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Occurrence of Antibody Against Rat Hepatic Sinusoidal Endothelial Cells in Sera of Patients with Autoimmune Hepatitis

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To determine whether an antibody against hepatic sinusoidal endothelial cells was present in sera of patients with autoimmune hepatitis (AIH) type 1, we measured the serum IgG bound to the glutaraldehyde-fixed cultured rat sinusoidal endothelial cells by enzyme-linked immunosorbent assay. IgG bound to the cells was detected significantly more in patients with autoimmune hepatitis type 1 (97.1%) than in those with primary biliary cirrhosis (13.0%), chronic hepatitis C (5.9%) or B (7.9%), or healthy controls (0%). IgG-F(ab')$_2$ fragments from autoimmune hepatitis patients also bound to the cells, and this binding was observed after absorption of the fragments with rat hepatoma cells, but not after absorption with bovine carotid endothelial cells. Culture of sinusoidal endothelial cells in the presence of IgG from AIH patients significantly reduced the number of viable attached cells. In conclusion, anti-sinusoidal endothelial cell antibody occurred in the sera from patients with autoimmune hepatitis type 1.

KEY WORDS: autoimmune hepatitis; sinusoidal cells; endothelial cells; autoantibody; anti-sinusoidal endothelial cell antibody.

Autoimmune hepatitis (AIH) is a form of chronic active hepatitis (CAH) that is characterized by marked hypergammaglobulinemia, occurrence of autoantibodies, responsiveness to corticosteroid therapy, and other features in common with diseases considered to have an autoimmune basis (1, 2). AIH is classified into three types (3): AIH type 1 is characterized by occurrence of anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (SMA), and AIH types 2 and 3 are characterized by liver–kidney microsomal antibody type 1 and the antibody to soluble liver antigen, respectively. However, the International Autoimmune Hepatitis Group did not recommend this subdivision (2). One of the most important characteristics of AIH is the effectiveness of immunosuppressive therapy in improvement of hepatocyte injury, suggesting that an autoimmune mechanism would play an important role in the development and perpetuation of hepatocyte injury. Although the precise mechanism of hepatocyte injury

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is still unclear, several reports have indicated the occurrence of autoantibodies against components of the hepatocyte plasma membrane (4–6), and these antibodies are proposed to be involved in the hepatocyte injury of AIH.

On the other hand, antibodies against vascular endothelial cells have recently been found in sera of patients with various autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and Wegener's granulomatosis (7–14), although the role of anti-endothelial cell antibody in the pathogenesis of the vascular damage associated with these diseases is still obscure. Since the clinical and laboratory findings of AIH overlap with those of other autoimmune diseases, it seems reasonable to assume that antibody to endothelial cells occurs in the sera of patients with AIH. In the present study, we have attempted to determine whether an antibody against hepatic sinusoidal endothelial cells (SEC) is present in sera from patients with AIH type 1.

MATERIALS AND METHODS

Patients. Serum samples were obtained from 35 patients with AIH type 1 (three males and 32 females), 38 patients with chronic hepatitis (CH) associated with HBV infection (CH-B) (29 males and nine females), 101 patients with CH associated with HCV infection (CH-C) (70 males and 31 females), 23 patients with primary biliary cirrhosis (PBC) (three males and 20 females), and 32 age-matched healthy controls (15 males and 17 females). Serum samples were drawn before start of treatment with corticosteroids, interferon, or ursodeoxycholic acid, and stored at −20°C until use. In some AIH patients, serum samples were also drawn after corticosteroid treatment. HBsAg and IgM anti-HAV were assayed by reversed passive hemaggulutinin test (RPHA) (Meguro Laboratories, Tokyo, Japan) and enzyme-linked immunosorbent assay (ELISA) (Dainabot, North Chicago, Illinois), respectively. Anti-HCV was assayed by second-generation ELISA (Dainabot, North Chicago, Illinois). When anti-HCV was positive, HCV-RNA was examined by the two-stage polymerase chain reaction (15). ANA was examined by indirect immunofluorescence on HEp-2 cell monolayers. SMA and anti-mitochondrial antibody (AMA) were examined by indirect immunofluorescence on rodent tissue sections. Positive tests for ANA, SMA, and AMA required a titer of >1:40. Diagnosis of CH-B and CH-C was made based on clinical, laboratory, and histological findings (16). Diagnosis of PBC was made by clinical and biochemical findings as well as liver histology (17). Diagnosis of AIH type 1 was made based on the criteria of the International Autoimmune Hepatitis Group, and all the patients had "definite" AIH according to the scoring system proposed by the group (2). In all patients with AIH type 1, ANA titer was 1:320 or higher, serum concentration of IgG was more than 2500 mg/100 ml, and histological examination of liver biopsy specimens revealed typical findings of moderate to severe, predominantly periportal, hepatitis with piecemeal necrosis, with no features suggestive of other etiologies (18). HBsAg, IgM anti-HAV and anti-HCV, and/or HCV-RNA were not detected in the patients with AIH type 1.

Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. In addition, experimental animals received humane care in compliance with guidelines of the Animal Research Committee of Tokyo University for the care and use of laboratory animals.

Materials. Peroxidase-conjugated protein A was purchased from Kirkegaard & Perry Laboratories Inc. (Gaithersburg, Maryland), and protein A-Sepharose was from Pharmacia Fine Chemicals (Uppsala, Sweden). Metrizamide, trypsin inhibitor, and bovine serum albumin (fraction V, BSA) were purchased from Sigma Chemical Co. (St. Louis, Missouri), and Hanks' balanced salt solution (HBSS), RPMI 1640 medium, fetal calf serum (FCS) trypsin-EDTA solution (0.05% w/v trypsin 0.53 mM EDTA) were from Gibco Laboratories (Grand Island, New York). Collagenase was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Acetylated low-density lipoprotein (LDL) labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate was purchased from Biomedical Technologies Inc. (Stoughton, Massachusetts). All the other chemicals used were of reagent grade.

Preparation of Rat Hepatic SEC. SEC were isolated from Sprague-Dawley rats (Nippon Bio-Supply Center, Tokyo, Japan), weighing 250–300 g, according to the method of Knook et al (19) with slight modifications. Briefly, rat liver was perfused with HBSS containing 0.05% w/v collagenase for 15 min at 37°C. Nonparenchymal cells were obtained by removing hepatocytes by centrifugation at 50 *g* for 6 min, followed by centrifugation over a two-layered metrizamide gradient (15% w/v and 11% w/v) at 2000 rpm for 40 min (Hitachi 05PR-22 centrifuge and 03 rotor). The cells in the boundary layer were collected and suspended in RPMI 1640 medium containing 20% v/v FCS and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) at a cell density of 1×10^6 cells/ml. After removing the contaminated Kupffer cells by the dish adherence procedure at 37°C for 60 min, the nonadherent cells (0.2 ml) were seeded onto 96-well plates that were previously coated with collagen type I (Corning 25860 COL1; Iwaki Glass Ltd., Tokyo). After 18 hr, 90% of the cultured cells showed incorporation of fluorescent-labeled acetylated LDL and characteristic features of SEC, such as pore and sieve plates, when examined by scanning electron microscopy. Contamination of Kupffer cells and fat-storing cells was less than 5%, as shown by peroxidase staining and staining with anti-desmin antibody, respectively. The purity of SEC in culture was more than 90%.

Antibody Against SEC. Antibody against SEC was determined in serum from the patients by an ELISA according to the method of Rosenbaum et al (8). Briefly, SEC after 18 hr of culture were fixed with 0.2% v/v glutaraldehyde for 15 min at room temperature and washed three times with phosphate buffered saline (PBS, pH 7.4) containing 1% w/v BSA (washing buffer). After the plates were blocked with washing buffer for 1 hr at 37°C, the cells were then incubated with 100 μl serum diluted 1:250 with washing buffer in

triplicate for 2 hr at room temperature. The optimal dilution of the serum was determined by preliminary experiments using serial dilutions from 1:125 to 1:2000. In some experiments, purified IgG-F(ab')$_2$ fragments were used instead of serum samples. After washing five times with washing buffer, the cells were incubated with 100 μl peroxidase-conjugated protein A, which was diluted 1:1000 with washing buffer for another hour at room temperature. After five washes, 100 μl 0.04% w/v *o*-phenylenediamine in 0.2 M phosphate/0.1 M citrate buffer (pH 4.8) containing 0.012% v/v hydrogen peroxide was added to each well. The reaction was stopped after 5 min by adding 100 μl of 2 N H_2SO_4. Absorbance was then measured at 492 nm (OD_{492}) with an multititer plate reader (MR-600, Dynatech, Chantilly, Virginia). The binding of IgG to the cells was expressed as the difference between the absorbances given by the well in the presence of SEC and by that in the absence of the cells.

