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Serum Autoantibody Against Interleukin-1α is Unrelated to the Etiology or Activity of Liver Disease but Can be Raised by Interferon Treatment

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Objective: **To clarify the clinical significance of serum levels of interleukin-1α autoantibody in liver disease and their change during interferon therapy for chronic hepatitis.** ***Methods:*** **By radioimmunoassay, we studied the incidence of serum interleukin-1α autoantibody in 838 healthy controls and 180 patients with liver disease and monitored the change in antibody titer during the interferon therapy for chronic hepatitis.** ***Results:*** **We detected the interleukin-1α autoantibody in 12.6% (106/838) of healthy controls. In patients with liver disease, we found the antibody in 15.6% (5/32) in patients with acute hepatitis, 16.3% (13/80) in patients with chronic hepatitis, 18.8% (9/48) in patients with liver cirrhosis, and 15% (3/20) in patients with autoimmune liver disease. The incidence was not related to either etiology or inflammatory activity of liver disease. Two of three chronic hepatitis patients with initially high serum levels of the antibody (>2000 ng/ml) showed transient increase in antibody titers during interferon therapy.** ***Conclusion:*** **The serum level of interleukin-1α autoantibody was unrelated to the etiology or activity of liver disease. Interferon therapy can cause transient elevation of serum interleukin-1α autoantibody levels.**

INTRODUCTION

A close relationship exists between the proinflammatory cytokines produced by peripheral blood mononuclear cells or monocytes *in vitro* and the pathophysiology of liver diseases (1–4). The production of cytokines by peripheral blood immunocompetent cells *in vivo* is regulated by various components in sera; various antagonists or anti-cytokine antibodies in sera may affect the production of cytokines by peripheral blood immunocompetent cells *in vivo*. Several antibodies to cytokines in human sera, such as interleukin-1α (IL-1α), tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), interferon-γ (IFN-γ), and interferon-α (IFN-α), have been described (5–8). A circulating antagonist to IL-1 and TNF has been identified (9, 10).

IL-1α is mainly secreted by cells of macrophage/monocyte lineage. A previous report showed a decreased secretion of IL-1α by monocytes in the presence of sera from patients with liver cirrhosis, which suggested the existence of some component in sera that interfered with the production of IL-1α (11). Svenson *et al.* (5) first reported the existence of an IL-1α autoantibody in normal human serum. Suzuki *et al.* (12) reported a higher incidence of IL-1α autoantibody in sera from patients with rheumatoid arthritis and from patients with systemic lupus erythematosus than from healthy controls.

Tilg *et al.* (13) recently described a close relationship between the serum level of IL-1 receptor antagonist and the inflammatory activity of hepatitis. However, little is known about the serum level of IL-1α autoantibody in humans with liver diseases or whether the serum level changes during IFN therapy for chronic hepatitis. We investigated the incidence of IL-1α autoantibody in sera of healthy controls and of patients with liver disease. We also studied the serum levels of IL-1α autoantibody during IFN therapy for chronic hepatitis B or C to determine if the titer changed during treatment.

MATERIALS AND METHODS

Subjects

We studied 838 healthy volunteers and 180 patients with acute or chronic liver diseases. All participants in this study gave their informed consent, and this study was approved by the ethical committee of the hospital. The healthy volunteers had normal serum levels of ALT, AST, and γ-glutamyl transpeptidase. The healthy volunteers had no history of significant disease, including liver disease. Diagnoses of liver disease were made by applying appropriate serological, virological, biochemical, and histological criteria (14, 15). Hepatitis B virus surface antigen, hepatitis B virus envelope antigen, and immunoglobulin M (IgM) antibody to hepatitis A virus were examined in sera by commercially available

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TABLE 1
Clinical Characteristics and the Incidence of IL-1α Autoantibody in Patients with Liver Diseases[a]

Diagnosis	N cases	Sex (F/M)	Hepatitis (A/B/C/nAnBnC)	ALT (IU/L)	G-gl (g/dL)	IL-1α/auto-Ab (%)
Acute hepatitis	32	15/17	20/5/6/1	1442 ± 852	1.7 ± 0.3	5/32 (15.6)
Chronic hepatitis	80	29/51	0/19/61/0	123 ± 82	1.3 ± 0.4	13/80 (16.3)
Liver cirrhosis	48	18/30	0/8/40/0	65 ± 21	1.8 ± 0.2	9/48 (18.8)
Autoimmune liver disease	20	—	—	—	—	3/20 (15)
Autoimmune chronic active hepatitis	8	4/4	0/0/0/8	98 ± 34	2.8 ± 0.3	1/8 (12.5)
Primary biliary cirrhosis	12	10/2	0/0/0/12	58 ± 11	2.1 ± 0.3	2/12 (16.7)
Total	180	76/104	20/32/107/21	—	—	30/180 (16.7)
Healthy controls	838	415/423	0/0/0/0	<30	ND	106/838 (12.6)

