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ORIGINAL ARTICLE

Clinical and pathological significance of serum anti-PD-1 autoantibodies in acute-onset and chronic autoimmune hepatitis

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Abstract

Background Acute-onset autoimmune hepatitis (A-AIH) poses diagnostic challenges because autoantibodies are frequently absent or serum IgG levels may only be mildly elevated. Autoantibodies against the immune checkpoint molecule PD-1 have been detected in patients with chronic AIH (C-AIH). To clarify the clinical and pathological significance of anti-PD-1 autoantibodies, we examined their association with the clinical features of patients with liver diseases, including A-AIH, C-AIH, primary biliary cholangitis (PBC), and drug-induced liver injury (DILI).

Methods We compared the clinical characteristics and serum anti-PD-1 autoantibody titers of patients with A-AIH, C-AIH, PBC, and DILI ($n = 72$, 45, 41, and 19, respectively). Correlations between autoantibody titers and biochemical parameters were assessed to identify the factors that discriminate between the diseases. To reduce non-specific IgG response, anti-PD-1 autoantibody titers were normalized to the serum IgG levels. The liver histology was evaluated in a subset of patients with AIH.

Results A-AIH caused severe liver parenchymal and portal inflammation. The anti-PD-1/IgG ratio showed positive correlations with AST and ALT, but only weak correlations with ALP and γ-GTP. Anti–PD-1/IgG ratio was the highest in A-AIH, followed by DILI, C-AIH and PBC. Histological findings showed that anti–PD-1/IgG ratio was associated with severe inflammation, but not with centrilobular necrosis.

Conclusions The serum anti-PD-1 autoantibody/IgG ratio was significantly elevated in A-AIH compared with that in C-AIH and PBC, and A-AIH was independently distinguished from C-AIH. These markers may assist with the assessment of acute-onset liver inflammation and clinical phenotyping of AIH.

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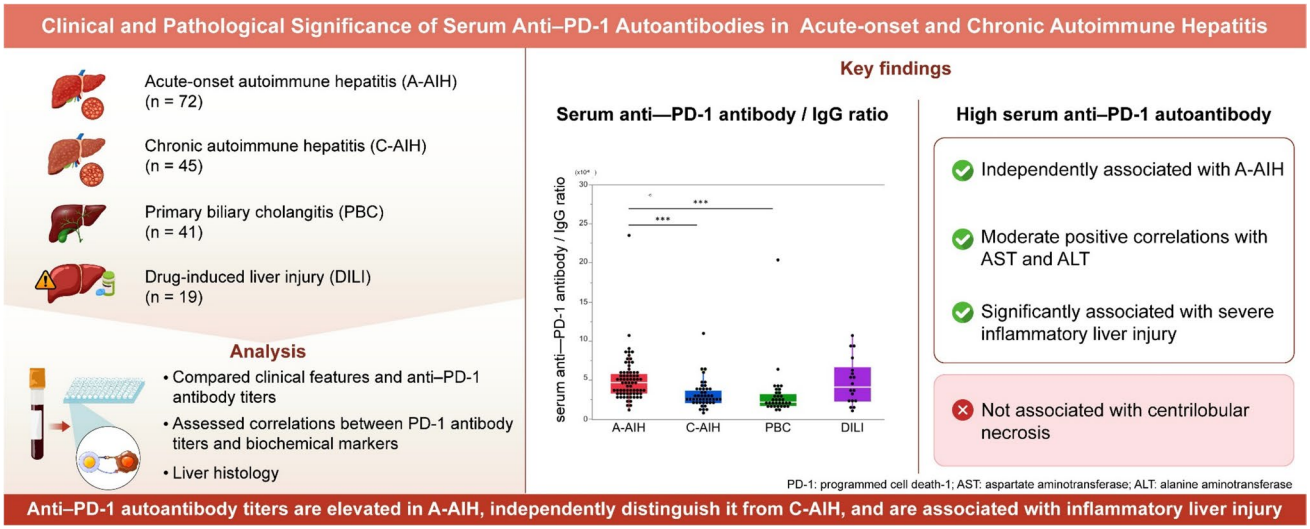
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Graphical Abstract



Keywords Acute liver injury · Chronic inflammatory liver disease · Primary biliary cholangitis · Immune checkpoint signaling · Immune tolerance · Interface hepatitis · Plasma cell infiltration · Emperipolesis · Diagnostic marker

Introduction

Autoimmune hepatitis (AIH) is classically a chronic inflammatory liver disease characterized by circulating autoantibodies, hypergammaglobulinemia on clinical examination, and interface hepatitis and prominent plasma cell infiltration on histological examination [1, 2]. However, 25–75% of AIH cases present with acute onset [3], and the number of patients with acute-onset AIH (A-AIH) presenting with acute hepatitis has been increasing in nationwide Japanese surveys [4]. Distinguishing A-AIH from chronic AIH (C-AIH) at first presentation is clinically important because A-AIH can progress rapidly and often requires urgent therapeutic decisions. Conventional serological markers, such as serum immunoglobulin G (IgG) levels and antinuclear antibody (ANA) titers, are frequently normal or non-discriminatory in the acute settings [5]. This diagnostic uncertainty underscores the need for biomarkers that reflect the immune activity underlying A-AIH.

Programmed cell death protein 1 (PD-1) is a key inhibitory receptor expressed on activated T cells that is essential for maintaining peripheral tolerance [6]. Disruption of the PD-1/programmed death-ligand 1 (PD-L1) axis leads to excessive T-cell activation and tissue inflammation, including AIH. A recent striking example is immune checkpoint inhibitor (ICI) therapy-related adverse events (irAEs), in which PD-1 blockade can induce severe immune-mediated hepatitis that closely resembles A-AIH with respect to histological findings, such as lobular necroinflammation, centrilobular damage, plasma cell–rich infiltrates, and immunophenotypic features of T cell activation [7, 8]. However, irAE-associated hepatitis has been reported to show fewer plasma cells, CD20-positive cells, and CD4-positive cells than classical AIH. These observations have generated interest in whether endogenous perturbation of PD-1 signaling might similarly contribute to immune activation in classical AIH.

Autoantibodies targeting PD-1 have been described in several autoimmune diseases, where they are thought to neutralize PD-1-mediated inhibitory signaling and promote T cell hyperactivation and heightened cytokine responses [9, 10]. We have previously reported that anti-PD-1 autoantibodies are prevalent in C-AIH [11, 12]. However, the clinical significance of anti-PD-1 autoantibodies in A-AIH has not yet been elucidated. Given the mechanistic parallels between ICI-induced hepatitis and A-AIH, it is plausible that endogenous anti-PD-1 responses amplify the intrahepatic immune activity and contribute to an acute inflammatory phenotype.

In this study, we investigated the serum anti-PD-1 autoantibody titers in patients with A-AIH, C-AIH, and primary biliary cholangitis (PBC). We hypothesized that anti-PD-1 autoantibody titers would be elevated in patients with A-AIH and would reflect intrahepatic immune activity. To test this hypothesis, we analyzed detailed clinical parameters and histological features and evaluated whether anti-PD-1 autoantibodies could serve as biomarkers for distinguishing these autoimmune liver diseases.

