Never	Ex (40–42)	Never	Never	Never
Satisfaction with clinical domain for IPAF	−	−	−	−	−	−
Satisfaction with serologic domain for IPAF	+	−	−	+	+	−
Antinuclear antibody, titers (pattern)	1:40 (Nu)	1:<40	1:<40	1:<40	1:40 (Sp)	1:<40
Autoantibody	7–2RNP	−	−	dsDNA	Jo-1	−
HRCT findings of ILD	UIP	PPFE	UIP	NSIP/OP	NSIP/OP	unclassifiable
Histopathological findings of lung biopsy	UIP	NA	NA	NA	OP	UIP
Satisfaction with the criteria for IPAF	−	−	−	+	+	−
Malignancy	−	−	−	−	HCC, Cancer of the tongue	−
KL-6, U/mL	1028	171	655	317	2050	322
SP-D, ng/mL	318	105	253	242	358	89
Therapy	Pirfenidone	−	−	−	Corticosteroid, tacrolimus	−
Long-term oxygen therapy	−	−	−	−	+	−
Outcome	Alive	Alive	Dead for exacerbation of lung disease	Alive	Dead for hepatocellular carcinoma	Dead for exacerbation of lung disease
Observation period (months)	23	67	31	61	15	53

Abbreviations; IPAF, interstitial pneumonia with autoimmune features; Nu, nucleolar; Sp, speckled; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; UIP, usual interstitial pneumonia; PPFE, pleuroparenchymal fibroelastosis; NSIP, non-specific interstitial pneumonia; OP, organizing pneumonia; HCC, hepatocellular carcinoma; Krebs von den Lungen-6; SP-D, surfactant protein-D; NA, not available.

Table 3
Comparison of clinical features stratified with anti-IFI16 antibody, anti-ARS antibody, and others.

	Anti-IFI16	(n = 5)	Anti-ARS	(*n* = 23)	None	(*n* = 266)	P value
Age, years	62	[56–69]	65	[54–70]	68	[61–74]	0.093
BMI, kg/m^2	23.1	[22.9–26.3]	24.7	[22.1–26.0]	23.5	[21.2–26.2]	0.61
Male, n (%)	2	(40)	12	(52)	184	(69)	0.11
Smoking, n (%)	2	(40)	13	(57)	195	(73)	0.060
Malignancy, n (%)	0	(0)	4	(17)	38	(14)	0.89
SpO_2, %	97	[94–97]	95	[94–97]	96	[95–97]	0.068
KL-6, U/mL	322	[317–655]	941	[565–1524]	834	[549–1380]	0.12
SP-D, ng/mL	242	[105–253]	186	[124–221]	207	[116–306]	0.58
%FVC, % predicted	75	[45–87]	75	[66–88]	76	[61–98]	0.78
%DLco, % predicted	55	[24–63]	54	[43–68]	51	[34–63]	0.51
UIP pattern on HRCT, n (%)	2	(40)	2	(9)	91	(34)	0.019
Immunosuppressive therapy, n (%)	0	(0)	16	(70)*	83	(31)	<0.001
Anti-fibrosis agent, n (%)	1	(20)	1	(4)	93	(35)*	<0.01
Long-term oxygen therapy, n (%)	0	(0)	4	(17)	65	(24)	0.48
Acute exacerbation, n (%)	2	(40)	1	(4)	35	(13)	0.098
Transplantation, n (%)	0	(0)	0	(0)	4	(2)	1
Mortality, n (%)	2	(40)	5	(22)	87	(33)	0.53
Follow-up time, days	1626	[972–1827]	1685	[1114–1827]	1726	[980–1827]	0.99

Abbreviations: IFI16, interferon gamma-inducible protein 16; ARS, aminoacyl-tRNA synthetase; BMI, body mass index; KL-6, Krebs von den Lungen-6; SP-D, surfactant protein-D; %FVC, percentage of predicted forced vital capacity; %DLco, percentage of predicted diffusing capacity for carbon monoxide; UIP, usual interstitial pneumonia; HRCT, high-resolution computed tomography. * Significant differences were found between the groups with Bonferroni adjustment.

anti-ARS antibodies, and more patients without anti-IFI16 or anti-ARS antibodies were treated with antifibrotic agents. Patients positive for anti-IFI16 antibody were not treated with immunosuppressive treatments or anti-fibrosis agents, except for one patient. The clinical characteristics of patients with anti-IFI16 antibodies and without anti-IFI16 antibodies among the 295 patients with IIPs are presented in Supplementary Table 4. No significant differences were found in serum markers, pulmonary function such as %FVC, or the type of therapy between patients positive and negative for anti-IFI16 antibodies.

3.5. Prognosis

Overall survival, acute exacerbation-free survival, and LTOT-free survival rates are shown in Fig. 3. The overall survival probabilities at 5 years after diagnosis were 50 %, 69 %, and 63 % in patients with anti-IFI16 antibodies, patients with anti-ARS antibodies, and others, respectively ($P = 0.60$). The acute exacerbation-free rates were 50 %, 96 %, and 84 % ($P = 0.15$), and the LTOT-free survival rates were 100 %, 76 %, and 72 %, respectively ($P = 0.39$). There were no significant differences; however, the overall survival and acute exacerbation-free rates in patients with anti-IFI16 antibodies seemed to be the lowest. In a direct comparison between patients with anti-IFI16 antibodies and anti-ARS antibodies, the overall survival probabilities at 5 years were 50 % vs. 69 % ($P = 0.38$), and the acute exacerbation-free rate of patients with anti-IFI16 antibodies was significantly lower (50 % vs. 96 %, $P = 0.031$) (Supplementary Fig. 2). When comparing those with or without anti-IFI16 antibodies following inclusion, one patient exhibited the coexistence of anti-IFI16 and anti-ARS in the anti-IFI16 positive group; the overall survival probabilities and the acute exacerbation-free rates at 5 years were 42 % vs. 63 % ($P = 0.31$) and 50 % vs. 85 % ($P = 0.15$), respectively (Supplementary Fig. 3). The comparison of patients with anti-IFI16 antibodies and IPAF patients is shown in Supplementary Fig. 4, after extracting IPAF patients who meet the classification for IPAF and were negative for anti-IFI16 antibodies. The overall survival and exacerbation-free rates were 42 % vs. 70 % ($P = 0.17$) and 50 % vs. 92 % ($P = 0.044$), respectively.