M, male; F, female; A, hepatitis A; B, hepatitis B; C, hepatitis C; nAnBnC, hepatitis non-A, non-B, non-C; IL-1α/auto-AB (%), the incidence of IL-1α autoantibody.

[a] Data are expressed as the mean ± SD. Statistical significance was evaluated by χ^2 test. The incidence of IL-1α autoantibody in all groups of patients were not significantly different from that of healthy controls.

radioimmunoassay (RIA) kits (Abbott Laboratories, North Chicago, IL), and anti-HCV antibody was determined by second generation ELISA (Abbott). The clinical characteristics of the patients with liver diseases and the incidence of IL-1α autoantibody are shown in Table 1. We examined whether the incidence of IL-1α autoantibody was different by sex or age; no statistical significance was found (data not shown).

Sixty patients with chronic hepatitis received natural IFN-α intramuscularly or natural IFN-β intravenously. Forty patients with chronic hepatitis C were given either 3, 5, or 6 × 10^6 units (MU) of natural IFN-α daily for 2 wk followed by 14–22 wk of administration three times a week. Ten chronic hepatitis B patients with hepatitis B virus envelope antigen and 10 chronic hepatitis C patients were given 3 or 6 MU of natural IFN daily for 8 wk. Their serum levels of IL-1α autoantibody were measured, at least, 1 month before, just before, 2 times during, and around 1 month after finishing IFN therapy. Sera were stored at −20°C until the assay.

Radioimmunoassay for IL-1α autoantibody

Iodination of recombinant human IL-1α (rhIL-1α; Otsuka Pharmaceutical Co., Tokushima, Japan) and purification of ^{125}I-labeled rhIL-1α were performed according to the method of Fraker and Speck (16). In brief, tubes were coated with iodogen solution, and 3 μg of rhIL-1α and 0.5 mCi (37 MBq) of Na^{125}I were mixed in each tube for 3 min on ice. The mixture was applied to a PD-10 column and eluted, and an aliquot of each 1.0-ml fraction was counted in a γ-spectrometer. Fractions with the strongest binding and highest specificity were stored at −4°C for the later use in the RIA.

RIA for IL-1α autoantibody was performed as follows. Fifty liters of duplicate samples of the patients' sera or the standard dilutions of monoclonal antibody to IL-1α (ANOC 301: purity >95%), ranging from 5 to 640 ng/ml, were mixed with ^{125}I-labeled rhIL-1α (10000 cpm/100 μl/tube). Each sample was mixed with 350 μl/tube of 0.1% bovine serum albumin/phosphate buffer saline (BSA/PBS) containing 5 mM ethylenediaminetetraacetic acid (EDTA)/0.05% NaN_3, and each standard dilution of rhIL-1α was mixed with 300 μl/tube of 0.1% BSA/PBS containing 5 mM EDTA/0.05% NaN_3 and 50 μl/tube of carrier buffer (2% bovine γ-globulin). Then, antigen-antibody binding reached equilibrium by incubating overnight at room temperature. After incubation, each sample was mixed with 500 μl/tube of 25% polyethylene glycol (MW 6000) and was incubated for 1 h. Then, the tubes were centrifuged at 3000 rpm for 15 min, and the precipitates were counted for 1 min in an automated γ-spectrometer. The IL-1α autoantibody titer was determined from the standard dilution curve (Fig. 1).