Materials and methods

Patients

The Japan Autoimmune Hepatitis Study Group (JAIHG) conducted this multicenter retrospective survey among nine hospitals (Okayama University Hospital, Kanazawa University Hospital, Kurume University Hospital, Ehime University Hospital, Teine Keijinkai Hospital, Fukushima Medical University Hospital, Toranomon Hospital, Keio University, Teikyo University Hospital). Seventy-three patients diagnosed with acute-onset AIH after ruling out AIH in the acute exacerbation phase by liver specialists between 1989 and 2018 were initially enrolled in this study. Ultimately, 72 patients with A-AIH and 45 patients with C-AIH were analyzed. The diagnoses of A-AIH and C-AIH were based on the criteria of the International AIH Group (IAIHG) (1999) [13], simplified criteria (2008) [14], and guidelines from the Ministry of Health, Labour and Welfare (MHLW) Research Program on Intractable Hepatobiliary Disease [15], with scoring for definite or probable AIH and/or empirical judgments by experienced hepatologists based on combined clinical, laboratory, serological, and histological data, as reported previously [16]. Patients with a history of abnormal liver enzyme levels or advanced liver fibrosis confirmed by liver biopsy or imaging were excluded.

Forty-one patients with PBC were enrolled before treatment. The diagnosis of PBC was made when two or more of the following three criteria were met: (1) cholestatic enzyme elevation (alkaline phosphatase and γ-glutamyl transpeptidase) persisting ≥ 6 months; (2) serum anti-mitochondrial antibodies detected by indirect immunofluorescence; and (3) characteristic histology on liver biopsy.

Drug-induced liver injury (DILI) was included as a disease control in 19 cases. The diagnosis of DILI was made by a hepatologist using the Council for International Organizations of Medical Sciences / the Roussel Uclaf Causality Assessment Method (CIOMS/RUCAM) score and the digestive disease week Japan (DDW-J) criteria (≥ 4 and/or ≥ 3, respectively).

All samples were collected during the patients' first visit to the participating institutions (i.e., before treatment initiation). The number of freeze–thaw cycles was kept to a minimum of no more than three.

Clinical and laboratory data

The demographic and biochemical data collected from the patients at the time of diagnosis were as follows: age, sex, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GTP), prothrombin time-international normalized ratio (PT-INR), IgG, immunoglobulin M (IgM), and ANA (Table 1).

Table 1 Clinical characteristics between A-AIH, C-AIH, PBC, and DILI

Variable	A-AIH ($n = 72$)	C-AIH ($n = 45$)	PBC ($n = 41$)	DILI ($n = 19$)
Sex (female), *n* (%)	61 (84.7)	37 (82.2)	34 (82.9)	63.2 (12)
Age at diagnosis (years)	56 (43–66)	63 (54.5–71)	59 (50–66)	49 (34–71)
Laboratory data				
T-Bil (mg/dL)*	8.05 (1.81–12.1)	1.25 (0.95–1.97)	0.65 (0.57–0.86)	1.11 (0.93–3.33)
AST (U/L)*	677.5 (375–1069.3)	155 (108.5–266.5)	36 (28.5–56)	95.5 (40.5–377.8)
ALT (U/L)*	798.5 (393.8–1305.8)	180 (119–289)	40 (24–66.5)	134.5 (48.8–353)
ALP (U/L)*	484.5 (373.8–708)	400 (270–548.5)	352 (275.5–587.5)	533.5 (299.3–649.8)
γ-GTP (U/L)*	247.5 (137.3–369)	129(75–253.5)	134 (85–250.5)	202.5 (70.8–407.8)
PT (%)**	71 (57.1–86)	92.5 (83.3–100)	111 (104.5–118)	96 (75–111.3)
PT-INR*	1.16 (1.06–1.29)	1.04 (0.99–1.09)	0.95 (0.93–0.98)	1.02 (0.95–1.22)
IgG (mg/dL)	1846.5 (1442.8–2386.5)	1938.3 (1582.2–2545.7)	1366.6 (1221.2–1851.2)	1201.1 (1056.9–1491.4)
IgM (mg/dL)	125 (89–177)	135.9 (87.9–235.4)	192 (134.5–303.6)	110.9 (80.6–142.2)
ANA titer ≥ 80, n (%)	38 (52.8)	25 (56.8) ***	18 (45.0) ***	4 (26.7) ***
IAIHG 1999 definite diagnosis, *n* (%)	22 (30.6)	10 (22.2)	–	–
IAIHG 2008 definite diagnosis, *n* (%)	7 (9.7)	16 (35.6)	–	–

Data are expressed as medians with interquartile ranges in parentheses for continuous variables, and as percentages with numbers in parentheses for categorical variables

AIH Autoimmune hepatitis, *PBC* Primary biliary cholangitis, *DILI* Drug-induced liver injury, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALP* Alkaline phosphatase, *γ-GTP* γ-glutamyl transpeptidase, *PT-INR* Prothrombin time-international normalized ratio, *IgG* Immunoglobulin G, *ANA* Antinuclear antibody

*Peak value, **Nadir value, ***Data were unavailable for one case each of C-AIH and PBC, and for four cases of DILI

Quantification of anti-PD-1 autoantibodies

Serum anti-PD-1 antibody titers were measured by indirect enzyme-linked immunosorbent assay (ELISA), according to the method described in our previous reports [17, 18], with some modifications.

Briefly, the plate and buffer system for ELISA (Ab-Match Universal Kit 5310) was purchased from MBL (Nagano, Japan). Ninety-six-well flat-bottom plates for ELISA provided with the kit were coated with 100 µL of 1 µg/mL recombinant PD-1 (Abnova, Taipei, Taiwan) at 4 °C overnight. The next day, the plates were washed twice with a wash buffer and blocked with a blocking buffer. After incubation for 1 h at 37 °C, the sera were added to the wells after the samples had been diluted 1:50 with sample diluent buffer. For quantitation, a calibration curve was created by serially diluting rabbit anti-PD-1 antibody (Abnova, Taipei, Taiwan) from 0.0025 µg/mL to 0.16 µg/mL, and serum data were fitted to this curve to calculate antibody concentrations. Serum-positive and negative controls were defined based on our previous results and were added to the assay to confirm the success of the experiments. After incubation for 1 h at room temperature, the wells were washed four times and 100 µL of 1:100 diluted peroxidase-labeled anti-human IgG (LGC Clinical Diagnostics, Inc, Milford, MA, USA) was applied to the wells. For the calibration curve, 100 µL of 1:100 diluted peroxidase-labeled anti-rabbit IgG was applied to the wells. The plates were then incubated for 1 h at room temperature. After incubation, the plates were washed four times and the substrate solution was added. The optical density was measured at 450 nm using a Model 680 microplate reader (Spectrophotometer Type 1510) (Fisher Scientific, Vantaa, Finland).

Given that autoantibodies are often correlated with non-specific IgG levels, we used the value obtained by dividing the anti-PD-1 antibody titers by the IgG levels to reduce non-specific B-cell responses for disease differentiation (Table 2).

Histological evaluation

We evaluated the histological findings of liver biopsy specimens obtained from 51 patients with AIH, including 14 with A-AIH and 37 with C-AIH. The cases from which liver biopsy specimens were obtained showed a significantly lower incidence of A-AIH and lower levels of liver inflammation related markers (Supplementary Table 1), likely because of the difficulty in performing liver biopsies in severe cases. The sections were stained with hematoxylin and eosin, and connective tissue, including Masson's trichrome and reticulin stains. All liver biopsy samples were independently evaluated by an experienced pathologist (K.H.) who was blinded to the clinical data, using identical histological scoring sheets covering parameters, such as fibrosis, central zone necrosis, perivenular necroinflammatory activity, lobular necrosis/inflammation, cobblestone appearance, plasma cell infiltration, and emperipolesis, as previously reported [16].