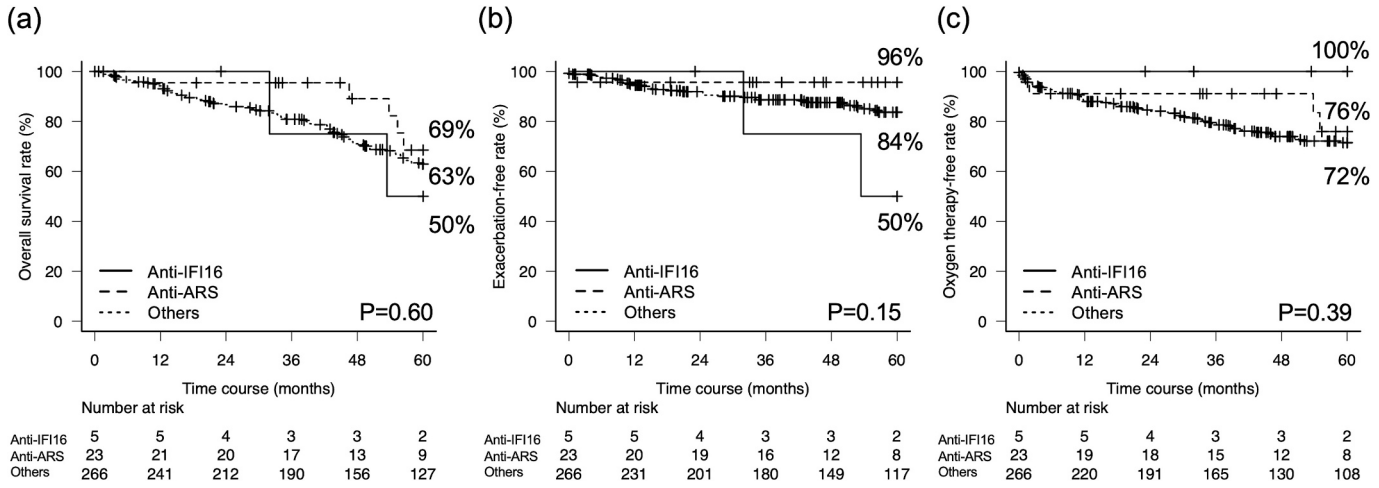

Fig. 3. Overall survival curves in patients with IIPs stratified by autoantibodies.
Overall survival curves (a), acute exacerbation-free curves (b), and long-term oxygen therapy-free curves (c) are shown in the figure. No significant differences were observed in the overall survival rate, acute exacerbation-free rate, or LTOT-free rate between each autoantibody; however, the overall survival rate and acute exacerbation-free rate in patients with anti-IFI16 antibodies appeared to be the lowest. Abbreviations: IIPs, idiopathic interstitial pneumonias; IFI16, interferon gamma-inducible protein 16; ARS, aminoacyl-tRNA synthetase; LTOT, long-term oxygen therapy.

3.6. Pathological findings

The patient (Patient 1 in Table 2) with anti-IFI16 antibody was pathologically diagnosed with UIP owing to the presence of fibroblastic foci, honeycomb, and patchy lesions. Immunohistochemistry showed that IFI16 was mainly expressed in the airway and alveolar epithelial cell nuclei. Notably, some interstitial lymphocytes and fibroblasts were also positive for IFI16 (Fig. 4a–c). IFI16 expression in airway epithelial cells of the patient positive for anti-IFI16 antibody detected in the cytoplasm and nucleus was apparently higher than that in patients with IPF or healthy controls (Fig. 4a). MxA, a type I interferon activation biomarker, was expressed in the alveolar macrophages of patients with UIP and healthy controls (Fig. 4e and f). In the patient positive for anti-IFI16 antibody, MxA-positive macrophages were modestly increased in the respiratory bronchioles, and some bronchiolar epithelial cells expressed MxA (Fig. 4d).

4. Discussion

In this study, we discovered a novel anti-tetramer antibody in patients with IIPs who did not meet the CTD criteria and identified IFI16 as its corresponding autoantigen. The frequency of immunosuppressant use was lower in patients with anti-IFI16 antibodies. Patients with anti-IFI16 antibodies appeared to have the lowest overall survival and acute exacerbation-free survival. Moreover, the lungs of the patient with anti-IFI16 antibody exhibit interferon activation. The prognosis for some IIPs with anti-IFI16 antibodies might be improved by suppressing interferon activation in lung lesions.

Notably, several autoantibodies are strongly associated with ILD. Antibodies against melanoma differentiation-associated gene 5 are associated with rapidly progressive ILD [24]. Anti-ARS antibodies, also known as myositis-specific autoantibodies, are strongly associated with ILD. They tend to show good responses to immunosuppressive therapy, including glucocorticoid; however, they are prone to frequent relapse, regardless of accompanying myositis [25]. Therefore, identifying these autoantibodies is beneficial in the daily clinical practice of ILD treatment because its accompanying CTDs, clinical course, and responsiveness to immunosuppressive therapy can be expected [11]. Other autoantibodies included in the serologic domain of the IPAF classification criteria, such as anti-double stranded DNA (anti-dsDNA), anti-Smith, anti-Ro/SSA, or anti-La/SSB, could be expected to meet the CTD classification criteria in the future or could provide a strong reason to suspect that inflammatory/immune abnormalities regarding CTDs may contribute to the pathophysiology of ILD.