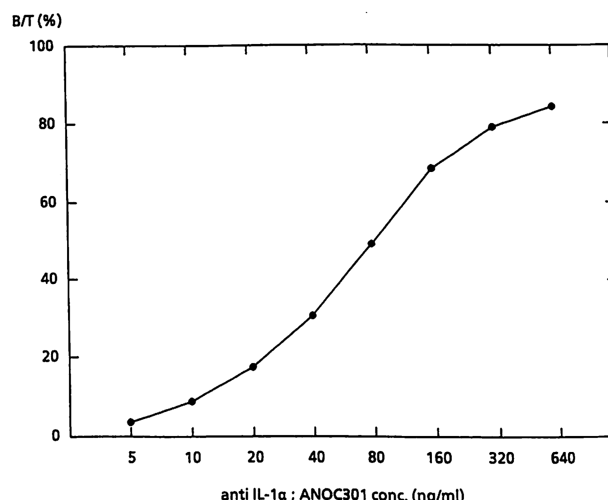


FIG. 1. Standard curve of IL-1α autoantibody by RIA. The standard dilutions of IL-1α monoclonal antibody ANOC 301, ranging from 5 to 640 ng/ml, were mixed with ^{125}I-labeled rhIL-1α. The mixture was centrifuged, and precipitates were counted by γ-spectrometer. *B/T(%)*, the percentage of bound ^{125}I-labeled IL-1α (B)/total ^{125}I-labeled IL-1α. The IL-1α antibody titer in each individual was determined by this curve.

Characterization of IL-1α autoantibody

To characterize IL-1α autoantibody, we used sera from healthy controls containing a high IL-1α autoantibody level. The serum was diluted and applied to a protein A column. After washing with the binding buffer, we obtained the IgG

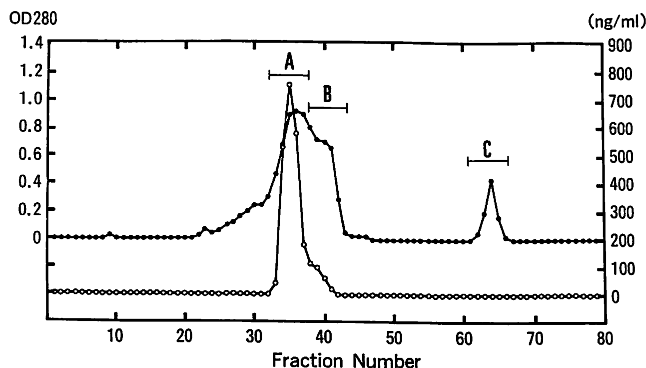


FIG. 2. Analysis of IL-1α autoantibody by gel filtration. The serum of a healthy control with a high IL-1α autoantibody level was fractionated. The titer of IL-1α autoantibody in each fraction was measured by RIA. SDS-PAGE analysis confirmed that the peak level of IL-1α autoantibody exists in the IgG fraction. ●, concentration of protein in each fraction; *OD 280*, the relative concentration of protein; ○, concentration of IL-1α autoantibody in each fraction as measured by RIA (ng/ml); *A*, immunoglobulin-G; *B*, albumin; *C*, amino acids.

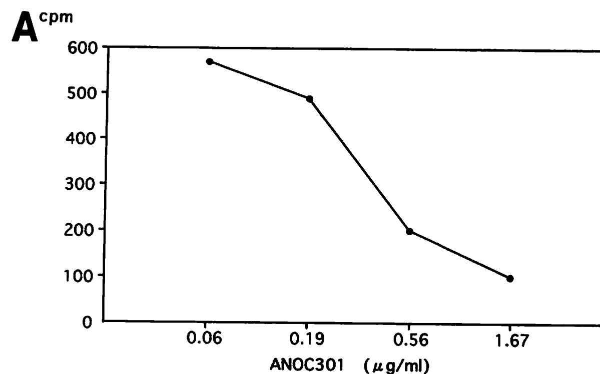
fraction using an elution buffer. RIA showed that a high titer of IL-1α autoantibody was present only in the IgG fraction. The serum was also fractionated by gel filtration (Fig. 2). The IL-1α autoantibody titer of each fraction was measured by RIA, and each fraction was applied to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE). We confirmed the peak level of IL-1α autoantibody in the IgG fraction. The affinity of the IL-1α autoantibody in several samples, examined by the Scatchard analysis, was between 2.1×10^{-10} and 1.2×10^{-9} M/L.