Statistical analysis

The results are presented as median and interquartile ranges for continuous variables and as percentages for categorical variables. Statistical analyses were performed using the JMP Student Edition 18.2.2 (SAS Institute Inc., Cary, NC, USA). The two groups were compared with the $\chi 2$ test and Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. The Spearman's correlation coefficient was used to assess the correlation between continuous variables in the two groups. Univariate analyses were performed to identify the variables associated with each disease group. In a multivariate logistic regression model, we included liver function-related total bilirubin, liver parenchymal inflammatory activity-related ALT, and biliary tract injury-related ALP as factors of clinical significance along with anti-PD-1 autoantibody titers/IgG as variables to identify independent factors. Although PT-INR and IgG are clinically important in AIH, PT-INR correlates with total bilirubin, and IgG is included in the anti-PD-1 autoantibody titers/IgG calculation formula; therefore, to avoid confounding factors, they were not included in the multivariate analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were also determined. To determine the optimal antibody titer for disease discrimination, we used the Youden index, which was calculated based on the receiver operating characteristic (ROC) curve. Sensitivity and specificity were determined based on the ROC curve. p-values < 0.05 were considered significant.

Results

Patient clinical characteristics

A total of 72 patients with A-AIH, 45 with C-AIH, 41 with PBC, and 19 with DILI were included; the median age at diagnosis was 56 years in patients with A-AIH, 63 years in patients with C-AIH, 59 years in patients with PBC, and 49 years in patients with DILI (Table 1).

Patients with A-AIH had higher total bilirubin, AST, and ALT levels than patients in the other groups, particularly AST and ALT levels (median values: 677.5 U/L and 798.5 U/L, respectively). In contrast, ALP and γ-GTP levels did not differ substantially among the four groups. Coagulation parameters revealed an increased PT-INR in patients with A-AIH, reflecting an impaired hepatic

Table 2 Clinical characteristics discriminating the diseases

Factor	Unit OR	OR (95% CI)	*P*	Unit OR	OR (95% CI)	*p*
A-AIH vs. C-AIH	Univariate			Multivariate		
Sex (female vs. male)	–	1.20 (0.43–3.24)	0.72			
Age (year)	1	0.98 (0.95–1.00)	0.05			
T-Bil (mg/dL)	1	1.38 (1.20–1.65)	< 0.001	1	1.22 (1.07–1.47)	**< 0.001**
AST (U/L)	10	1.02 (1.01–1.04)	< 0.001			
ALT (U/L)	10	1.02 (1.01–1.03)	< 0.001	10	1.01 (0.99–1.02)	0.08
ALP (U/L)	10	1.01 (0.99–1.03)	0.07	10	0.99 (0.98–1.01)	0.76
γ-GTP (U/L)	10	1.02 (0.99–1.04)	0.09			
PT-INR	0.1	1.33 (1.08–1.73)	0.006			
IgG (mg/dL)	10	0.99 (0.99–1.00)	0.79			
IgM (mg/dL)	10	0.98 (0.94–1.00)	0.10			
ANA titer ≥ 80	–	0.85 (0.40–1.80)	0.67			
Anti-PD-1 autoantibody titers (μg/mL)	0.01	1.29 (1.14–1.48)	< 0.001			
Anti-PD-1 autoantibody titers/IgG	1.0×10^{-6}	1.84 (1.40–2.53)	< 0.001	1.0×10^{-6}	1.52 (1.14–2.16)	**0.003**
A-AIH vs. PBC						
Sex (female vs. male)	–	1.14 (0.39–3.18)	0.80			
Age (year)	1	0.98 (0.95–1.01)	0.17			
T-Bil (mg/dL)	1	21.9 (5.20–151.1)	< 0.001	1	102 (1.38–43,237)	**0.02**
AST (U/L)	10	1.46 (1.22–1.96)	< 0.001			
ALT (U/L)	10	1.55 (1.27–2.14)	< 0.001	10	1.55 (1.19–2.61)	**< 0.001**
ALP (U/L)	10	1.01 (0.99–1.03)	0.09	10	0.91 (0.76–0.99)	**0.02**
γ-GTP (U/L)	10	1.01 (0.99–1.03)	0.19			
PT-INR	0.1	5.59 (2.98–12.6)	< 0.001			
IgG (mg/dL)	10	1.02 (1.01–1.04)	< 0.001			
IgM (mg/dL)	10	0.94 (0.90–0.97)	< 0.001			
ANA titer ≥ 80	–	1.37 (0.63–2.99)	0.43			
Anti-PD-1 autoantibody titers (μg/mL)	0.01	1.50 (1.27–1.83)	< 0.001			
Anti-PD-1 autoantibody titers/IgG	1.0×10^{-6}	1.66 (1.27–2.30)	< 0.001	1.0×10^{-6}	0.81 (0.42–1.08))	0.15
C-AIH vs. PBC						
Sex (female vs. male)	–	0.95 (0.31–2.93)	0.93			
Age (year)	1	1.01 (0.98–1.05)	0.47			
T-Bil (mg/dL)	1	18.1 (4.64–99.1)	**< 0.001**	1	24.9 (2.97–508.7)	**0.001**
AST (U/L)	10	1.66 (1.36–2.17)	**< 0.001**			
ALT (U/L)	10	1.42 (1.24–1.69)	**< 0.001**	10	1.40 (1.19–1.78)	**< 0.001**
ALP (U/L)	10	1.00 (0.99–1.01)	0.98	10	0.96 (0.92–0.99)	**0.01**
γ-GTP (U/L)	10	0.99 (0.98–1.02)	0.83			
PT-INR	0.1	59.9 (12.4–476.5)	**< 0.001**			
IgG (mg/dL)	10	1.03 (1.02–1.05)	**< 0.001**			
IgM (mg/dL)	10	0.97 (0.95–1.00)	0.06			
ANA titer ≥ 80	–	1.61 (0.68–3.85)	0.28			
Anti-PD-1 autoantibody titers (μg/mL)	0.01	1.20 (1.02–1.49)	**0.02**			
Anti-PD-1 autoantibody titers/IgG	1.0×10^{-6}	1.00 (0.83–1.24)	0.98	1.0×10^{-6}	0.90 (0.71–1.23)	0.44
A-AIH vs. DILI						
Sex (female vs. male)	–	3.23 (1.02–10.1)	3.23			
Age (year)	1	1.01 (0.98–1.04)	0.53			
T-Bil (mg/dL)	1	1.25 (1.09–1.53)	**< 0.001**	1	1.19 (1.04–149)	**0.009**
AST (U/L)	10	1.02 (1.01–1.04)	**< 0.001**			
ALT (U/L)	10	1.02 (1.01–1.03)	**< 0.001**	10	68.4 (2.14–7748.8)	**0.01**
ALP (U/L)	10	0.99 (0.98–1.01)	0.47	10	0.99 (0.97–1.00)	0.06
γ-GTP (U/L)	10	0.99 (0.98–1.02)	0.67			

Table 2 (continued)

