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Usefulness of liver infiltrating CD86-positive mononuclear cells for diagnosis of autoimmune hepatitis

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Abstract

AIM: Although the pathogenic mechanism underlying autoimmune hepatitis (AIH) remains unclear, the immune system is thought to be critical for the progression of the disease. Cellular immune responses may be linked to the hepatocellular damage in AIH. Recently, much attention has been focused on the critical functions of costimulatory molecules expressed on mononuclear cells in the generation of effective T cell-mediated immune responses. Analysis of costimulatory molecule expressed on mononuclear cells from the patients with AIH may give us insight into the pathogenic mechanism of hepatocellular damage in AIH.

METHODS: Peripheral blood mononuclear cells (PBMC) were taken from the patients with AIH (34 cases) and healthy controls (25 cases). Liver infiltrating mononuclear cells (LIMCs) were taken from the patients with AIH (18 cases), the patient with chronic hepatitis C (CH-C) (13 cases) and the patients with fatty liver (2 cases). Using flow cytometry, the cells were analyzed for the expression of costimulatory molecules, such as CD80, CD86, and CD152 (CTLA-4). The results were compared with clinical data such as the level of gammaglobulin, histological grade, presence or absence of corticosteroids administration and the response to corticosteroids.

RESULTS: The levels of CD80+, CD86+ and CD152+ PBMC were significantly reduced in the patients with AIH as compared with healthy controls. By contrast, those cells were significantly higher in LIMC than in PBMC of the patients with AIH. Especially, the level of CD86+ LIMC showed a marked increase irrespective of the degree of disease activity in the patients with AIH, although CD86+ cells were rarely present in PBMC. The levels of CD86+ cells were present in significantly higher frequency in patients with AIH than in the patients with CH-C. Furthermore, the patients with AIH with high levels of CD86+ LIMC showed good responses to corticosteroids, whereas 2 cases of AIH with low levels of CD86+ LIMC did not respond well.

CONCLUSION: These results suggest that LIMC over-expressing costimulatory molecules such as CD80 and CD86 appears to play a role in the pathogenesis of AIH. Especially, CD86 molecule expressed on the LIMC may be useful for the diagnosis of AIH and for the prediction of the therapeutic effects of corticosteroids on AIH.

Key words: Autoimmune hepatitis; Costimulatory molecule; CD86 molecule; Peripheral blood mononuclear cells; Liver infiltrating mononuclear cells; Flow cytometry

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INTRODUCTION

In autoimmune liver diseases including autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), autoimmune mechanisms are thought to affect the disease progression and manifestation. Although the mechanism of hepatocellular damage in AIH and PBC is still unclear, it is speculated that the immunological disorder is linked to host immunological targeting of hepatocytes in the liver[1-3]. It has been recently reported that polymorphism of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and the susceptibility to AIH and PBC were associated[4]. Among T cell-mediated immune responses, especially those mediated by CD4+ or CD8+ T cells, are likely to be associated with the hepatocellular damage in AIH[5,6]. We have previously reported that the expression of bcl-2, an anti-apoptotic molecule, in CD4+ Th1 cells was increased in peripheral blood and in the liver of the patients with

AIH[7]. Moreover, it was reported that T cells bearing certain T cell receptor clonotypes were expanded in the liver of the patients with AIH[8]. Although extensive efforts have been made to identify the target antigens expressed on the hepatocyes in AIH, the real target antigen has not been identified yet.

For the generation of effective T cell-mediated immune responses, much attention has been paid on the critical functions of a series of costimulatory molecules expressed on the cell surface of T or B cells. The T cell receives signals from antigen-presenting cells (APC) through the interaction of the T cell receptor (TCR) and major histocompatibility complex (MHC) in an antigen-dependent manner. However, these signals alone are insufficient to generate effective T cell-mediated immune responses and often lead to T cell anergy. The aberrant expression of costimulatory molecules was reported in some liver diseases other than AIH[9-14]. Although it is clear that the costimulatory molecules play a crucial role in T cell activation, few reports have addressed the role of these costimulatory molecules in AIH. In the present study, to elucidate the role of costimulatory molecules on mononuclear cells in AIH, some of the costimulatory molecules expressed on PBMC and LIMC were analyzed using flow cytometry and the results were evaluated in terms of the clinical status of the patients with AIH.