For comparison of the results of the assays performed on different days, results were expressed by arbitrary cutoff index calculated as (cutoff index) = (the binding of IgG from a sample)/(cutoff value), where the cutoff value was mean + 3 SD of the binding of IgG from 32 healthy controls. A panel of sera from five healthy controls was incubated in each plate to adjust the variations between assays. Values of the cutoff index over 1.0 were considered as positive for the antibody to SEC.

Preparation of F(ab')$_2$ Fragment of Serum IgG. The F(ab')$_2$ fragment of serum IgG was prepared from serum of the four randomly selected AIH patients, who showed positive IgG binding to SEC, and of three healthy controls. After isolation of serum IgG by protein A-Sepharose column chromatography (20), the F(ab')$_2$ fragment was prepared by pepsin digestion using an ImmunoPure F(ab')$_2$ preparation kit (Pierce, Rockyford, Illinois). The purity of the F(ab')$_2$ fragments, assessed by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, was over 99%. The purified F(ab')$_2$ fragments were stored at −20°C until use. The protein concentration was determined by the method of Lowry et al (21). When the serum F(ab')$_2$ fragments were applied to the ELISA for anti-SEC antibody, peroxidase-conjugated goat IgG-F(ab')$_2$ fragment against human IgG-F(ab')$_2$ (Cappel Laboratories, Cochranville, Pennsylvania) was used to detect the F(ab')$_2$ fragments that bound to the cells.

Cellular Specificity of IgG–F(ab')$_2$ Binding. Specificity of the antibody against various types of cells was assessed by ELISA using purified F(ab')$_2$ fragments, as described above. The following rat cell lines from by Riken Cell Bank (Tsukuba-city, Japan) were used: NRK and NRK49F (fibroblast-like cells derived from a rat kidney) and LYM-1 (sticky cells from a rat lymph node). Rat hepatocytes in primary culture were prepared as described previously (22). Rat hepatoma cell line, dRLh84, was from the Japanese Cancer Research Resources Bank (Tokyo, Japan). Bovine carotid endothelial cells (CEC) were prepared according to the method of Emori et al (23). The cells in confluent monolayers, obtained by subculture in uncoated 96-well plates (Corning, New York), were fixed with 0.2% v/v glutaraldehyde for 15 min at room temperature and were incubated with the F(ab')$_2$ fragments. The F(ab')$_2$ fragments bound to the cells were measured by ELISA, as described above. ELISA was independently performed for each cell line.

Absorption Studies. Absorption studies were performed using rat hepatocytes, rat hepatoma cell line dRLh84, and bovine CEC. Six micrograms of F(ab')$_2$ fragments (4 μg protein/ml) were incubated with the monolayer of each cell preparation, which had been fixed with 0.2% v/v glutaraldehyde, at 4°C for 24 hr in 35-mm plates. This absorption process was repeated three times using new cell monolayers for each incubation. Reactivity of the absorbed F(ab')$_2$ fragments to corresponding cell monolayers was assessed by measuring specific binding by ELISA to confirm that the absorbed F(ab')$_2$ fragments no longer reacted with corresponding cell monolayers. Reactivity of absorbed F(ab')$_2$ fragments to SEC was then assessed by incubating them with SEC and measuring the bound F(ab')$_2$ fragments by ELISA.

Effect of IgG on SEC Attachment to Culture Dish. After culture for 18 hr, SEC were incubated in the presence or absence of 600 μg/ml of serum IgG for 30 hr. The number of the cells attached to the culture dish were counted by hemacytometer after the cells were washed with PBS three times and detached from the dish by treatment with trypsin–EDTA solution (24). The number of attached SEC cultured in the absence of IgG were used as the 100% standard. Thus, the relative percentage of the cells attached to the dish was calculated as (relative percentage) = 100 × (number of the attached cells cultured in the presence of serum IgG)/(number of the attached cells cultured in the absence of serum IgG).