Factor	Unit OR	OR (95% CI)	*P*	Unit OR	OR (95% CI)	*p*
PT-INR	0.1	0.99 (0.86–1.19)	0.91			
IgG (mg/dL)	10	1.03 (1.02–1.05)	**< 0.001**			
IgM (mg/dL)	10	1.02 (0.97–1.09)	0.48			
ANA titer ≥ 80	–	3.07 (0.95–11.9)	0.06			
Anti-PD-1 autoantibody titers (μg/mL)	0.01	1.29 (1.11–1.56)	**< 0.001**			
Anti-PD-1 autoantibody titers/IgG	1.0×10^{-6}	1.03 (0.87–1.29)	0.76	1.0×10^{-6}	0.98 (0.77–1.30)	0.86
C-AIH vs. DILI						
Sex (female vs. male)	–	2.70 (0.80–9.20)	0.11			
Age (year)	1	1.03 (1.00–1.06)	0.07			
T-Bil (mg/dL)	1	0.93 (0.77–1.11)	0.40	1	0.98 (0.79–1.28)	0.88
AST (U/L)	10	1.00 (0.99–1.01)	0.97			
ALT (U/L)	10	0.99 (0.99–1.01)	0.95	10	0.99 (0.98–1.01)	0.94
ALP (U/L)	10	0.99 (0.98–1.00)	0.13	10	0.98 (0.97–1.00)	0.16
γ-GTP (U/L)	10	0.99 (0.97–1.01)	0.24			
PT-INR	0.1	0.89 (0.72–1.05)	0.17			
IgG (mg/dL)	10	1.04 (1.02–1.07)	**< 0.001**			
IgM (mg/dL)	10	1.04 (0.99–1.11)	0.11			
ANA titer ≥ 80	–	3.62 (1.06–14.7)	**0.04**			
Anti-PD-1 autoantibody titers (μg/mL)	0.01	1.02 (0.855–1.23)	0.87			
Anti-PD-1 autoantibody titers/IgG	1.0×10^{-6}	0.72 (0.54–0.92)	**0.007**	1.0×10^{-6}	0.73 (0.54–0.94)	**0.02**
PBC vs. DILI						
Sex (female vs. male)	–	2.83 (0.82–10.0)	0.10			
Age (year)	1	1.04 (0.99–1.08)	0.09			
T-Bil (mg/dL)	1	0.12 (0.02–0.44)	**< 0.001**	1	0.06 (0.001–0.64)	**0.01**
AST (U/L)	10	0.81 (0.64–0.93)	**< 0.001**			
ALT (U/L)	10	0.83 (0.70–0.93)	**< 0.001**	10	0.83 (0.64–0.98)	**0.01**
ALP (U/L)	10	0.99 (0.98–1.00)	0.16	10	1.02 (0.9–1.08)	0.18
γ-GTP (U/L)	10	0.99 (0.97–1.01)	0.31			
PT-INR	0.1	0.20 (0.05–0.61)	**< 0.001**			
IgG (mg/dL)	10	1.02 (1.00–1.04)	**0.03**			
IgM (mg/dL)	10	1.12 (1.04–1.24)	**< 0.001**			
ANA titer ≥ 80	–	2.25 (0.65–9.23)	0.21			
Anti-PD-1 autoantibody titers (μg/mL)	0.01	0.90 (0.73–1.04)	0.17			
Anti-PD-1 autoantibody titers/IgG	1.0×10^{-6}	0.83 (0.64–1.01)	0.06	1.0×10^{-6}	1.00 (0.74–1.28)	0.97

AIH autoimmune hepatitis, *PBC* primary biliary cholangitis, *DILI* drug-induced liver injury, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *PT-INR* prothrombin time-international normalized ratio, *IgG* immunoglobulin G, *PD-1* Programmed cell death-1

reserve. Serum IgG levels were elevated in both the A-AIH and C-AIH groups, whereas they were relatively lower in the PBC group, and IgM levels tended to be higher in the PBC group. High-titer ANA positivity (≥ 80) was detected in 52.8% of patients with A-AIH, 56.8% of those with C-AIH, 45.0% of those with PBC and 26.7% of those with DILI, indicating no significant differences among them ($p = 0.19$). According to the IAIHG 1999 criteria, 30.6% of patients with A-AIH and 22.2% of patients with C-AIH were classified as having definite AIH, whereas 9.7% and 35.6% met the IAIHG 2008 criteria.

Correlation of clinical characteristics and the titers of serum anti-PD-1 autoantibodies

When correlations between serum anti-PD-1 antibody titers and hepatobiliary enzyme data were examined across all cases, the autoantibody titers showed moderate positive correlations with AST and ALT (Spearman's $\rho = 0.45$ for both, $p < 0.001$), whereas correlations with ALP ($\rho = 0.25$, $p = 0.001$) and γ-GTP ($\rho = 0.12$, $p = 0.135$) were weak (Supplementary Table 2). These results indicate that serum anti-PD-1 antibody titers are more closely associated with

hepatocellular inflammation than with biliary tract injury. The anti-PD-1 antibody titers were positively correlated with IgG levels and were higher in patients with ANA positivity > ×80 than in others.

Serum anti-PD-1 antibody titers were significantly higher in patients with A-AIH (median [range] 0.0915 [0.021–0.383] μg/mL) than in those with C-AIH (0.0564 [0.014–0.137] μg/mL) and PBC (0.031 [0.015–0.278] μg/mL) (Fig. 1a). However, the A-AIH titer was comparable to that of DILI. Given that autoantibodies are often correlated with non-specific IgG, we used the anti-PD-1 antibody/IgG ratio in subsequent analyses to reduce non-specific B-cell responses (Fig. 1b).

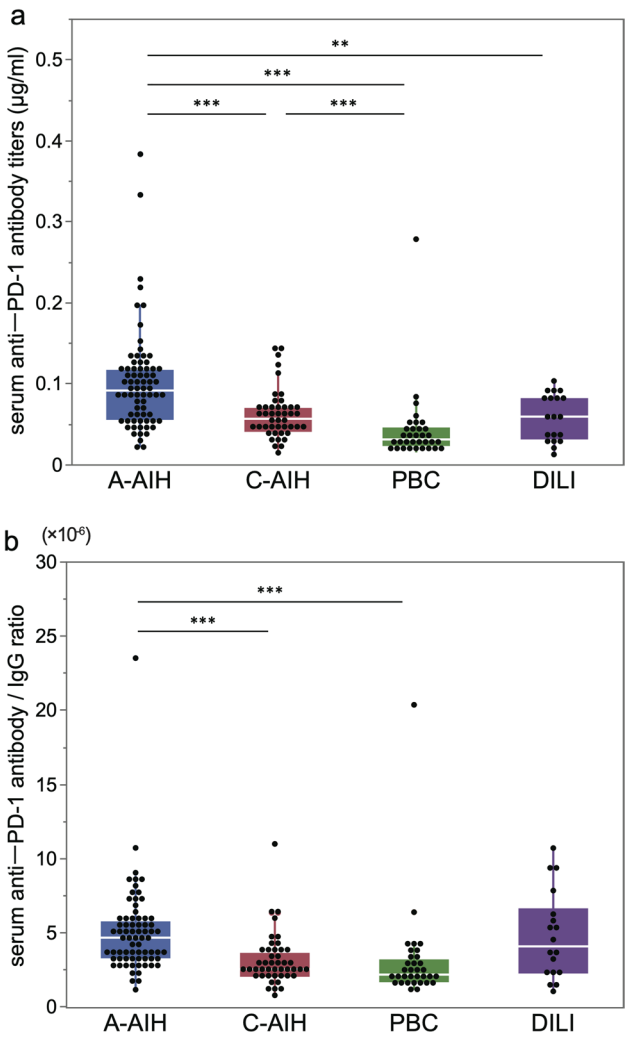


Fig. 1 Serum anti-PD-1 autoantibody levels and autoantibody/IgG levels in patients with A-AIH, C-AIH, PBC, and DILI. Serum anti-PD-1 autoantibody titers (**a**) and the autoantibody titer/IgG ($\times 10^{-6}$) levels (**b**) were measured in patients with *A-AIH* acute-onset autoimmune hepatitis, *C-AIH* chronic autoimmune hepatitis, *PBC* primary biliary cholangitis, and DILI (drug-induced liver injury). Box plots represent the interquartile range and the horizontal line indicates the median. Individual data points are indicated by black dots. P-values are indicated in the figure; ***$p < 0.001$