MATERIALS AND METHODS

Patients

Thirty-four patients with AIH serologically and histologically diagnosed at Kagawa University Hospital were enrolled in the present study. All patients had anti-nuclear antibody (ANA) in their sera and 19 of 34 (55.88%) patients showed hyper-gammaglobulinemia (> 2.0 g/dL). Based on the criteria proposed by International Autoimmune Hepatitis Group, all patients satisfied the score over probable AIH. PBMCs were analyzed in 34 patients with AIH. LIMCs were also analyzed in 18 of 34 patients at the liver biopsy for diagnosis. As a disease control, PBMC and LIMC of thirteen patients with chronic hepatitis C (CH-C) were analyzed. Twenty-five healthy individuals without any symptoms of liver injury were selected as normal controls for the analysis of PBMC. All of these studies were conducted with informed consent at the time of the enrollment for this study in all patients.

Flow cytometric analysis of PBMC

PBMCs from patients and healthy individuals were separated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation (Histopaque; Sigma Chemical Co., St Lois, MO). Immediately after separating PBMC, cells were washed twice in ice-cold phosphate-buffered saline (PBS), and adjusted to 4×10^5 cells per well of a 96-well U-bottom cell culture plate in flow microfluorometry medium [FMF medium: Hanks' balanced salt solution (without Ca, Mg and phenol red) containing 2 g/L bovine serum albumin, and 1 g/L sodium azide]. After centrifugation at 1200 r/min for 5 min, the supernatant was removed and cells were incubated for 30 min with FITC-conjugated or phycoerythrin (PE)-conjugated antibodies. The combinations of antibodies used were anti-CD80-FITC (Pharmingen, San Diego, USA)/anti-CD152-PE (Pharmingen), anti-CD86-FITC (Pharmingen). FITC-conjugated IgG (Dainippon Pharmaceutical Co., Ltd. Osaka, Japan) and PE-conjugated IgG (Dako A/G, Denmark) were used as the negative controls. After being washed twice, the pellets were resuspended with FMF medium, and the fluorescence detections were performed with a flow cytometer, COULTER EPICS XL. Prior to the analysis, FL1, FL2 and color compensation were adjusted so that no CD4CD8 double-positive cells were detected. The gate was set for accumulation of PBMC and ten thousand events were acquired for each analysis. Flow cytometric analysis was done immediately after isolating PBMC.

Flow cytometric analysis of LIMC

Liver specimens from the 18 patients with AIH, 10 patients with CH-C and 2 patients with fatty liver were obtained using a 16 G biopsy needle for diagnosis. Most of the patients with AIH underwent laparoscopy to obtain the liver specimen and observe the change of the liver surface for diagnosis. After incubating the liver biopsy specimen in RPMI-1640 containing 1 g/L collagenase for 2 h to destroy the connective tissue, LIMCs were separated by Ficoll-Hypaque density gradient centrifugation at 3000 r/min for 10 min. Isolated LIMCs were stained with FITC- or PE-conjugated antibodies and analyzed using flow cytometry. The cells positive for CD69, a marker for activated T cells, were also analyzed in LIMC. An anti-CD69-PE (Dako Japan A/G) was used. Three thousand events were acquired for each analysis.

Clinical markers

Levels of alanine aminotransferase (ALT) (IU/L) and gammaglobulin (g/dL) and titer of ANA were monitored. These laboratory data were compared with the level of LIMC positive for costimulatory molecules obtained in the present study. Inflammation and fibrosis in histological analysis was graded according to the classification documented by Knodell *et al*[15]. Histological staging and grading were classified based on the classification documented by Desmet *et al*[16].

Statistical analysis

Statistical analysis was performed using Macintosh software, Statview II (version 4.2) and a Mann-Whitney *U* test (non-parametric analysis). $P < 0.05$ was considered statistically significant.