Statistical Analysis. The data represent mean ± SD unless otherwise stated. For statistical analysis, the χ^2 test and Student's *t* test were used, and differences at $P < 0.05$ were considered to be statistically significant.

RESULTS

IgG Antibody to SEC. IgG antibody to SEC (anti-SEC) was detected in 97.1% of patients with AIH (34/35) (Figure 1). In contrast, only 7.9% of patients with CH-B (3/38), 5.9% of patients with CH-C (6/101), and 13.0% of patients with PBC (3/23) were positive for IgG antibody to SEC. No healthy control subject had antibody to SEC. The differences in rates of positivity between AIH and all the other groups is highly significant ($P < 0.001$) by χ^2 test.

Anti-SEC positivity did not correlate with the presence or titers of ANA. Apart from the AIH group, 42 patients with CH-C and 12 with CH-B were ANA positive but only five and one, respectively, of these had anti-SEC. Conversely, 1/59 CH-C, 1/26 CH-B, and 3/23 PBC patients who were ANA negative were found to be positive for anti-SEC. There was also no correlation between anti-SEC and SMA in that, of 18 anti-SEC-positive AIH patients tested, seven were SMA negative. The all three PBC patients with anti-SEC had AMA, two of whom were also seropositive for anti-HCV and HCV-RNA.

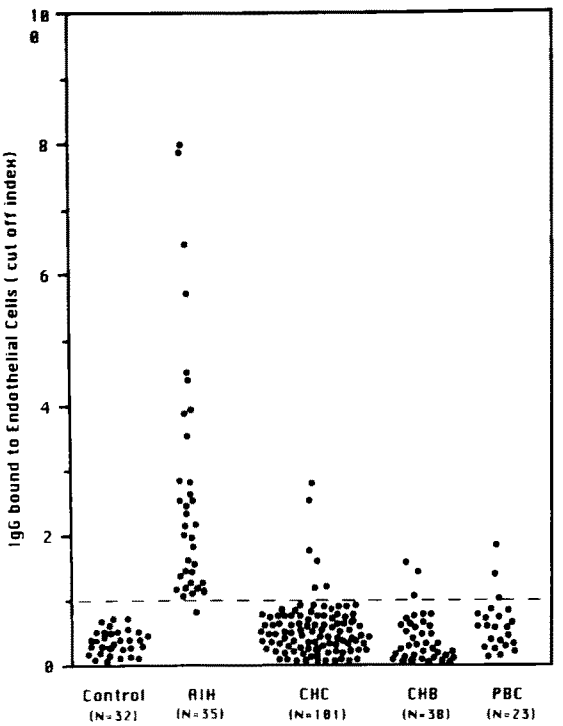

Fig 1. IgG bound to hepatic sinusoidal endothelial cells (SEC) in the sera of patients with chronic liver diseases. Rat SEC in primary culture were fixed with 0.2% v/v glutaraldehyde and incubated with 1:250 diluted serum from patients with autoimmune hepatitis type 1 (AIH), chronic hepatitis (CH) associated with HCV infection (CHC), CH associated with HBV infection (CHB), primary biliary cirrhosis (PBC), or healthy controls (Control). IgG bound to the cells was measured by ELISA as described in the text. The results were expressed as a cutoff index as described in the text. The dotted line represents the upper limit of normal range (cutoff value) given by mean +3 SD of the absorbances of healthy controls. *N* = size of a group.