Factors distinguishing A-AIH, C-AIH, PBC, and DILI

Next, we examined the clinical factors that distinguished between A-AIH, C-AIH, PBC, and DILI (Table 2). Multivariate analysis demonstrated that higher T-Bil and serum anti-PD-1 autoantibody/IgG ratio were independently associated with A-AIH in comparison with C-AIH. To discriminate these two conditions, the optimal titer was 2.821 as determined from the ROC curve (sensitivity, 0.64; specificity, 0.86).

We further evaluated the factors that distinguished A-AIH from PBC. Multivariate analysis identified higher ALT and PT-INR levels as independent factors associated with A-AIH.

Next, we examined the clinical factors that distinguished C-AIH from PBC. Multivariate analysis demonstrated that higher T-Bil, ALT levels were independently associated with C-AIH.

Finally, we examined the clinical factors that distinguished DILI from the others. Although DILI and A-AIH share the common feature of acute-onset, there was a significant difference in the degree of ALT and T-Bil levels.

Correlation of anti-PD-1 autoantibody/IgG ratio and ANA

To clarify the difference between anti-PD-1 autoantibody/IgG and ANA, we next analyzed the relationship between their positivity in patients with A-AIH, C-AIH and PBC (Table 3). For this comparison, we defined the positive anti-PD-1 autoantibody/IgG ratio as > 2.821, which was calculated by the Youden index using data from patients with

Table 3 Correlation of anti-PD-1 autoantibody/IgG and ANA

	ANA < ×80		ANA ≥ ×80	
	Anti-PD-1 Negative	Anti-PD-1 Positive	Anti-PD-1 Negative	Anti-PD-1 Positive
A-AIH	6 (17.7%)	28 (82.4%)	4 (10.5%)	34 (89.5%)
C-AIH	12 (63.2%)	7 (36.8%)	16 (64%)	9 (36%)
PBC	12 (63.1%)	7 (36.8%)	11 (84.6%)	2 (15.4%)
		$p < 0.001$		$p < 0.001$

A-AIH and C-AIH, as mentioned above. Even in patients with low ANA titers, anti-PD-1 autoantibody/IgG ratio was positive in 82.4% of patients with A-AIH.

Liver histological findings in AIH

Given that anti-PD-1 autoantibody titers were correlated with liver parenchymal damage-related clinical features and were significantly different between the A-AIH and C-AIH groups, we next compared their histological features. Representative cases of these two entities are shown in Fig. 2. Compared to the C-AIH group ($n = 37$), the A-AIH group ($n = 14$) showed greater lobular inflammatory activity (Table 4). Specifically, perivenular necroinflammatory activity was more frequent and the lobular necrosis/inflammation score (excluding centrilobular necrosis (CN)) was higher in patients with A-AIH. Prominent plasma cells were more common in patients with A-AIH.

In the portal/periportal compartments, the portal plasma cell score was higher in the A-AIH group, whereas ductular reactions were more frequent in the C-AIH group. In contrast, no between-group differences were observed for the CN, which is a lesion thought to reflect hemodynamic disturbances in addition to inflammatory injury.

Correlation of liver histological findings and serum anti-PD-1 autoantibody titers/IgG ratio in AIH

Next, we examined the relationship between serum anti-PD-1 autoantibody titers, anti-PD-1 autoantibody/IgG ratio, and the histological features of AIH. Anti-PD-1 autoantibody titers were significantly associated with parenchymal inflammatory activity. Higher anti-PD-1 autoantibody titers were observed in patients with pronounced lobular necrosis/inflammation and prominent plasma cell infiltration, whereas no significant association was detected with CN ($p = 0.35$) (Fig. 3a). These findings indicate that anti-PD-1 does not simply reflect liver parenchymal damage, but instead reflects immune-mediated hepatocellular injury.

Next, we evaluated the histological features of the portal areas. Anti-PD-1 autoantibody titers were significantly higher in patients with marked portal inflammation, portal plasmacytosis, and interface hepatitis, whereas no significant differences were observed in patients with emperipolesis (Fig. 3b). Anti-PD-1 autoantibody/IgG ratio showed almost the same pattern, but the lobular necro-inflammatory activity showed a relatively weak difference (Fig. 3c, d).

We next evaluated the relationship between the degree and pattern of B-cell infiltration, although the sample size was small (n=20), and the study was conducted exclusively in patients with C-AIH (Supplementary Fig. 1). Although the B-cell infiltration pattern was not significantly correlated with the autoantibody levels, strong parenchymal B-cell infiltration was relatively correlated with high anti-PD-1 autoantibody titers. (Supplementary Table 3).

Collectively, these results suggest that serum anti-PD-1 autoantibodies specifically track the degree of intrahepatic acute-onset inflammatory response rather than secondary hemodynamic injury, although the sample size was small. Further investigations with a large number of biopsy samples are needed.

Discussion

In this study, we identified a distinct immunological feature of A-AIH, namely, a marked elevation in serum anti-PD-1 autoantibodies, which is closely associated with intrahepatic inflammatory activity.

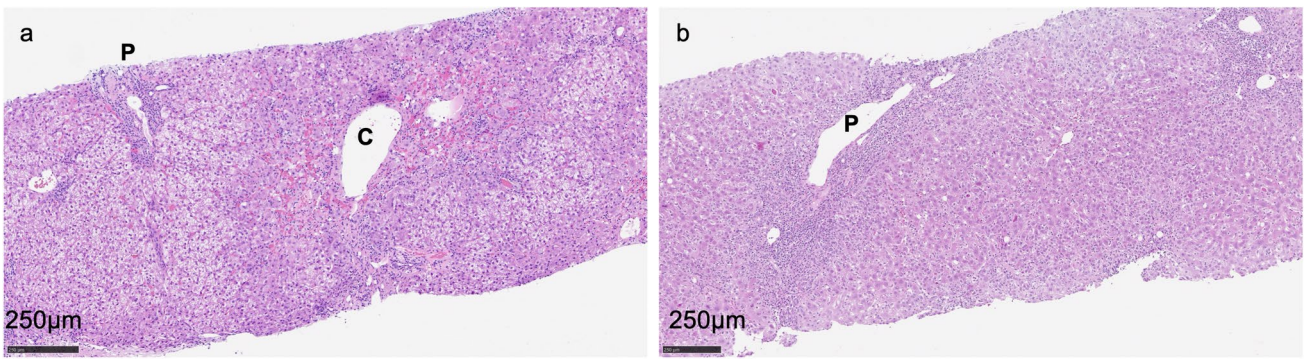


Fig. 2 Typical histological findings of A-AIH and C-AIH. **a** Hematoxylin and eosin (H&E) staining of a biopsy specimen obtained from patients with A-AIH. Centrilobular zonal necrosis surrounding the central vein (C), characterizing an acute presentation, is present. Mild inflammation was observed in the portal tract (P), but no distinct enlargement was observed. **b** H&E staining of a C-AIH biopsy specimen. The portal tract (P) shows cellular enlargement, severe inflammation, and interface hepatitis, indicating chronic hepatitis. Spotty necrosis and apoptosis were observed in the parenchyma