RESULTS

Flow cytometric analysis of PBMC

For the analysis of costimulatory molecules, we focused on several costimulatory molecules critical for the induction of effective T and B cell-mediated immune responses. Initially, we examined the expression of those molecules on PBMC in patients with AIH. The level of positive cells for each surface molecule on PBMC was compared between the patients and healthy controls (Table 1). The results revealed that the patients with AIH had significantly

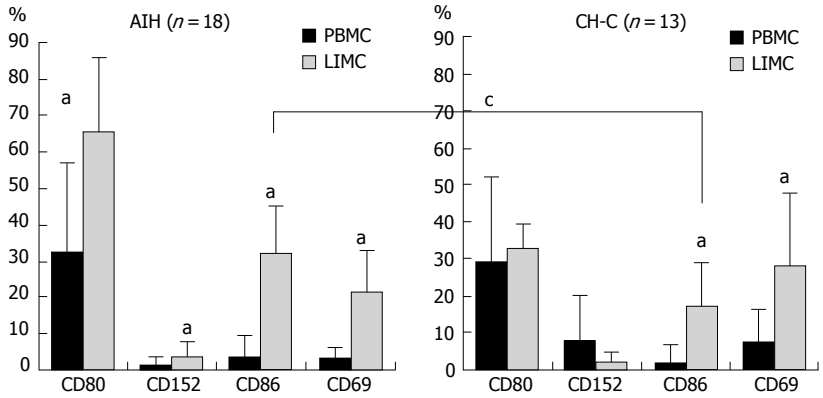

Figure 1 Isolation of PBMCs and LIMCs from peripheral blood and liver biopsy specimens in the patients with AIH and CH-C by using density gradient centrifugation. The cells were stained with anti-CD80, anti-CD86, anti-CD152 and anti-CD95 antibodies and analyzed using flow cytometry. The percentage of cells positive for those costimulatory molecules is shown. CD80+, CD86+ and CD152+ cells were significantly higher in LIMCs compared to PBMC in patients with AIH. The difference of CD86+ cells between LIMCs and PBMCs was more striking than that of CD80+ cells between LIMCs and PBMCs. (a) represents the significant difference ($P < 0.05$) from the data of PBMCs and (c) shows the significant difference ($P < 0.05$) of LIMCs between AIH and CH-C.

Table 1 Analysis of costimulatory molecules expressed on PBMCs (mean ± SD)

	Control n = 25	AIH Patients n = 34	P value
CD80	41.3 % ± 7.04 %	32.7 % ± 23.5 %	0.008
CD86	1.15 % ± 1.19 %	0.78 % ± 0.75 %	0.04
CD152 CTLA-4	13.8 % ± 10.9 %	2.32 % ± 2.80 %	0.01

The P values calculated by a Mann-Whitney's U-test are shown. Compared to the healthy controls, CD80+, CD86+ and CD152+ PBMC of the patients with AIH are shown to be significantly fewer.

fewer CD80+, CD86+ and CD152+ (CTLA-4+) cells in PBMC as compared with the healthy controls. Among the cells analyzed, CD86+ cells were present in low frequency in PBMC of both the patients and healthy controls.

Flow cytometric analysis of LIMCs

CD80 and CD86 are expressed on professional APC and activated lymphocytes, and have unique expression pattern[17]. In the patients with AIH, the predominant location of inflammation and damage is seen in the liver. Therefore, it might be reasonable to think that those decreased cells in the blood, such as CD80+, CD86+ or CD152+ cells, accumulate in the liver and are likely to be important to analyze the expression of costimulatory molecules on LIMCs as well as on PBMCs. Although it may be necessary to compare the expression of costimulatory molecules on LIMCs between the patients and healthy controls, it is ethically difficult to take mononuclear cells from the liver of healthy controls. Therefore, the frequency of LIMC positive for each costimulatory molecule was compared between PBMC and LIMC in patients with AIH and CH-C in the present study (Figure 1). In both AIH and CH-C, the ratios of CD69+ cells in LIMCs were significantly higher than that in PBMCs, suggesting infiltration of many activated T cells into the liver in both AIH and CH-C. In AIH, the ratios of CD80+ and CD86+ cells were significantly higher in LIMCs compared to PBMCs. The difference of CD86+ cells between LIMCs and PBMCs was more striking than that of CD80+ cells between LIMCs and PBMCs. Although the ratio of CD152+ cells was significantly higher in LIMCs than that in PBMCs, both the ratios were very low. In CH-C, the ratio of CD86+ cells was significantly higher in LIMCs than that in PBMC, but the ratios of CD80+ and CD152+ cells in LIMC were not significantly different from those in PBMC. Although the ratios of CD86+ cells were significantly higher both in AIH and in CH-C, the ratio of CD86+ cells in LIMCs was significantly higher in AIH than that in CH-C. Taken collectively, the most dramatic and apparent difference between PBMCs and LIMCs was the marked increase of CD86+ cells in LIMCs of AIH. By contrast, the levels of CD86+ cells in LIMCs of patients with fatty liver were low (Table 2).