Furthermore, there may be unidentified autoantibodies that are not currently included in the serologic domain of IPAF but may be used to predict inflammatory processes, as this guidance allows for the inclusion of unreported autoantibodies in the future [10]. In our routine screening for autoantibodies using the IP assay, we identified various unknown autoantibodies in patients with IIPs. Therefore, we identified a novel autoantibody in patients with IIPs and proved it was an anti-IFI16 antibody.

IFI16, a member of the PYHIN family, directly senses intracellular viral-derived DNA, leading to the recruitment of stimulator of interferon genes (STING) and activation of interferon regulatory factor (IRF) 3 and nuclear factor kappa B [26]. IFI16 is primarily located in the nucleus. Following viral DNA recognition and binding, IFI16 can induce the activation of the canonical inflammasome by recruiting apoptosis-

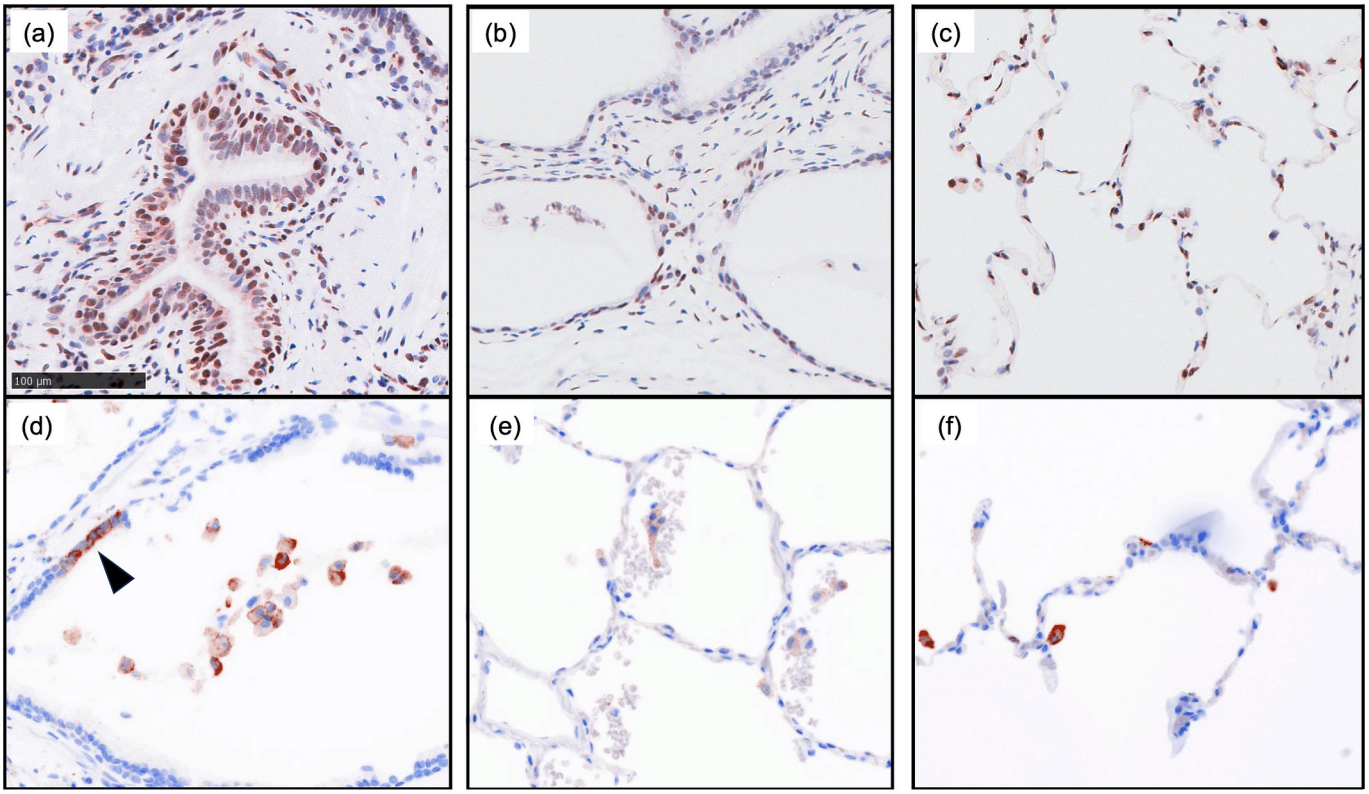

Fig. 4. Immunohistochemistry staining of lung specimens.
(a-c) Immunohistochemical staining with anti-IFI16 antibody. (d-f) Immunohistochemical staining with anti-MxA antibody. (a, d) lung specimen of idiopathic interstitial pneumonia with anti-IFI16 antibody (Patient 1 in Table 2). (b, e) lung specimen diagnosed with idiopathic pulmonary fibrosis. (c, f) lung specimen obtained surgically for pneumothorax (healthy control). Original magnification ×300. IFI16 was mainly expressed in the nuclei of cells in the airway and alveolar epithelial cells in each lung specimen; however, it was much more expressed in the cytoplasm and nuclei of the lung specimen of the patient with anti-IFI16 antibody-positive. MxA was expressed in alveolar macrophages and part of bronchiole epithelial cells (arrowhead). Abbreviations: IFI16, interferon gamma-inducible protein 16; MxA, myxovirus resistance protein A.

associated speck-like protein containing a caspase recruitment domain and pro-caspase 1 and the production of type 1 interferon through the STING-IRF axis. Furthermore, following the self-DNA recognition, IFI16 produces pro-inflammatory cytokines and type 1 interferon through the same pathways [27]. Aberrant expression of IFI16 can lead to extracellular leakage, amplifying inflammatory signals and producing protective autoantibodies [27].