Table 4 Comparison of lobular and portal/periportal histopathological findings between acute-onset and chronic autoimmune hepatitis

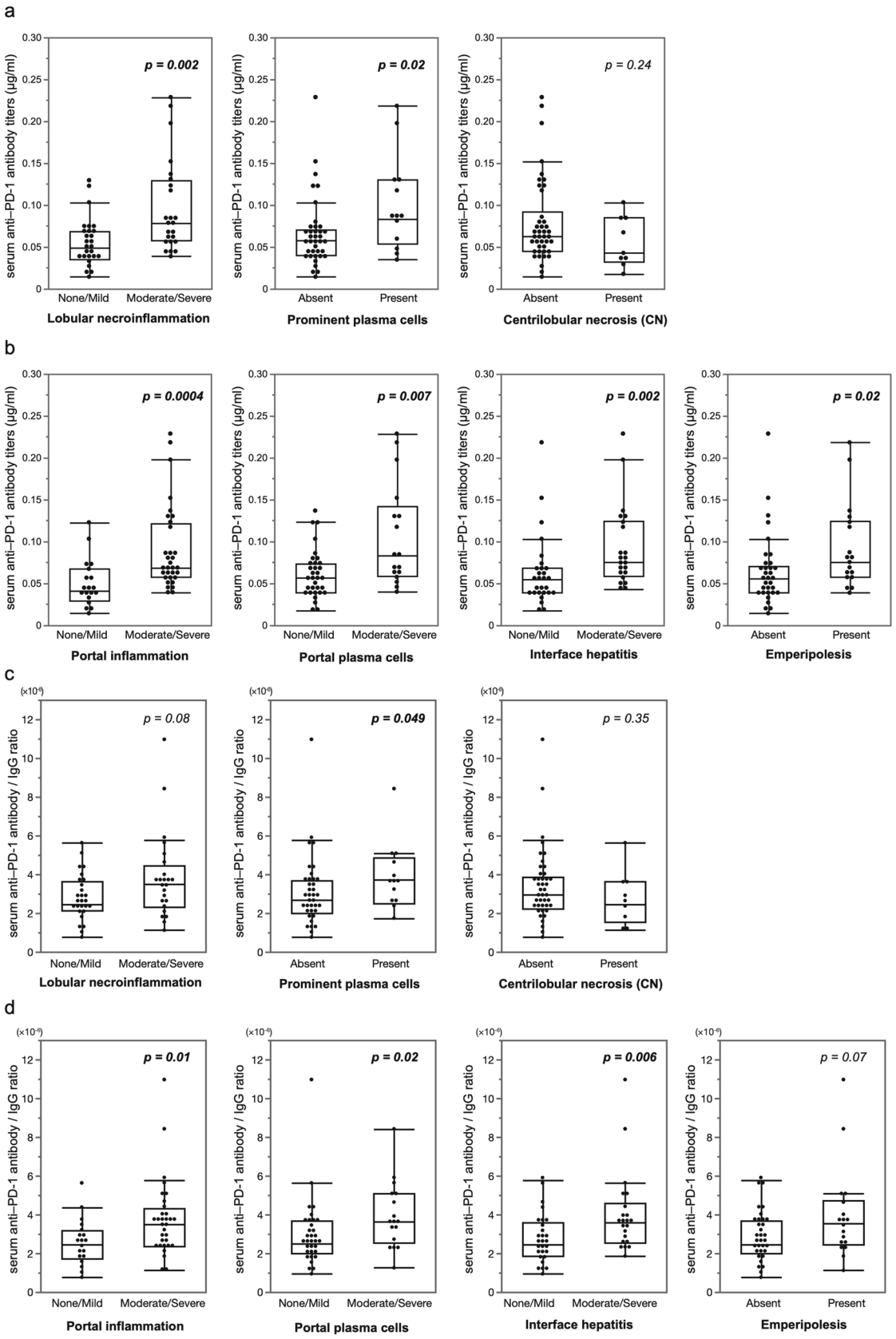
	A-AIH ($n = 14$)	C-AIH ($n = 37$)	p
Lobular changes			
Centrilobular necrosis (CN)	4 (28.6%)	5 (13.5%)	0.208
Perivenular necroinflammatory activity	11 (78.6%)	17 (46.0%)	**0.037**
Centrilobular fibrosis, 0/1/2/3	4/7/3/0	13/9/10/5	0.300
Lobular necrosis/inflammation (not including CN), 0/1/2/3	0/4/6/4	0/23/12/2	**0.029**
Necrosis type, bridging/spotty	4/10	1/36	**0.006**
Prominent plasma cells	9 (64.3%)	4 (10.8%)	**< 0.001**
Cobblestone appearance of hepatocytes, 0/1/2/3	8/1/3/2	27/6/3/1	0.185
Hepatic rosette formation in cobblestone appearance areas	3 (33.3%)	4 (13.3%)	0.170
Portal/periportal changes			
Portal inflammation, 0/1/2/3	0/3/8/3	0/16/17/4	0.303
Portal plasma cells, 0/1/2/3	0/4/9/1	8/21/7/0	**0.003**
Interface hepatitis, 0/1/2/3	0/6/5/3	2/20/10/4	0.558
Hepatic rosette formation in periportal areas	7 (50%)	10 (27.8%)	0.136
Portal fibrosis, F0/F1/F2/F3/F4	1/6/4/3/0	3/16/4/9/5	0.408
Bile duct injury	5 (35.7%)	11 (29.7%)	0.681
Ductular reaction	2 (14.3%)	17 (46.0%)	**0.037**
Cholestasis	0 (0%)	0 (0%)	–
Steatosis	0 (0%)	7 (18.9%)	0.080
Emperipolesis	6 (42.9%)	12 (32.4%)	0.487
Granulomas	0 (0%)	4 (10.8%)	0.200
Mitosis	0 (0%)	0 (0%)	–
Sinusoidal dilation, 0/1/2/3	14/0/0/0	33/4/0/0	0.200
Nodular transformation	0 (0%)	0 (0%)	–

All features were scored as follows: 0, none; 1, mild; 2, moderate; 3, severe. *AIH* autoimmune hepatitis, *CN* centrilobular necrosis

We found that the anti-PD-1 autoantibody titers were correlated with transaminase levels, PT-INR and histological inflammatory reactions. Antibody production may be related to hepatic parenchymal damage and, presumably, the resulting increase in the release of autoantigens. However, no association was observed with CN, which is a histological feature of inflammatory reactions and blood flow defects, suggesting that the anti-PD-1 autoantibodies reflect strong inflammation rather than simple parenchymal destruction. However, further analyses with a larger number of cases are required to confirm these findings. In addition, although the number of patients with DILI was small, they showed high anti-PD-1 autoantibody levels comparable to those seen in A-AIH. This suggests that the autoantibody levels are not specific to the autoimmune response, and that they correlate with severe inflammation associated with the immune response. These results indicate that anti-PD-1 autoantibodies can serve as biomarkers of a strong immune-related inflammatory response.