Relationship between the level of CD86+ LIMCs and clinical markers

Since the most dramatic change of frequency between PBMCs and LIMCs was the increase of CD86+ cells in patients with AIH, relationship between the level of CD86+ LIMC and the clinical parameters was examined in patients with AIH (Table 2). In most of the patients with AIH tested, the levels of CD86+ LIMC were elevated by more than 20%. Three patients (No. 1-3 in Table 2) were already being treated with corticosteroids when the biopsy was performed. Even in these 3 patients, the aberrant expression of CD86 molecule on LIMCs was observed. The level of CD80+ or CD86+ LIMC did not show any significant correlation with that of ALT [correlation coefficient (r): CD80 *vs* ALT = -0.160; CD86 *vs* ALT = -0.166]. Furthermore, no significant correlation of the level of CD86+ LIMC with the level of serum gammaglobulin, ANA titer and HAI score was observed. These results suggested that LIMCs in patients with AIH are over-expressing CD80 or CD86 molecule irrespective of the degree of hepatocellular damage or disease activity. Among 18 patients analyzed for the expression of costimulatory molecule on LIMCs, clinical course after the administration of corticosteroids could be followed up in 10 patients. Administration of corticosteroids was effective in decreasing the level of transaminase in 8 of 10 patients. All of these 8 patients showed the high levels of CD86+ LIMC (> 20%). By contrast, 2 patients who did not respond satisfactorily to corticosteroids showed low levels of CD86+ LIMC (11.1% and 5.9%, respectively).

Representative AIH cases reactive and non-reactive to corticosteroids

Clinical course, laparoscopic appearance of the liver surface and liver histology of two AIH cases reactive and non-reactive to corticosteroids are shown in Figures 2 and 3. The

Table 2 Analysis of CD86+ cells in liver infiltrating mononuclear cells

	γ -globulin (g/dL)	ALT (U/L)	ANA	Histology	HAI	CD86 (%)	CS	Reactivity to CS
No. 1	2.1	34	×40	CH (A0, F2)	9	44.0	On	Reactive
No. 2	1.1	55	×8	CH (A0, F1)	3	43.5	On	Reactive
No. 3	1.6	44	×20	CH (A2, F3)	13	27.5	On	NT
No. 4	2.0	151	×160	CH (A3, F3)	16	42.2	Off	Reactive
No. 5	2.0	99	×40	CH (A0, F1)	3	43.7	Off	NT
No. 6	2.0	105	×40	CH (A2, F2)	3	33.8	Off	NT
No. 7	2.3	71	×640	CH (A1, F1)	5	41.2	Off	NT
No. 8	2.4	18	×320	CH (A1, F2)	2	34.0	Off	NT
No. 9	1.6	50	×320	LC (A1, F4)	18	19.1	Off	NT
No. 10	2.7	84	×1280	CH (A2, F2)	14	11.1	Off	Non-reactive
No. 11	1.3	131	×5120	CH (A2, F2)	14	28.9	Off	Reactive
No. 12	2.4	93	×640	CH (A2, F2)	12	40.0	Off	Reactive
No. 13	1.7	363	×2560	CH (A2, F2)	15	27.3	Off	Reactive
No. 14	1.4	123	×40	CH (A1, F1)	2	5.9	Off	Non-reactive
No. 15	1.4	50	×20	LC (A3, F4)	18	25.7	Off	NT
No. 16	2.1	34	×20	CH (A2, F2)	12	29.4	Off	NT
No. 17	2.3	65	×40	LC (A2, F4)	20	24.1	Off	Reactive
No. 18	1.9	17	×1280	CH (A2, F3)	12	23.0	Off	Reactive
No. 19	1.2	45	(-)	Fatty liver	NT	11.8	Off	NT
No. 20	1.3	47	(-)	Fatty liver	NT	10.7	Off	NT