Anti-IFI16 antibodies were first detected in patients with systemic lupus erythematosus (SLE) and have since been reported in patients with other CTDs, such as systemic sclerosis (SSc) or Sjogren's syndrome. The rate of anti-IFI16 antibodies in patients with SSc was 18–29 % [28,29], and the frequency of anti-IFI16 antibodies in patients with Sjogren's syndrome was 29 % [30]. Despite routinely screening sera from Japanese patients with CTDs, including those with SLE, SSc, and Sjogren's syndrome, using an IP assay, we rarely detected anti-IFI16 antibodies that immunoprecipitated tetrameric peptides at approximately 80–100 kDa, among patients with CTDs. The low frequency of anti-IFI16 antibodies detected in our institute, compared with the relatively high frequency reported in patients with CTDs, is attributed to differences in detection methods. Anti-IFI16 antibody was first detected using IP assay [31], but most reports on anti-IFI16 antibody use ELISA as a detection method [30,32]. IFI16 has four isoforms corresponding to each tetrameric peptide band precipitated in the IP assay [33,34]. Moreover, it has been reported that anti-IFI16 antibodies detected using ELISA have different reactivity against different epitopes of IFI16 [32]. Therefore, determining the presence of anti-IFI16 antibodies using ELISA alone may result in a high false-positive rate, leading to relatively high frequencies of anti-IFI16 antibodies among patients with CTDs. In this study, we confirmed the presence of an anti-IFI16 antibody by IP assay using whole protein extracted from HeLa cells and recombinant IFI16 expressed in an in vitro transcription/translation system. Therefore, the likelihood of a false-positive result for the anti-IFI16 antibody was extremely low.

Regarding the clinical significance of the anti-IFI16 antibody, no association was found with age, sex, smoking history, malignancy, or HRCT patterns of ILD among patients with IIPs. Serum markers such as KL-6 or surfactant protein-D and pulmonary function tests also showed no significant differences between patients with anti-IFI16 antibodies, those with anti-ARS antibodies, and those with other IIPs. At the time of IIP diagnosis, no unique clinical characteristics were observed in patients with anti-IFI16 antibodies. Apart from one patient who also tested positive for the anti-ARS antibody and was treated with glucocorticoids and immunosuppressants, the other patients with anti-IFI16 antibodies were not treated with immunosuppressants. Patients with anti-IFI16 antibodies showing no clinical features other than lung disease were diagnosed with IIPs and observed without any medication unless there was a diagnosis of IPF, which is usually treated with an anti-fibrosis agent. In our cohort, the overall survival and acute exacerbation-free rates of patients with anti-IFI16 antibodies seemed to be the lowest among all patients with IIPs. In comparing patients with anti-IFI16 antibodies and anti-ARS antibodies, the acute exacerbation-free rate was significantly lower in patients with anti-IFI16 antibodies. The difference in prognosis could be attributed to the use of immunosuppressants for lung disease. The recognition of anti-synthetase syndrome has led to routine screening for anti-ARS antibodies in patients with IIP. If these patients are positive for anti-ARS antibodies, they are likely to be treated with glucocorticoids and/or immunosuppressants [35]. The major cause of death due to ILD is an acute exacerbation of the lung, and antifibrotic agents such as nintedanib can reduce the frequency of this acute exacerbation [36,37]. The poor prognosis of patients with anti-IFI16 antibodies may be due to the low frequency of treatment with immunosuppressants and antifibrotic agents.

ILD can precede the diagnosis of CTDs [6,38]. However, patients with anti-IFI16 antibodies did not exhibit any clinical features associated with CTD and were not diagnosed with specific CTD during the entire observation period. Among the six patients with anti-IFI16 antibodies, two with other IPAF-associated autoantibodies, such as an anti-dsDNA antibody or anti-Jo-1 antibody, were classified as having IPAF because they met the serologic and morphological domains. However, the others were not classified as having IPAF. In our cohort, patients with anti-IFI16 antibodies were associated with ILD, irrespective of other accompanying CTDs. To date, there are few reports on the association between anti-IFI16 antibodies and ILD. In patients with ILD and SSc, a previous report suggested that the %DLco of patients with anti-IFI16 antibody-positive SSc was significantly lower than that of those with anti-IFI16 antibody-negative SSc [28]. This study mainly focused on digital ischemia in patients with SSc and used ELISA to detect anti-IFI16 antibodies, which could result in false positives. Our study is the first to focus on the association between anti-IFI16 antibodies and IIPs.

Pathological findings showed that IFI16 expression was primarily observed in airway epithelial cells and interstitial fibroblasts in lung lesions using the anti-IFI16 antibody. Even in normal human tissues, the IFI16 protein can be expressed in the epithelial cells of non-lymphoid tissues, including the trachea, gastrointestinal tract, and skin. It is predominantly expressed in surface epithelial cells, and staining is strongest in the basal epithelial layers [39]. We could not quantitatively compare the expression level of IFI16; however, it appeared to be higher in the airway epithelium of the patient positive for anti-IFI16 antibody than in patients with IPF (without anti-IFI16 antibody) or healthy control. MxA was also expressed in the airway epithelial cells of the patient positive for anti-IFI16 antibody, which appeared to be more intense than that in patients with IPF or healthy control. MxA expression is elevated in infectious diseases and type 1 interferon-driven autoimmune diseases such as SLE [40]. IFI16 acts as a sensor of viral infection followed by induction of the interferon pathway; therefore, some triggers, such as viral infection followed by the overexpression of IFI16, might trigger the pathogenesis of anti-IFI16 antibody-positive ILD [41].