The mechanisms underlying the production of these inflammation-related autoantibodies remain unclear; however, previous studies have shown that serum autoantibodies against immune checkpoint molecules are found in patients with autoimmune diseases or cancer and that their titers reflect disease severity and clinical courses, in agreement with our findings [11, 12, 19]. Various mechanisms have been proposed to explain the autoantibody production. One such mechanism is increased expression or release of autoantigens. A soluble form of PD-1 can be generated by alternative splicing or proteolytic cleavage and has been shown to be correlated with the active disease state of AIH [20]. PD-1-positive memory helper CD4 T cells, which show autoreactivity, have also been reported to be increased in AIH [21]. Upregulation of PD-1 may induce autoreactivity against it.

It is also important to determine how antibody responses arise following PD-1 upregulation. Recent single-cell transcriptomic and T-cell receptor analyses of circulating self-antigen-specific CD4+ T cells from patients with autoimmune liver disease showed that autoreactive CD4+ T cells have a distinct B-cell helper transcriptional profile characterized by PD-1 expression [22]. Impaired PD-1 signaling has been implicated in the unrestrained activation of autoreactive T cells and the expansion of T follicular helper (Tfh) cells, promoting autoantibody production [23]. Tfh cells are specialized subpopulations primarily located within


a
serum anti–PD-1 antibody titers (μg/ml)
p = 0.002
None/Mild
Moderate/Severe
Lobular necroinflammation
p = 0.02
Absent
Present
Prominent plasma cells
p = 0.24
Absent
Present
Centrilobular necrosis (CN)
b
p = 0.0004
Portal inflammation
p = 0.007
Portal plasma cells
p = 0.002
Interface hepatitis
p = 0.02
Emperipolesis
c
serum anti–PD-1 antibody / IgG ratio
(×10⁻⁶)
p = 0.08
Lobular necroinflammation
p = 0.049
Prominent plasma cells
p = 0.35
Centrilobular necrosis (CN)
d
p = 0.01
Portal inflammation
p = 0.02
Portal plasma cells
p = 0.006
Interface hepatitis
p = 0.07
Emperipolesis

◂**Fig. 3** Relationship between serum anti-PD-1 autoantibody titers, anti-PD-1 autoantibody titers/IgG ratios and histological features of AIH Serum anti-PD-1 autoantibody titers were significantly elevated in patients exhibiting pronounced intrahepatic inflammatory activity. **a** Comparison of serum anti-PD-1 autoantibody titers between patients with none/mild and moderate/severe lobular necrosis/inflammation ($p = 0.002$). Serum anti-PD-1 autoantibody titers were stratified according to the presence of prominent plasma cell infiltration ($p = 0.02$). No significant differences were observed in the presence of centrilobular necrosis (CN) ($p = 0.24$). **b** Serum anti-PD-1 autoantibody titers were significantly higher in patients with moderate or severe portal inflammation ($p = 0.0004$). Serum anti-PD-1 autoantibody titers were significantly higher in patients with moderate or severe portal plasma cell infiltration ($p = 0.007$). Serum anti-PD-1 autoantibody titers were significantly higher in patients with moderate or severe interface hepatitis ($p = 0.002$). Serum anti-PD-1 autoantibody titers were significantly higher in patients with the presence of emperipolesis ($p = 0.02$). **c** Serum anti-PD-1 autoantibody titers/IgG levels tended to be higher in patients with moderate/severe lobular necrosis/inflammation ($p = 0.08$). Serum anti-PD-1 autoantibody titers/IgG levels were significantly higher in patients with prominent plasma cell infiltration ($p = 0.049$). No significant differences were observed in the presence of centrilobular necrosis (CN) ($p = 0.35$). **d** Serum anti-PD-1 autoantibody titers/IgG levels were significantly higher in patients with moderate/severe portal inflammation ($p = 0.01$). Serum anti-PD-1 autoantibody titers/IgG levels were significantly higher in patients with moderate/severe portal plasma cell infiltration ($p = 0.02$) Serum anti-PD-1 autoantibody titers/IgG levels were significantly higher in patients with moderate/severe interface hepatitis ($p = 0.006$) Serum anti-PD-1 autoantibody titers/IgG levels tended to be higher in patients with the presence of emperipolesis ($p = 0.07$) Data are shown as box-and-whisker plots indicating the median, interquartile range, and full range. Significant p values are shown in bold

germinal centers and play a crucial role in mediating the differentiation of B cells into autoantibody-producing cells [24]. Indeed, animal models with disrupted PD-1 signaling develop severe AIH with Tfh-driven germinal center reactions [25]. However, whether the PD-1 signaling pathway is downregulated in the patient lymphocytes was not examined in the present study and warrants further investigation.

In terms of whether the autoantibodies detected in our assay were functional, our findings suggest that endogenous anti-PD-1 autoantibodies represent a mechanism of functional PD-1 inhibition, resulting in immune activation in A-AIH and probably DILI. A recent comprehensive study on plasma autoantibodies showed that some antibodies can impair the function of their target antigens [26]. The anti-PD-1 autoantibodies may attenuate PD-1 signaling, leading to enhanced effector T-cell activation and amplification of intrahepatic inflammatory cascades. Such amplification of intrahepatic immune responses can trigger acute disease flares, particularly in individuals with an underlying autoimmune predisposition as well as in cases of DILI.

From a clinical perspective, measurement of anti-PD-1 autoantibodies has several potential applications. In agreement with previous reports, our present analysis indicates that a definitive diagnosis of AIH in A-AIH is very difficult. One previous report compared the serological characteristics of patients with low simplified AIH score (31%) and those with high score patients (69%) in A-AIH [27]. The low-scoring group had low IgG, ANA, and anti-SMA antibody levels. Patients with low scores were difficult to diagnose with A-AIH. A Japanese nationwide survey also indicated that ANA < 80 was present in approximately one-third of A-AIH patients. The low diagnostic performance of the IAIHG criteria in our analysis is partly because testing for autoantibodies other than ANA is not covered by health insurance in Japan, and no data were available for autoantibodies. It is unclear why these patients develop AIH in the liver. The present analysis showed that the anti-PD-1 antibody/IgG ratios were significantly elevated in A-AIH, even in ANA-low patients (82.4% positive), which contributed to the discrimination between A-AIH and C-AIH.

Our present analysis revealed that the histological findings and anti-PD-1 autoantibody titers correlated with previous findings; thus, we concluded that the antibody reflects the histological features that are characteristic of A-AIH [16]. Although anti-PD-1 autoantibodies did not show independent significance in distinguishing AIH from PBC, this was likely due to the significant differences in biliary enzymes or transaminase levels. In particular, anti-mitochondrial antibodies are powerful biomarkers for distinguishing AIH from PBC, whereas anti-PD-1 autoantibodies are less useful. Anti-PD-1 autoantibodies are particularly useful in cases of atypical AIH, including in patients with relatively low IgG levels or conventional autoantibodies, where diagnostic uncertainty often arises.

This study had several limitations. The most important limitation was the absence of functional assays, which prevented us from concluding whether anti-PD-1 antibodies exert direct biological effects on PD-1 signaling, resulting in the lymphocytes functions. Second, the multicenter retrospective observational study design introduced a potential selection bias, and the sample size limited generalizability. The diagnosis of A-AIH relies partly on the empirical judgment of expert hepatologists, raising the possibility of misclassification. Additionally, there are no longitudinal data on the dynamic changes with treatment, prediction of flares, or outcomes, such as liver failure or transplant-free survival. The duration of serum collection was long, and the number of serum freeze–thaw cycles was heterogeneous. Moreover, histological analysis was performed in only one part of the cohort, particularly for A-AIH, with only 14 patients included. Finally, the assay used for anti-PD-1 measurement has not been standardized, and external validation in independent cohorts is lacking, although a small number of DILI cases were included. Prospective multicenter validation and longitudinal monitoring with a large number of patients, including those with other acute and chronic liver diseases, are essential for establishing its clinical utility.