Comparison of the level of CD86+ LIMC and clinical data in patients with AIH (18 cases) and fatty liver (2 cases). LIMCs taken from the liver biopsy specimen of patients with AIH (18 cases) and 2 patients with fatty liver were stained with antibody against CD86 and analyzed by using flow cytometry. Three thousand events were acquired for each analysis. Patients with AIH showed high levels of CD86+ LIMC irrespective of the levels of γ-globulin, ALT, histological activity, and the presence or absence of the administration of corticosteroids (CS). Response to CS in patients with AIH was shown to be associated with the level of CD86+ LIMC. NT: not tested.

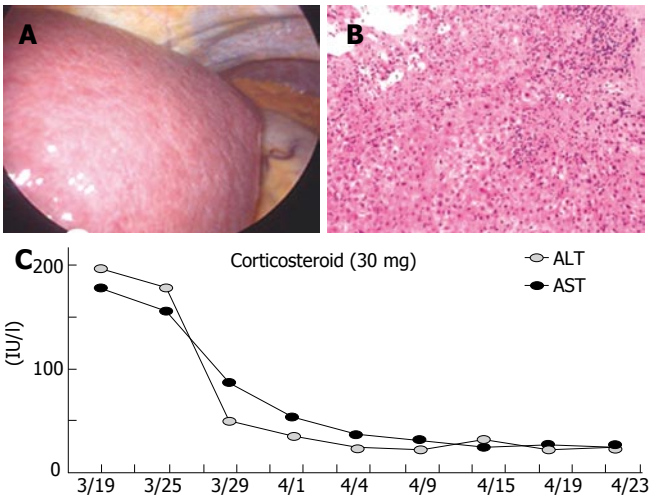

Figure 2 Laparoscopic findings, liver biopsy and clinical course after the administration of corticosteroids of a patient with AIH reactive to corticosteroids. **A:** Laparoscopic findings showing many reddish markings on the liver surface, indicating that the ongoing hepatitis was active; **B:** microscopic observation of the liver showing formation of rosette and bridging necrosis, infiltration of a large number of lymphocytes and plasma cells; and **C:** time-course of the levels of ALT and AST after the administration of corticosteroids. The levels of ALT and AST were rapidly decreased to normal ones after administration of corticosteroids.

former case was a 58-year-old female (No. 11 in Table 2) who showed a good response to the treatment of corticosteroids (Figure 2). At the time of diagnosis, she had ALT 131 IU/dL, AST 139 IU/dL, T-Bil 1.1 mg/dL, ALB 2.9 g/dL, IgG 2730 mg/dL and ANA ×5120. Laparoscopic findings of the liver showed many reddish markings on the surface of the liver, indicating that the ongoing hepatitis was active (Figure 2A). Histological findings were compatible to AIH showing the formation of rosette and bridging necrosis, infiltration of a large number of lymphocytes and many plasma cells (Figure 2B). The level of CD86+ LIMC was high, 28.9 %. The levels of ALT and AST were rapidly decreased to normal ones after the administration of corticosteroids (Figure 2C). By contrast, the latter case was a 59-year-old female (No. 10 in Table 2) who was not reactive to the treatment of corticosteroids (Figure 3). At the time of diagnosis, she had ALT 50 IU/dL, AST 29 IU/dL, T-Bil 1.9 mg/dL, ALB 2.9 g/dL, IgG 2880 mg/dL, and ANA ×1280. Laparoscopic findings of the liver showed many reddish markings and small lymph cysts on the surface of the liver (Figure 3A). Histological findings revealed the bridging necrosis, infiltration of a large number of lymphocytes and plasma cells (Figure 3B). The level of CD86+ LIMC was relatively low, 11%. Although the levels of ALT and AST were slightly improved after the administration of corticosteroids, those were elevated again despite the treatment.