IL-1α receptor assay

Neutralizing activity of IL-1α autoantibody in sera was examined by IL-1 receptor assay as we have previously described (17). ^{125}I-labeled IL-1α was prepared by the same way as described for IL-1α RIA. The specific activity of ^{125}I-labeled IL-1α was 130–150 μCi/g. BALB/3T3 fibroblasts (the American Type Culture Collection) were maintained in a Dulbecco's minimum essential medium (Nissui Pharm. Co. Ltd., Tokyo, Japan) containing 10% fetal calf serum (Hyclone Inc., Utah). Cells (1×10^6) were incubated with 500 μl of culture medium containing ^{125}I-labeled IL-1α (10^5 cpm) and 10% sera from the patients in 12-well plates (Corning Glass Works, NY) for 2 hours at 4°C. Nonspecific binding was determined in the presence of 1 μg/ml of unlabeled IL-1α. The cell monolayers were then rinsed with 1 ml of PBS three times then solubilized with 1 ml of a mixture of 1% SDS and 0.2 N NaOH. The radioactivity was counted in a γ-counter. The standard (Fig. 3*A*) was prepared by 0.06, 0.19, 0.56, and 1.67 ng/ml of monoclonal antibody to IL-1α (ANOC 301).

Statistical analysis

Laboratory data of the patients are expressed as the mean values and SD. The incidence in the two groups was compared by the χ^2 test. A p value less than 0.05 was considered to be statistically significant.



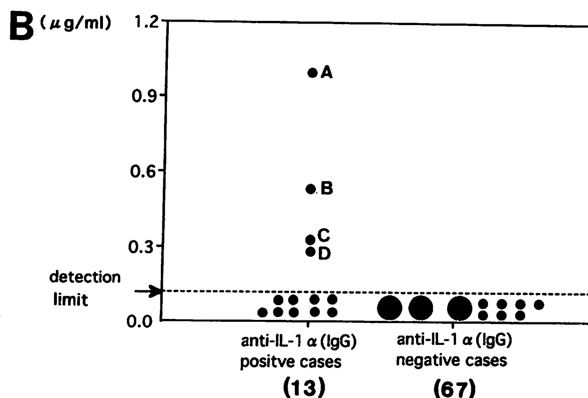


FIG. 3. *A*, Standard of IL-1 receptor assay. BALB/3T3 fibroblasts were incubated with ^{125}I-labeled IL-1α for 2 h in the presence of 0.06, 0.19, 0.56, and 1.67 ng/ml of monoclonal antibody to IL-1α (ANOC301), which has neutralizing activity for IL-1α. Cells were rinsed and solubilized, and then the radioactivity was counted using a γ-counter. *B*, Neutralizing activity of IL-1α autoantibody in sera. BALB/3T3 fibroblasts were incubated with ^{125}I-labeled IL-1α in the presence of sera from 80 patients with chronic hepatitis. Cells were rinsed and solubilized, and then the radioactivity was counted. Data are calculated as the titer corresponding to the monoclonal antibody to IL-1α (ANOC301) as shown in *A*. Sera from four patients with high concentrations of IL-1α autoantibody [(*A*) 4620, (*B*) 3020, (*C*) 2180, and (*D*) 1750 ng/ml] showed neutralizing activity. A large, closed circle denotes 20 patients, and a smaller closed circle denotes one patient.

The standard curve, titers of IL-1α autoantibody, and neutralizing activity of IL-1α autoantibody were expressed as the mean value of duplicate cultures.

RESULTS

Standard curve of IL-1α autoantibody in RIA and analysis by gel filtration

In Figure 1, the standard curve of IL-1α autoantibody as measured by RIA is shown. From this standard curve, we determined concentrations, ranging from 5 to 640 ng/ml. In Figure 2, the result of gel filtration analysis of IL-1α autoantibody was shown. The peak IL-1α autoantibody fractions were located in the immunoglobulin-G fraction.

Serum levels of IL-1α autoantibody in patients with liver disease and in healthy controls

We investigated the incidence of IL-1α autoantibody in patients with acute or chronic liver disease (Table 1). The incidence of IL-1α autoantibody in patients with liver dis-