This study has some limitations. First, the number of patients with anti-IFI16 antibodies was low, which may have affected the ability to detect significant differences in clinical characteristics or disease course. Second, because this was a retrospective study, the treatment was at the attending physician's discretion, and the protocols were not standardized. Most cases in this study were diagnosed as IIPs without pathological examination, leading to a tendency to classify them as unclassifiable IIPs based on HRCT. Additionally, patient backgrounds could be heterogeneous. Third, a few patients with anti-IFI16 antibodies also had other autoantibodies, such as anti-7-2 ribonucleoprotein or anti-dsDNA antibodies. We could not compare the clinical course of patients positive for a single anti-IFI16 antibody with the other IIP subgroups. Further accumulation of patients positive for anti-IFI16 antibodies and analysis of their clinical characteristics, including their response to immunosuppressants, are needed to clarify the clinical significance of anti-IFI16 antibodies.

In conclusion, we have identified an anti-IFI16 antibody associated with ILD. This antibody is not IIP-specific but could suggest a poor pulmonary prognosis factor for IIPs without therapeutic intervention. Considering the potential for an autoimmune-mediated inflammatory background, the response to immunosuppressants in patients with anti-IFI16 is worth exploring. Future studies are required to validate these results and to modify the serological domain of the IPAF criteria for determining the proper therapy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2024.110372.

CRediT authorship contribution statement

Tsuneo Sasai: Writing – original draft. **Ran Nakashima:** Writing – review & editing, Supervision, Conceptualization. **Tomohiro Handa:** Resources, Data curation. **Shogo Matsuda:** Resources, Data curation. **Takuya Kotani:** Resources, Data curation. **Hiromi Tomioka:** Resources, Data curation. **Ryo Tachikawa:** Resources, Data curation.

Keisuke Tomii: Resources, Data curation. **Kiminobu Tanizawa:** Resources, Data curation. **Yasuhiro Nohda:** Resources, Data curation. **Toshiaki Kogame:** Methodology. **Mirei Shirakashi:** Writing – review & editing. **Ryosuke Hiwa:** Writing – review & editing. **Hideaki Tsuji:** Writing – review & editing. **Shuji Akizuki:** Writing – review & editing. **Hajime Yoshifuji:** Writing – review & editing. **Tsuneyo Mimori:** Writing – review & editing. **Kenji Kabashima:** Writing – review & editing. **Akio Morinobu:** Writing – review & editing, Project administration.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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References

[1] American Thoracic Society, European Respiratory Society, American thoracic S, European respiratory S, American thoracic society/European Respiratory Society international multidisciplinary consensus classification of the idiopathic interstitial pneumonias, Am. J. Respir. Crit. Care Med. 165 (2002) 277–304, https://doi.org/10.1164/ajrccm.165.2.ats01.

[2] W.D. Travis, U. Costabel, D.M. Hansell, T.E. King, D.A. Lynch, A.G. Nicholson, C. J. Ryerson, J.H. Ryu, M. Selman, A.U. Wells, J. Behr, D. Bouros, K.K. Brown, T. V. Colby, H.R. Collard, C.R. Cordeiro, V. Cottin, B. Crestani, M. Drent, R.F. Dudden, J. Egan, K. Flaherty, C. Hogaboam, Y. Inoue, T. Johkoh, D.S. Kim, M. Kitaichi, J. Loyd, F.J. Martinez, J. Myers, S. Protzko, G. Raghu, L. Richeldi, N. Sverzellati, J. Swigris, D. Valeyre, ATS/ERS Committee on idiopathic interstitial pneumonias, an official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias, Am. J. Respir. Crit. Care Med. 188 (2013) 733–748, https://doi.org/10.1164/rccm.201308-1483ST.

[3] B. Ley, H.R. Collard, T.E. King Jr., Clinical course and prediction of survival in idiopathic pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 183 (2011) 431–440, https://doi.org/10.1164/rccm.201006-0894CI.

[4] S. Homma, M. Bando, A. Azuma, S. Sakamoto, K. Sugino, Y. Ishii, S. Izumi, N. Inase, Y. Inoue, M. Ebina, T. Ogura, K. Kishi, T. Kishaba, T. Kido, A. Gemma, Y. Goto, S. Sasaki, T. Johkoh, T. Suda, K. Takahashi, H. Takahashi, Y. Taguchi, H. Date, H. Taniguchi, T. Nakayama, Y. Nishioka, Y. Hasegawa, N. Hattori, J. Fukuoka, A. Miyamoto, H. Mukae, A. Yokoyama, I. Yoshino, K. Watanabe, Ministry of Health, Labour and Welfare, the study group on diffuse pulmonary disorders, scientific research/research on intractable diseases, and Japanese respiratory society, Japanese guideline for the treatment of idiopathic pulmonary fibrosis, Respir. Investig. 56 (2018) 268–291, https://doi.org/10.1016/j.resinv.2018.03.003.

[5] J.H. Park, D.S. Kim, I.N. Park, S.J. Jang, M. Kitaichi, A.G. Nicholson, T.V. Colby, Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes, Am. J. Respir. Crit. Care Med. 175 (2007) 705–711, https://doi.org/10.1164/rccm.200607-912OC.

[6] M. Kono, Y. Nakamura, N. Enomoto, D. Hashimoto, T. Fujisawa, N. Inui, M. Maekawa, T. Suda, T.V. Colby, K. Chida, Usual interstitial pneumonia preceding collagen vascular disease: a retrospective case control study of patients initially diagnosed with idiopathic pulmonary fibrosis, PLoS One 9 (2014) e94775, https://doi.org/10.1371/journal.pone.0094775.