As aforementioned, these 2 cases showed similar aspects of laboratory data, laparoscopic findings and histological findings. Nevertheless, one with a high level of CD86+ LIMC was responsive to corticosteroids, and the other with a low level of CD86+ LIMC was not. In these 2 cases, the differences were seen in the response to corticosteroids and in the level of CD86+ LIMC, but not in other clinical markers.

DISCUSSION

Although the mechanism of hepatocellular damage in AIH has not been well understood, many lines of evidence have demonstrated the presence of immunological disorders in AIH[5]. For example, wide ranges of circulating auto-antibodies are observed in the sera of patients with AIH[18-21]. Moreover, it has been shown that

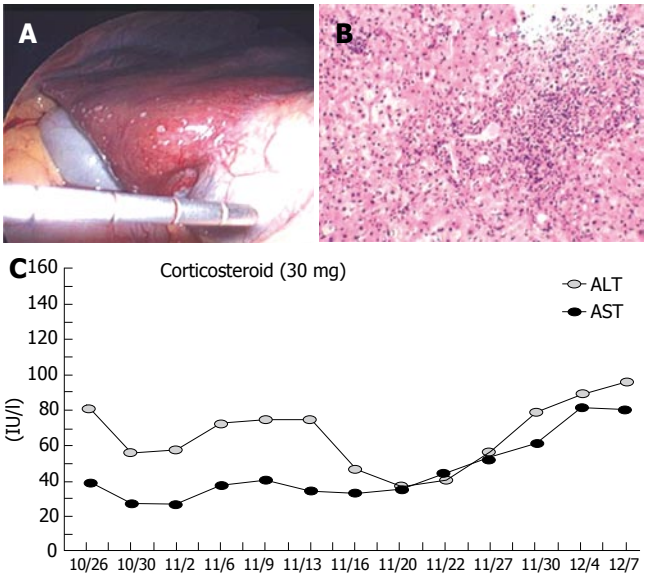

Figure 3 Laparoscopic findings, liver biopsy and clinical course after the administration of corticosteroids of a AIH patient non-reactive to corticosteroids. **A:** Laparoscopic view showing many reddish markings on the surface of the liver and small lymph cysts; **B:** microscopic findings of the liver showing bridging necrosis, infiltration of a large number of lymphocytes and plasma cells; and **C:** the time-course of the levels of ALT and AST after the administration of corticosteroids. The levels of ALT and AST were not improved to normal level after administration of corticosteroids.

the susceptibility to AIH was associated with HLA class II, DR3 and DR4 alleles[3,22,23]. Some reports have also suggested the association of cytotoxic T lymphocytes (CTL) with the hepatocellular injury in AIH using animal models[24-26].

Helper T cells (Th) receive a TCR signal from APC via HLA class II molecules. Helper T cells are divided into two subtypes, Th1 and Th2, according to their cytokine secretion profile[27]. Both Th1 and Th2 differentiate from Th0 cells. In the treatment of the patients with AIH, corticosteroids are effective in decreasing hepatocellular damage. This effect is thought to be due to the action of corticosteroids in inhibiting Th0 activation, leading to inhibition of CTL responses and antibody production[28,29]. CTL and Th receive signals from APC via the TCR-MHC complex[30]. However, this signal alone is not enough to activate T cells, and the expression of costimulatory molecules plays a crucial role in full T cell activation. Nevertheless, few reports have analyzed the expression of costimulatory molecules on mononuclear cells in AIH. In the present study, flow cytometry was used to analyze the expression of costimulatory molecules on mononuclear cells, because it is a sensitive method for detecting positive cells. PBMCs expressing CD80+, CD152+ (CTLA-4+) and CD86+ were shown to be significantly lower in the patients with AIH as compared with the healthy controls. However, in the patients with AIH, these cells existed more frequently in LIMCs than that in PBMCs. Interestingly, the most dramatic difference in frequency between PBMCs and LIMCs was detected in the ratios of cells positive for CD86 molecule, formerly designated as B7-2. Although CD86+ cells were barely detected in PBMCs obtained from the patients and healthy controls, they were considerably high in LIMCs obtained from the patients with AIH.