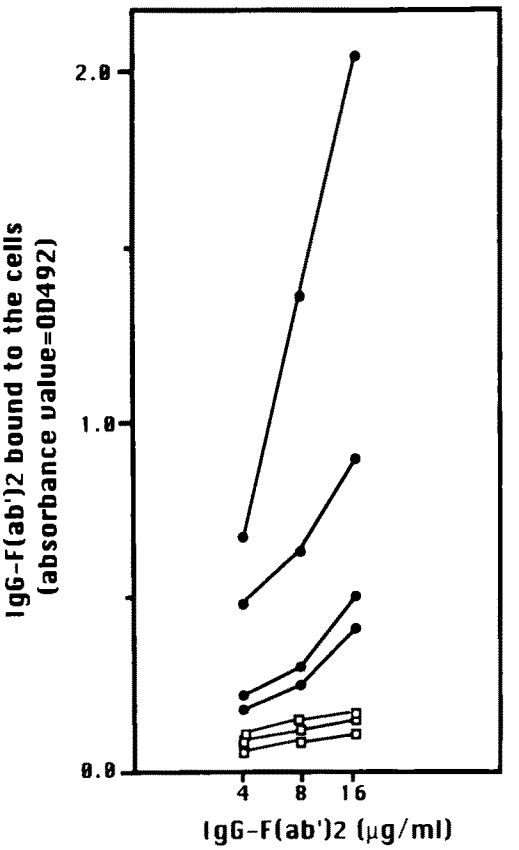

Fig 2. Binding of serum IgG-F(ab')$_2$ fragments from AIH patients to SEC. SEC were incubated with various concentrations of F(ab')$_2$ of serum IgG from patients with AIH (solid circles) or healthy controls (open squares), and the F(ab')$_2$ fragments bound to the cells were measured by ELISA as described in the text. Each point represents the mean value measured in triplicate.

Characterization of Serum IgG Bound to SEC. To exclude the possible involvement of Fc receptors in binding of IgG to SEC (25), we also examined the reactivity of IgG-F(ab')$_2$ fragments with SEC by ELISA. The absorbance values given by bound F(ab')$_2$ fragments were higher in AIH patients than in healthy controls at each concentration of F(ab')$_2$ tested (Figure 2). Moreover, the increase in the absorbance values was correlated with the increase in the concentration of F(ab')$_2$ tested in each patient with AIH (Figure 2).

Cellular Specificity of Antibody. Reactivities of the F(ab')$_2$ fragments with different types of cells were examined by ELISA. For the rat cell lines of NRK, NRK-49F, and LYM-1, the absorbance values given by F(ab')$_2$ binding to the glutaraldehyde-fixed cells did not differ significantly between AIH patients and healthy controls (Table 1). However, those to the glutaraldehyde-fixed dRLh84 cells and CEC were significantly higher in AIH patients than in healthy controls (Table 1). Those to the fixed rat hepatocytes in primary culture were higher in AIH patients than healthy controls, although the difference between the both group was not significant.

Absorption Studies. Before absorption, the absorbance values given by bound F(ab')$_2$ fragments to SEC were significantly higher in AIH patients than in healthy controls (Table 2). Even after extensive absorption of F(ab')$_2$ with rat hepatocytes or dRLh84, the binding of F(ab')$_2$ to SEC was still significantly higher in AIH patients than in healthy control (Table 2). After extensive absorption with CEC, however, it did not differ significantly between these two groups (Table 2).

Effect of IgG on SEC Attachment to Culture Dish. After SEC had been cultured for 30 hr in the pres-

TABLE 1. BINDING OF F(ab')$_2$ FRAGMENTS TO RAT NONENDOTHELIAL CELL LINES, RAT HEPATOCYTES, RAT HEPATOMA CELL LINE, AND BOVINE CAROTID ENDOTHELIAL CELLS*

Group (N)	*NRK*	*NRK-49F*	*LYM-1*	*RH*	*dRLh84*	*CEC*
Control (3)	0.137 ± 0.032	0.112 ± 0.025	0.091 ± 0.020	0.077 ± 0.008	0.317 ± 0.012	0.096 ± 0.003
AIH (4)	0.216 ± 0.083	0.128 ± 0.005	0.113 ± 0.019	0.135 ± 0.085	0.470 ± 0.048	0.223 ± 0.056
P	NS	NS	NS	NS	<0.01	<0.05

*Binding of F(ab')$_2$ fragments to the cells was measured by ELISA as described in the text. Results were expressed as mean ± SD of OD_{492} and the difference was compared between control and AIH groups in each cell line or cultured cell. NRK and NRK-49F, cell lines derived from a rat kidney; LYM-1, cell line from a rat lymph node; RH, rat hepatocytes in primary culture; dRLh84, a rat hepatoma cell line; CEC, bovine carotid endothelial cells; NS, not significant; *N*, size of a group.

ence or absence of 0.6 mg/ml serum IgG, the number of cells still attached to the dish was then counted. Serum IgG from seven randomly selected AIH patients, who showed a positive result for IgG binding to SEC, reduced the number of cells attached to the dish significantly ($P < 0.01$) when compared with serum IgG from eight healthy controls (Figure 3). In contrast, serum IgG from nine patients with CH-B or CH-C did not reduce the number of attached cells (Figure 3).