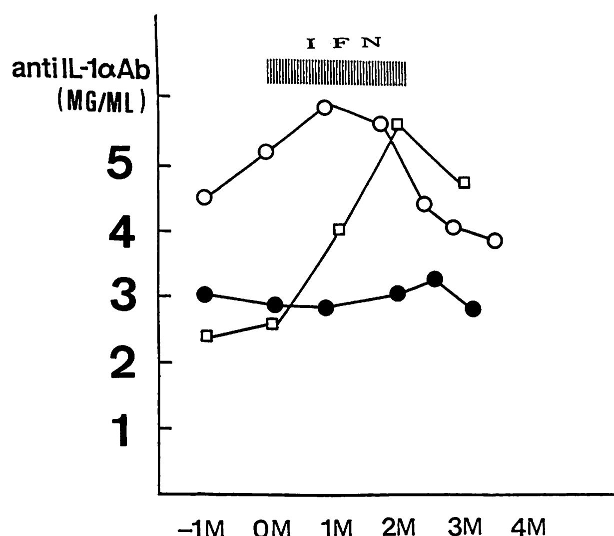


FIG. 4. Serum levels of IL-1α autoantibody during IFN therapy in three cases. The concentration of IL-1α autoantibody in sera from three cases of chronic hepatitis whose initial IL-1α autoantibody titers were greater than 2000 ng/ml was investigated. *−1M*, 1 month before IFN therapy; *0 M*, just before IFN therapy; *1M-4M*, 1–4 months after starting IFN therapy; □, a patient with chronic hepatitis B who received 6 MU of IFN-α daily for 8 wk; ○, a patient with chronic hepatitis C who received 3 MU of IFN-α daily for 8 wk; ●, a patient with chronic hepatitis C who received 6 MU of IFN-α daily for 8 wk.

ease ranged between 12.5 and 18.8% and was not significantly different from the incidence in healthy controls (12.6%) (Table 1). Sex and age were not significantly related to the incidence of IL-1α autoantibody in healthy controls, although the incidence in individuals under 30 yr old tended to be lower than those over 30 yr old (data not shown).

Serum levels of IL-1α autoantibody during IFN therapy for chronic hepatitis

When we examined changes in serum levels of IL-1α autoantibody in 60 patients with chronic hepatitis, nine patients (two hepatitis B and seven hepatitis C) were found to be positive for IL-1α autoantibody. The antibody titers were 12, 13, 14, 20, 69, 980, 2180, 3020, and 4620 ng/ml, respectively. As shown in Figure 4, the serum levels of IL-1α autoantibody in three patients were greater than 2000 ng/ml initially. In two of these three patients, serum levels of IL-1α autoantibody significantly increased during IFN therapy and no significant increase was shown in one. The elevated levels of IL-1α autoantibody returned to lower levels after IFN therapy was completed. However, the remaining six patients, whose initial titers were less than 1000 ng/ml before IFN therapy, showed no significant increase in antibody titer during the course of the therapy (data not shown). All 51 patients without detectable levels of IL-1α autoantibody before IFN therapy continued to demonstrate no detectable titers during and after IFN treatment (data not shown).

Neutralizing activity of IL-1α autoantibody

To examine neutralizing activity of IL-1α autoantibody in sera, we performed IL-1α receptor assay using BALB/3T3 mice. Sera from 80 patients with chronic hepatitis were examined. In patients received IFN therapy, sera obtained before IFN therapy were used. Among the 80 samples, 13 were positive for IL-1α autoantibody (Table 1). Sera from four patients with high concentrations of IL-1α autoantibody (4620, 3020, 2180, and 1750 ng/ml) blocked binding of ^{125}-labeled IL-1α to the IL-1 receptor, showing neutralizing activity against IL-1α (Fig. 3*B*). Sera from patients with IL-1α autoantibody less than 1000 ng/ml or without detectable titers showed no detectable neutralizing activity.

DISCUSSION

In this investigation, we measured the serum level of IL-1α autoantibody in healthy controls and in patients with liver disease. We found that IL-1α autoantibody was present in an average of 16.7% (30/180) of the patients with liver disease, which was not significantly different from the incidence of healthy controls (12.6%, 106/838). The mean titer of the IL-1α autoantibody in patients with liver disease was 1106 ± 1682 ng/ml (mean ± SD) and was not significantly different from that of healthy controls (919 ± 1885 ng/ml) (data not shown). The incidence of IL-1α autoantibody in all groups of patients with different etiology was not significantly different from that in healthy controls (Table 1).

Several autoantibodies such as anti-nuclear antibody, anti-smooth muscle antibody, and anti-mitochondrial antibody have been associated with autoimmune chronic active hepatitis or primary biliary cirrhosis (18), particularly when they are present in high titer (19). Previous reports have linked IL-1α autoantibody to the activity of such autoimmune diseases as rheumatoid arthritis (12) and Crohn's disease (20); however, our findings in this study were different. We noted that the incidence of IL-1α autoantibody in patients with autoimmune liver disease was not significantly different from that of healthy controls or from that of patients with viral liver disease. We caution that the number of patients with autoimmune liver disease was small (eight autoimmune chronic active hepatitis and 12 primary biliary cirrhosis) in our study; a larger sample population, perhaps a multi-center investigation, is necessary to reach a final conclusion.