In conclusion, serum anti-PD-1 autoantibodies are elevated in A-AIH and are associated with intrahepatic inflammatory activity. Measurement of anti-PD-1 autoantibodies may improve the diagnosis of A-AIH and provide a potential biomarker reflecting acute-onset liver inflammation-related activity. Further prospective and mechanistic studies are required to clarify their pathogenic and clinical significance.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12072-026-11152-x.

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Author contributions Akinobu Takaki designed and planned this study. Takahi Oda, Akinobu Takaki, Kazuyuki Matsumoto, Nozomi Miyake, Masahiko Sue, Takuya Adachi, Yasuto Takeuchi and Hideki Onishi conducted the experiments. Akinobu Takaki, Kenichi Harada, Teruko Arinaga-Hino, Masanori Abe, Takeshi Matsui, Atsushi Takahashi, Hiromasa Ohira, Yoshiyuki Suzuki, M. Nakamoto, and Atsushi Tanaka acquired data. Takahi Oda, Akinobu Takaki, and Motoyuki Otsuka analyzed the data and wrote the manuscript.

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Data availability The data presented in this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy concern.

Declarations

Conflict of interest The authors declare no conflict of interest.

Informed consent in studies with human subjects All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients included in the study.

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References

1. Czaja AJ. Autoimmune hepatitis: evolving concepts and treatment strategies. Dig Dis Sci. 1995;40:435–456
2. Czaja AJ, Carpenter HA. Sensitivity, specificity, and predictability of biopsy interpretations in chronic hepatitis. Gastroenterology. 1993;105:1824–1832
3. Mack CL, Adams D, Assis DN, Kerkar N, Manns MP, Mayo MJ, et al. Diagnosis and management of autoimmune hepatitis in adults and children: 2019 practice guidance and guidelines from the American Association for the Study of Liver Diseases. Hepatology. 2019;72:671–722
4. Takahashi A, Ohira H, Abe K, Zeniya M, Abe M, Arinaga-Hino T, et al. Increasing incidence of acute autoimmune hepatitis: a nationwide survey in Japan. Sci Rep. 2020;10: 14250
5. Fujiwara K, Yasui S, Yokosuka O. Autoimmune acute liver failure: an emerging etiology for intractable acute liver failure. Hepatol Int. 2013;7:335–346
6. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol. 2007;19:813–824
7. Peeraphatdit TB, Wang J, Odenwald MA, Hu S, Hart J, Charlton MR. Hepatotoxicity from immune checkpoint inhibitors: a systematic review and management recommendation. Hepatology. 2020;72:315–329
8. De Martin E, Michot JM, Papouin B, Champiat S, Mateus C, Lambotte O, et al. Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. J Hepatol. 2018;68:1181–1190
9. Shi H, Ye J, Teng J, Yin Y, Hu Q, Wu X, et al. Elevated serum autoantibodies against co-inhibitory PD-1 facilitate T cell proliferation and correlate with disease activity in new-onset systemic lupus erythematosus patients. Arthritis Res Ther. 2017;19: 52
10. Zamani MR, Aslani S, Salmaninejad A, Javan MR, Rezaei N. PD-1/PD-L and autoimmunity: a growing relationship. Cell Immunol. 2016;310:27–41
11. Matsumoto K, Miyake Y, Matsushita H, Ohnishi A, Ikeda F, Shiraha H, et al. Anti-programmed cell death-1 antibody as a new serological marker for type 1 autoimmune hepatitis. J Gastroenterol Hepatol. 2014;29:110–115
12. Miyake Y, Yamamoto K, Matsushita H, Abe M, Takahashi A, Umemura T, et al. Multicenter validation study of anti-programmed cell death-1 antibody as a serological marker for type 1 autoimmune hepatitis. Hepatol Res. 2014;44:1299–1307
13. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol. 1999;31:929–938
14. Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008;48:169–176
15. Onji M, Zeniya M, Yamamoto K, Tsubouchi H. Autoimmune hepatitis: diagnosis and treatment guide in Japan, 2013. Hepatol Res. 2013;44:368–370
16. Nguyen Canh H, Harada K, Ouchi H, Sato Y, Tsuneyama K, Kage M, et al. Acute presentation of autoimmune hepatitis: a multicentre study with detailed histological evaluation in a large cohort of patients. J Clin Pathol. 2017;70:961–969
17. Matsumoto K, Ohara T, Fujisawa M, Takaki A, Takahara M, Tanaka N, et al. The relationship between PD-L1 expression of surgically resected and fine-needle aspiration specimens in patients with pancreatic cancer. J Gastroenterol. 2019;2019(54):1019–1028
18. Sasaki Y, Matsumoto K, Takaki A, Adachi T, Takahara M, Ozato K, et al. Anti-PD-1 autoantibody predicts survival of patients with

hepatocellular carcinoma receiving atezolizumab/bevacizumab. Gastro Hep Advances. 2024;3:1138–1147
19. Tan Q, Wang D, Yang J, Xing P, Yang S, Li Y, et al. Autoantibody profiling identifies predictive biomarkers of response to anti-PD1 therapy in cancer patients. Theranostics. 2020;10:6399–6410
20. Hadley T, Gillespie S, Espinoza H, Prince J, Gronbaek H, Chandrakasan S, et al. Soluble PD-1 levels are increased with disease activity in paediatric-onset autoimmune hepatitis and inflammatory bowel disease. Autoimmunity. 2020;53:253–260
21. Renand A, Cervera-Marzal I, Gil L, Dong C, Garcia A, Kervagoret E, et al. Integrative molecular profiling of autoreactive CD4 T cells in autoimmune hepatitis. J Hepatol. 2020;73:1379–1390
22. Cardon A, Guinebretiere T, Dong C, Gil L, Ado S, Gavlovsky PJ, et al. Single-cell profiling of circulating autoreactive CD T cells from patients with autoimmune liver diseases suggests tissue imprinting. J Nat Commun. 2026;16:1161
23. Sagrero-Fabela N, Chavez-Mireles R, Salazar-Camarena DC, Palafox-Sanchez CA. Exploring the role of PD-1 in the autoimmune response: insights into its implication in systemic lupus erythematosus. Int J Mol Sci. 2024;25: 7726
24. Li L, Xu P, Zhou Q, Xu J. The function of T follicular helper cells in autoimmune liver diseases. J Immunol Res. 2020;2020:5679254
25. Aoki N, Kido M, Iwamoto S, Nishiura H, Maruoka R, Tanaka J, et al. Dysregulated generation of follicular helper T cells in the spleen triggers fatal autoimmune hepatitis in mice. Gastroenterology. 2011;140:1322-1333.e5
26. Dai Y, Aizenbud L, Qin K, Austin M, Jaycox JR, Cunningham J, et al. Humoral determinants of checkpoint immunotherapy. Nature. 2025;644:527–536
27. Muratori P, Granito A, Lenzi M, et al. Limitation of the simplified scoring system for the diagnosis of autoimmune hepatitis with acute onset. Liver Int. 2021;41:529–534

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