DISCUSSION

The immunological mechanisms underlying hepatic injury in AIH are still not clearly elucidated, although autoantibodies have been postulated to be involved in the pathogenesis of AIH (4–6, 26–29). ANA and SMA are autoantibodies characteristic of AIH type 1 (1–3). However, they may not contribute to the pathogenesis of AIH, because they can not react with hepatocytes as far as the plasma membrane is intact. The antibody to human asialoglycoprotein receptor, a glycoprotein that is located specifically on the hepatocyte plasma membrane, has occurred more frequently in patients with AIH than in those with other liver diseases (30), suggesting that a hepatocyte-specific antibody might mediate the hepatic injury in AIH. However, antibodies against nonparenchymal cells in the liver have not been reported yet. Since an antibody against vascular endothelial cells had been found in the serum of patients with various kinds of autoimmune diseases (7–14), we investigated whether an antibody against SEC would be present in the serum of patients with AIH type 1.

In the present study, we have detected IgG bound to cultured and glutaraldehyde-fixed SEC using an ELISA method. Since SEC are known to have Fc receptors on their surface, this receptor may interact with the Fc portion of IgG, aggregated IgG, or immune complex (25, 31). To exclude a possibility of the Fc receptor-mediated binding of IgG to SEC, binding of IgG-F(ab')$_2$ fragments prepared from the sera of AIH patients was examined. These IgG-F(ab')$_2$ fragments bound to SEC in a concentration-dependent manner. We therefore concluded that the IgG bound to the SEC was mediated through its F(ab) domain. Thus, it is rational to assume that the IgG reactive with SEC would be the antibody to the cells: anti-SEC antibody.

Anti-SEC antibody was detected in as much as 97.1% of patients with AIH type 1, whereas it was detected in less than 8% of patients with chronic viral hepatitis and 13% of patients with PBC. These findings could indicate that anti-SEC antibody would be one of the antibodies that characterize AIH type 1.

TABLE 2. ABSORPTION WITH RAT HEPATOCYTES, RAT HEPATOMA CELLS, AND BOVINE CAROTID ENDOTHELIAL CELLS*

	OD_{492}			
		After absorption with		
Group (N)	*Before absorption*	*RH*	*dRLh84*	*CEC*
Control (3)	0.182 ± 0.030	0.216 ± 0.016	0.218 ± 0.014	0.199 ± 0.032
AIH (4)	0.518 ± 0.243	0.340 ± 0.095	0.303 ± 0.071	0.179 ± 0.034
P	<0.001	<0.01	<0.01	NS

*Binding of F(ab')$_2$ fragments to the cells was measured by ELISA in a plate as described in the text. Results were expressed as mean ± SD of OD_{492} and the difference was compared between control and AIH groups. RH, rat hepatocytes in primary culture; dRLh84, rat hepatoma cell line; CEC, bovine carotid endothelial cells; NS, not significant; *N*, size of group.

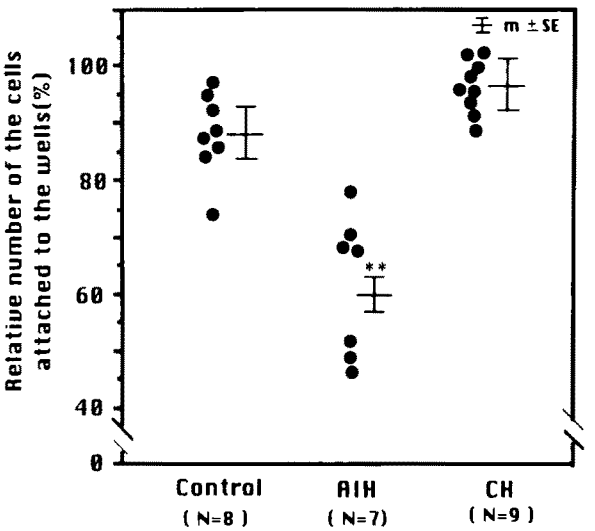

Fig 3. Effects of IgG on the SEC attachment to culture dish. SEC were cultured for 30 hr in the presence of serum IgG from patients with AIH, chronic hepatitis (CH) (six patients with CH-C and three patients with CH-B), or healthy controls (Control), or in the absence of serum IgG (standard condition). The number of cells attached to the wells was then counted, and the results were expressed as relative percentage of the standard condition as described in the text. Each vertical bar represents mean ± SE. N = size of a group. $^{**}P < 0.01$ when compared with healthy controls.