CD80 and CD86 molecules have unique expression patterns on professional APC and activated lymphocytes[31]. CD80 is constitutively expressed at low levels on lymphoid cells, whereas CD86 expression is rapidly increased upon activation[32]. Our results regarding the high level of CD86+ LIMC in the patients with AIH suggested that activated APC or lymphocytes might be enriched in the liver of the patients with AIH. Furthermore, the aberrant expression of CD86 was observed irrespective of the presence of hepatocellular damage and the treatment with corticosteroids, and the enhanced expression of CD86 was not observed in other liver diseases. These results suggest that the costimulatory molecules, such as CD80 and CD86, are continuously expressed on mononuclear cells in the liver even when the hepatocellular damage is not present. In addition, we often observe the relapse of liver dysfunction during the time course of tapering the dose of corticosteroids. Therefore, these lead us to speculate that excessive antigen presentation by APC to T cells, via the interaction between the CD80/86 and CD28 molecules, might contribute to the hepatocellular damage in AIH and administration of corticosteroids may block the signal transduction between APC and T cells. Indeed, it is important to identify which subtypes of PBMC or LIMC really express CD86 molecule. However, we have not identified it yet. Because the expression of CD86 on PBMCs was extremely low and the amount of LIMCs purified from a tiny liver specimen was quite a few, it was technically difficult to identity a specific subtype of the cells. The more detail analysis of this subtype and the function CD86+ cells in AIH awaits further elucidation in the next study.

Recently, extensive efforts have been made to elucidate the critical function of costimulatory molecules in various diseases. The aberrant expression of costimulatory molecules on mononuclear cells has been reported in a variety of autoimmune diseases, such as lupus, multiple sclerosis (MS), and experimental autoimmune encep halomyelitis[33-35]. The enhanced expression of CD80 and CD86 has also been reported in liver diseases, such as fulminant hepatic failure, primary biliary cirrhosis (PBC), primary sclerosing cholangitis, hepatitis C and hepatocellular carcinoma[9-14]. However, all of these reports focused on the CD80 or CD86 molecules expressed on either hepatocytes or bile ducts. There are few reports expressing the role of costimulatory molecules in AIH. The costimulatory molecules, such as CD80, CD86 and CTLA-4 (CD152), are essentially expressed on mononuclear cells. To our best of knowledge, this is the first descriptive report showing the aberrant expression of CD86 molecule on LIMCs of patients with AIH. Recently, anti-CD86 antibody or soluble CD152 (CTLA-4) was used as blocking agents for CD86 function in the treatment of autoimmune diseases[36-38]. Similarly, our data suggest that anti-CD86 antibody might be used to stabilize liver function for the treatment of AIH.

In PBC, presence of anti-mitochondria M2 antibody is a useful diagnostic marker[39,40]. In AIH, presence of ANA and the scoring system proposed by the International

Autoimmune Hepatitis Group are currently being used for the diagnosis of AIH[41]. However, some cases are still difficult to classify as definite or probable AIH. Actually, the cases with low levels of gammaglobulin (< 2.0 g/dL) were present in 8 of 18 cases whose LIMCs were examined in the present study. In contrast, all cases except No. 14 in Table 2 showed high levels of CD86+ LIMC. Furthermore, 2 patients who did not respond satisfactory to corticosteroids showed low levels of CD86+ LIMC. It is well known that non-response to the treatments of corticosteroids is uncommon in AIH. Therefore, these 2 cases might not be a real AIH although they were clarified as AIH by the scoring system. Thus, the detection of CD86+ LIMC may be useful for the diagnosis of AIH and this may lead to be helpful for the prediction of therapeutic effects of corticosteroids on AIH in the future.

In summary, our results showed lower proportions of CD80+, CD86+ and CD152+ (CTLA-4+) PBMC in the patients with AIH as compared with the healthy controls. These cells are present in greater frequency in LIMCs, suggesting that the aberrant expression of these costimulatory molecules on LIMCs might be associated with pathogenic mechanism of AIH. Especially, the level of CD86+ LIMC is likely to be helpful for the diagnosis of AIH and for the prediction of therapeutic effects of corticosteroids on AIH.

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