[7] I. Marie, P.Y. Hatron, S. Dominique, P. Cherin, L. Mouthon, J.F. Menard, Short-term and long-term outcomes of interstitial lung disease in polymyositis and dermatomyositis: a series of 107 patients, Arthritis Rheum. 63 (2011) 3439–3447, https://doi.org/10.1002/art.30513.

[8] S.Y. Ji, F.Q. Zeng, Q. Guo, G.Z. Tan, H.F. Tang, Y.J. Luo, Z.Q. Tang, Y.F. Han, Predictive factors and unfavourable prognostic factors of interstitial lung disease in patients with polymyositis or dermatomyositis: a retrospective study, Chin. Med. J. 123 (2010) 517–522. https://www.ncbi.nlm.nih.gov/pubmed/20367973. https://doi.org/10.3760/cma.j.issn.0366-6999.2010.05.002.

[9] H.C. Kim, J.H. Lee, E.J. Chae, J.S. Song, J.W. Song, Long-term clinical course and outcome of interstitial pneumonia with autoimmune features, Respirology. 25 (2020) 636–643, https://doi.org/10.1111/resp.13665.

[10] A. Fischer, K.M. Antoniou, K.K. Brown, J. Cadranel, T.J. Corte, R.M. du Bois, J. S. Lee, K.O. Leslie, D.A. Lynch, E.L. Matteson, M. Mosca, I. Noth, L. Richeldi, M. E. Strek, J.J. Swigris, A.U. Wells, S.G. West, H.R. Collard, V. Cottin, "ERS/ATS task force on undifferentiated forms of CTD-ILD", an official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features, Eur. Respir. J. 46 (2015) 976–987, https://doi.org/10.1183/13993003.00150-2015.

[11] J. Graham, I. Bauer Ventura, C.A. Newton, C. Lee, N. Boctor, J.V. Pugashetti, C. Cutting, E. Joerns, H. Sandhu, J.H. Chung, C.K. Garcia, M. Kadoch, I. Noth, A. Adegunsoye, M.E. Strek, J.M. Oldham, Myositis-specific antibodies identify a distinct interstitial pneumonia with autoimmune features phenotype, Eur. Respir. J. (2020), https://doi.org/10.1183/13993003.01205-2020.

[12] I.N. Targoff, Humoral immunity in polymyositis/dermatomyositis, J. Invest. Dermatol. 100 (1993) 116S–123S, https://doi.org/10.1111/1523-1747.ep12356607.

[13] K. Huang, R. Aggarwal, Antisynthetase syndrome: a distinct disease spectrum, J. Scleroderma Relat. Disord. 5 (2020) 178–191, https://doi.org/10.1177/2397198320902667.

[14] G. Raghu, H.R. Collard, J.J. Egan, F.J. Martinez, J. Behr, K.K. Brown, T.V. Colby, J. F. Cordier, K.R. Flaherty, J.A. Lasky, D.A. Lynch, J.H. Ryu, J.J. Swigris, A.U. Wells, J. Ancochea, D. Bouros, C. Carvalho, U. Costabel, M. Ebina, D.M. Hansell, T. Johkoh, D.S. Kim, T.E. King, Y. Kondoh, J. Myers, N.L. Müller, A.G. Nicholson, L. Richeldi, M. Selman, R.F. Dudden, B.S. Griss, S.L. Protzko, H.J. Schünemann, ATS/ERS/JRS/ALAT committee on idiopathic pulmonary fibrosis, an official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management, Am. J. Respir. Crit. Care Med. 183 (2011) 788–824, https://doi.org/10.1164/rccm.2009-040GL.

[15] G. Raghu, M. Remy-Jardin, J.L. Myers, L. Richeldi, C.J. Ryerson, D.J. Lederer, J. Behr, V. Cottin, S.K. Danoff, F. Morell, K.R. Flaherty, A. Wells, F.J. Martinez, A. Azuma, T.J. Bice, D. Bouros, K.K. Brown, H.R. Collard, A. Duggal, L. Galvin, Y. Inoue, R.G. Jenkins, T. Johkoh, E.A. Kazerooni, M. Kitaichi, S.L. Knight, G. Mansour, A.G. Nicholson, S.N.J. Pipavath, I. Buendía-Roldán, M. Selman, W. D. Travis, S. Walsh, K.C. Wilson, American Thoracic Society, European Respiratory Society, Japanese respiratory society, and Latin American Thoracic Society, diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline, Am. J. Respir. Crit. Care Med. 198 (2018) e44–e68, https://doi.org/10.1164/rccm.201807-1255ST.

[16] G. Raghu, Idiopathic pulmonary fibrosis: guidelines for diagnosis and clinical management have advanced from consensus-based in 2000 to evidence-based in 2011, Eur. Respir. J. 37 (2011) 743–746, https://doi.org/10.1183/09031936.00017711.

[17] T.L. Reddy, M. Tominaga, D.M. Hansell, J. von der Thusen, D. Rassl, H. Parfrey, S. Guy, O. Twentyman, A. Rice, T.M. Maher, E.A. Renzoni, A.U. Wells, A. G. Nicholson, Pleuroparenchymal fibroelastosis: a spectrum of histopathological and imaging phenotypes, Eur. Respir. J. 40 (2012) 377–385, https://doi.org/10.1183/09031936.00165111.