There were three CH-C and one CH-B patient showing both anti-SEC and ANA. The former cases, but not the latter one, fulfilled the diagnostic criteria for AIH except for the positive markers for HCV infection. Since HCV infection has been known to be linked to the emergence of autoimmune phenomena, this might be explained by such a linkage (16, 32). However, the possibility that these patients might have underlying AIH would not be ruled out.

To examine the cellular specificity of $F(ab')_2$ fragments from AIH patients who had anti-SEC antibody, we measured their binding to three rat cell lines, which were derived from a kidney and a lymph node. We demonstrated that the binding of $F(ab')_2$ fragments to these cell lines did not differ significantly between AIH patients and healthy controls, indicating that AIH patients did not have an antibody to these cell lines. Unlike previous reports (4–6, 26–29), the binding of $F(ab')_2$ fragments from AIH patients to rat hepatocytes in primary culture did not differ significantly from that from healthy controls. It is possible that many of the relevant antigens on the hepatocytes had been denatured by glutalaldehyde fixation (33). The binding of the $F(ab')_2$ fragments to a rat hepatoma cell line dRLh84 was greater in AIH patients than in controls, suggesting that the $F(ab')_2$ fragments from AIH patients might contain an antibody reactive with components of the plasma membrane of these hepatoma cells (4–6, 26–29). Even after the extensive absorption of the $F(ab')_2$ fragments with dRLh84 cells and rat hepatocytes, however, the binding of the $F(ab')_2$ fragments to SEC was still greater in AIH patients. These findings indicate that the anti-SEC antibody could be different from the antibodies against components of the hepatocyte plasma membrane (4–6, 26–29) unless affected by the fixation procedure. We observed significant binding of the $F(ab')_2$ fragments to cultured bovine CEC, one of the widely used endothelial cell preparations, in AIH patients. The binding of the $F(ab')_2$ fragments to SEC was no longer detected after extensive adsorption of the $F(ab')_2$ fragments by bovine CEC, suggesting that anti-SEC antibody might be directed to antigens presented in common with rat SEC and bovine CEC.

It has been recently reported that an anti-endothelial cell antibody was not frequently detected in patients with collagen diseases when examined by flow cytometric analysis using unfixed endothelial cells, indicating that the antibodies against intracellular components might have been detected by ELISA using glutaraldehyde-fixed endothelial cells (34). However, these findings are in contrast to those of Cines et al, who detected frequent occurrence of anti-endothelial cell antibody by a similar flow cytometric analysis with unfixed cells (9). We demonstrated that the binding of $F(ab')_2$ fragments to fixed rat cell lines did not differ significantly between AIH patients and healthy controls, although the binding of $F(ab')_2$ to fixed SEC was greater in AIH patients. Thus, we concluded that the antibodies against intracellular components would be negligible in the serum IgG from AIH patients in the present study.

There has been a debate whether anti-endothelial antibodies exert cytotoxicic activity against cultured endothelial cells (7–14). In the present study, the number of SEC attached to the wells decreased after 30-hr cultures with the serum IgG from AIH patients. Since cultured cells in monolayer are known to be easily detached from the dishes after being damaged (35), these data suggest that the anti-SEC antibody might be cytotoxic against cultured rat SEC. However, further studies are needed to elucidate the mechanisms of this cytotoxicity and its possible roles in the pathogenesis of AIH.

In conclusion, the present study demonstrates the presence of a circulating IgG class antibody against hepatic SEC in the sera of most of the patients with

AIH type 1. This antibody rarely occurred in patients with chronic viral hepatitis and PBC and seemed to be different from antibodies reactive with the components of the hepatocyte plasma membrane. This antibody may be characteristic of AIH type 1. The antigen determinant of this antibody and the roles of this antibody in the pathogenesis of AIH need further investigation.

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