[18] H.R. Collard, C.J. Ryerson, T.J. Corte, G. Jenkins, Y. Kondoh, D.J. Lederer, J.S. Lee, T.M. Maher, A.U. Wells, K.M. Antoniou, J. Behr, K.K. Brown, V. Cottin, K. R. Flaherty, J. Fukuoka, D.M. Hansell, T. Johkoh, N. Kaminski, D.S. Kim, M. Kolb, D.A. Lynch, J.L. Myers, G. Raghu, L. Richeldi, H. Taniguchi, F.J. Martinez, Acute exacerbation of idiopathic pulmonary fibrosis. An international working group report, Am. J. Respir. Crit. Care Med. 194 (2016) 265–275, https://doi.org/10.1164/rccm.201604-0801CI.

[19] T. Mimori, J.A. Hardin, J.A. Steitz, Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders, J. Biol. Chem. 261 (1986) 2274–2278. https://www.ncbi.nlm.nih.gov/pubmed/3511059. https://doi.org/10.1016/S0021-9258(17)35929-X.

[20] E. Fukuda, H. Tanaka, K. Yamaguchi, M. Takasaka, Y. Kawamura, H. Okuda, A. Isotani, M. Ikawa, V.S. Shapiro, J. Tsuchida, Y. Okada, A. Tsujimura, Y. Miyagawa, S. Fukuhara, Y. Kawakami, M. Wada, Y. Nishimune, N. Goshima, Identification and characterization of the antigen recognized by the germ cell mAb TRA98 using a human comprehensive wet protein array, Genes Cells 26 (2021) 180–189, https://doi.org/10.1111/gtc.12832.

[21] N. Goshima, Y. Kawamura, A. Fukumoto, A. Miura, R. Honma, R. Satoh, A. Wakamatsu, J. Yamamoto, K. Kimura, T. Nishikawa, T. Andoh, Y. Iida, K. Ishikawa, E. Ito, N. Kagawa, C. Kaminaga, K. Kanehori, B. Kawakami, K. Kenmochi, R. Kimura, M. Kobayashi, T. Kuroita, H. Kuwayama, Y. Maruyama, K. Matsuo, K. Minami, M. Mitsubori, M. Mori, R. Morishita, A. Murase, A. Nishikawa, S. Nishikawa, T. Okamoto, N. Sakagami, Y. Sakamoto, Y. Sasaki, T. Seki, S. Sono, A. Sugiyama, T. Sumiya, T. Takayama, Y. Takayama, H. Takeda, T. Togashi, K. Yahata, H. Yamada, Y. Yanagisawa, Y. Endo, F. Imamoto, Y. Kisu, S. Tanaka, T. Isogai, J. Imai, S. Watanabe, N. Nomura, Human protein factory for converting the transcriptome into an in vitro-expressed proteome, Nat. Methods 5 (2008) 1011–1017, https://doi.org/10.1038/nmeth.1273.

[22] Y. Toda, K. Kono, H. Abiru, K. Kokuryo, M. Endo, H. Yaegashi, M. Fukumoto, Application of tyramide signal amplification system to immunohistochemistry: a potent method to localize antigens that are not detectable by ordinary method, Pathol. Int. 49 (1999) 479–483, https://doi.org/10.1046/j.1440-1827.1999.00875.x.

[23] Y. Kanda, Investigation of the freely available easy-to-use software 'EZR' for medical statistics, Bone Marrow Transplant. 48 (2013) 452–458, https://doi.org/10.1038/bmt.2012.244.

[24] R. Nakashima, Y. Imura, S. Kobayashi, N. Yukawa, H. Yoshifuji, T. Nojima, D. Kawabata, K. Ohmura, T. Usui, T. Fujii, K. Okawa, T. Mimori, The RIG-I-like receptor IFIH1/MDA5 is a dermatomyositis-specific autoantigen identified by the

anti-CADM-140 antibody, Rheumatol. (Oxf. Engl.). 49 (2010) 433–440, https://doi.org/10.1093/rheumatology/kep375.
[25] K. Tanizawa, T. Handa, R. Nakashima, T. Kubo, Y. Hosono, K. Watanabe, K. Aihara, K. Ikezoe, A. Sokai, Y. Nakatsuka, Y. Taguchi, K. Hatta, S. Noma, Y. Kobashi, A. Yoshizawa, T. Oga, T. Hirai, K. Chin, S. Nagai, T. Izumi, T. Mimori, M. Mishima, The long-term outcome of interstitial lung disease with anti-aminoacyl-tRNA synthetase antibodies, Respir. Med. 127 (2017) 57–64, https://doi.org/10.1016/j.rmed.2017.04.007.
[26] L. Unterholzner, S.E. Keating, M. Baran, K.A. Horan, S.B. Jensen, S. Sharma, C. M. Sirois, T. Jin, E. Latz, T.S. Xiao, K.A. Fitzgerald, S.R. Paludan, A.G. Bowie, IFI16 is an innate immune sensor for intracellular DNA, Nat. Immunol. 11 (2010) 997–1004, https://doi.org/10.1038/ni.1932.
[27] V. Caneparo, S. Landolfo, M. Gariglio, M. De Andrea, The absent in melanoma 2-like receptor IFN-inducible protein 16 as an inflammasome regulator in systemic lupus erythematosus: the dark side of sensing microbes, Front. Immunol. 9 (2018) 1180, https://doi.org/10.3389/fimmu.2018.01180.
[28] Z.H. McMahan, A.A. Shah, D. Vaidya, F.M. Wigley, A. Rosen, L. Casciola-Rosen, Anti-interferon-inducible protein 16 antibodies associate with digital gangrene in patients with scleroderma, arthritis, Rheumatol. 68 (2016) 1262–1271, https://doi.org/10.1002/art.39558.
[29] S. Costa, M. Mondini, V. Caneparo, A. Afeltra, P. Airò, F. Bellisai, P. Faggioli, R. Gerli, M. Lotzniker, P.L. Meroni, G. Morozzi, A. Radice, V. Riccieri, M. Scarsi, G. D. Sebastiani, R.A. Sinico, A. Tincani, M. Gariglio, S. Landolfo, Detection of anti-IFI16 antibodies by ELISA: clinical and serological associations in systemic sclerosis, Rheumatol. (Oxf. Engl.). 50 (2011) 674–681, https://doi.org/10.1093/rheumatology/keq372.
[30] A.N. Baer, M. Petri, J. Sohn, A. Rosen, L. Casciola-Rosen, Association of antibodies to interferon-inducible Protein-16 with markers of more severe disease in primary Sjogren's syndrome, Arthritis Care Res. 68 (2016) 254–260, https://doi.org/10.1002/acr.22632.
[31] H.P. Seelig, H. Ehrfeld, M. Renz, Interferon-gamma-inducible protein p16. A new target of antinuclear antibodies in patients with systemic lupus erythematosus, Arthritis Rheum. 37 (1994) 1672–1683, https://doi.org/10.1002/art.1780371117.
[32] M. Mondini, M. Vidali, M. De Andrea, B. Azzimonti, P. Airò, R. D'Ambrosio, P. Riboldi, P.L. Meroni, E. Albano, Y. Shoenfeld, M. Gariglio, S. Landolfo, A novel autoantigen to differentiate limited cutaneous systemic sclerosis from diffuse cutaneous systemic sclerosis: the interferon-inducible gene IFI16, Arthritis Rheum. 54 (2006) 3939–3944, https://doi.org/10.1002/art.22266.
[33] R.W. Johnstone, M.H. Kershaw, J.A. Trapani, Isotypic variants of the interferon-inducible transcriptional repressor IFI 16 arise through differential mRNA splicing, Biochemistry. 37 (1998) 11924–11931, https://doi.org/10.1021/bi981069a.
[34] J. Borucka, K. Sterzyńska, D. Kaźmierczak, M. Świerczewska, M. Nowacka, K. Wojtowicz, A. Klejewski, M. Nowicki, M. Zabel, R. Ramlau, R. Januchowski, The significance of interferon gamma inducible protein 16 (IFI16) expression in drug resistant ovarian cancer cell lines, Biomed. Pharmacother. 150 (2022) 113036, https://doi.org/10.1016/j.biopha.2022.113036.
[35] Y. Kondoh, S. Makino, T. Ogura, T. Suda, H. Tomioka, H. Amano, M. Anraku, N. Enomoto, T. Fujii, T. Fujisawa, T. Gono, M. Harigai, H. Ichiyasu, Y. Inoue, T. Johkoh, H. Kameda, K. Kataoka, Y. Katsumata, Y. Kawaguchi, A. Kawakami, H. Kitamura, N. Kitamura, T. Koga, K. Kurasawa, Y. Nakamura, R. Nakashima, Y. Nishioka, O. Nishiyama, M. Okamoto, F. Sakai, S. Sakamoto, S. Sato, T. Shimizu, N. Takayanagi, R. Takei, T. Takemura, T. Takeuchi, Y. Toyoda, H. Yamada, H. Yamakawa, Y. Yamano, Y. Yamasaki, M. Kuwana, joint committee of Japanese Respiratory Society and Japan College of Rheumatology, Guide for the diagnosis and treatment of interstitial lung disease associated with connective tissue disease, Respir. Investig. 59 (2021) (2020) 709–740, https://doi.org/10.1016/j.resinv.2021.04.011.
[36] J. Salonen, M. Purokivi, R. Bloigu, R. Kaarteenaho, Prognosis and causes of death of patients with acute exacerbation of fibrosing interstitial lung diseases, BMJ Open Respir. Res. 7 (2020), https://doi.org/10.1136/bmjresp-2020-000563.
[37] H.R. Collard, L. Richeldi, D.S. Kim, H. Taniguchi, I. Tschoepe, M. Luisetti, J. Roman, G. Tino, R. Schlenker-Herceg, C. Hallmann, R.M. du Bois, Acute exacerbations in the INPULSIS trials of nintedanib in idiopathic pulmonary fibrosis, Eur. Respir. J. 49 (2017), https://doi.org/10.1183/13993003.01339-2016.
[38] M. Kono, Y. Nakamura, K. Yoshimura, Y. Enomoto, Y. Oyama, H. Hozumi, N. Enomoto, T. Fujisawa, N. Inui, E. Hamada, T.V. Colby, M. Maekawa, T. Suda, Nonspecific interstitial pneumonia preceding diagnosis of collagen vascular disease, Respir. Med. 117 (2016) 40–47, https://doi.org/10.1016/j.rmed.2016.05.030.
[39] W. Wei, C.J. Clarke, G.R. Somers, K.S. Cresswell, K.A. Loveland, J.A. Trapani, R. W. Johnstone, Expression of IFI 16 in epithelial cells and lymphoid tissues, Histochem. Cell Biol. 119 (2003) 45–54, https://doi.org/10.1007/s00418-002-0485-0.
[40] X. Feng, H. Wu, J.M. Grossman, P. Hanvivadhanakul, J.D. FitzGerald, G.S. Park, X. Dong, W. Chen, M.H. Kim, H.H. Weng, D.E. Furst, A. Gorn, M. McMahon, M. Taylor, E. Brahn, B.H. Hahn, B.P. Tsao, Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus, Arthritis Rheum. 54 (2006) 2951–2962, https://doi.org/10.1002/art.22044.
[41] F. Gugliesi, M. Bawadekar, M. De Andrea, V. Dell'Oste, V. Caneparo, A. Tincani, M. Gariglio, S. Landolfo, Nuclear DNA sensor IFI16 as circulating protein in autoimmune diseases is a signal of damage that impairs endothelial cells through high-affinity membrane binding, PLoS One 8 (2013) e63045, https://doi.org/10.1371/journal.pone.0063045.