We also investigated the serum level of IL-1α autoantibody in patients with chronic or acute hepatitis both during the convalescence phase and during the active inflammation phase, and the antibody titers did not change depending on the disease activity. In each patient, there was consistently almost the same level of IL-1α autoantibody in the circulation (data not shown). We suppose that the serum level of IL-1α autoantibody is not related to the pathophysiology of liver disease. The result that the sera containing high titers of this antibody blocked binding of ^{125}I-labeled IL-1α to

IL-1 receptor on the surface of BALB/3T3 fibroblasts suggests the neutralizing activity of this antibody *in vivo*. This finding is consistent with the results by Suzuki *et al.* (21), who confirmed neutralizing activity of IL-1α autoantibody.

A recent report by Tilg *et al.* (13) showed that the titer of IL-1 receptor antagonist paralleled with the activity of hepatic inflammation. We speculate that elevated levels of IL-1 receptor antagonist in hepatitis (13) may be caused by IFNs, because IFNs induce IL-1 receptor antagonist *in vivo* (22). However, IL-1α autoantibody was unrelated to hepatic inflammation in our study. We suppose that autoantibodies to proinflammatory cytokines exist in the circulation and play important roles in decreasing harmful effects of excessive cytokines in bacteremia or endotoxemia, because previous papers describing autoantibodies against TNFα (6), neutrophil attractant protein-1, and monocyte chemoattractant protein-1 (23) showed a close association between the incidence or the titer of these autoantibodies and bacteremia or endotoxemia. The IL-1α autoantibody may have some additional effect in combination with IL-1 receptor antagonist (24), TNFα autoantibody (6), and TNF soluble receptor (25) for the protection of lethal shock in such situation. Another explanation is that IL-1α autoantibody is particularly associated with the pathophysiology of rheumatoid arthritis but is not associated with other diseases, such as systemic lupus erythematosus, PSS (12), and viral hepatitis, as has been shown in this study. Caruso *et al.* (7) reported increased titer of autoantibody against IFN-γ in viral infection. Ikeda *et al.* (26) showed increased frequency and titer of anti-IFNα2a antibody in patients with acute hepatitis. Because we mainly studied patients with viral hepatitis in this study, autoantibodies to IFNs might have been frequently detected.

Several recent studies demonstrated increased levels of serum anti-IFNα autoantibody during IFN-α therapy for chronic hepatitis B and C (27–29). We also investigated whether the frequency and the titer of IL-1α autoantibody changed during IFN therapy in patients with chronic hepatitis B or C. Sera from 10 patients with chronic hepatitis B and 50 patients with chronic hepatitis C were tested for the concentration of IL-1α autoantibody. The sera from nine patients were positive before IFN therapy and remained so during and after IFN therapy. No other patients developed this antibody during and after IFN therapy.

However, as shown in Figure 4, two patients with initially high serum levels of IL-1α autoantibody (one hepatitis B and one hepatitis C; higher than 2000 ng/ml) showed an increase in the antibody titer during IFN therapy. By accident, these two patients had received IFN-α 3 or 6 MU daily for 8 wk. Because two of the six patients with initially low titer of this antibody (20 and 69 ng/ml, respectively) received 6 MU of IFN daily for 8 wk and showed no increase in antibody titer, the protocol of IFN therapy might not be an important factor in the changes of antibody titer. Neither the presence nor the titer of this antibody were related to the therapeutic efficacy of IFN therapy.

Because several organ-associated or non-organ-associated autoantibodies, such as anti-thyroid antibody and antinuclear antibody, increase during IFN treatment for hepatitis B or C (30–33), we speculated that the elevated serum levels of IL-1α autoantibody may similarly be a manifestation of an enhanced autoantibody production induced by IFN. We previously reported a patient with chronic hepatitis B who showed greatly increased serum levels of IL-6 and γ-globulin during IFN therapy (34). In this case, we speculated that IFN might have amplified the subclinical immunological background of the patient. The present study is consistent with this idea because two of the three patients with initially high IL-1α autoantibody levels showed increased titer of this antibody during IFN treatment.

The physiological significance of IL-1α autoantibody in human liver disease still remains to be clarified. From the results of our study, we propose that elevated serum levels of IL-1α autoantibody during the IFN therapy represent an accelerated production of autoantibody induced by IFN in patients with liver disease.

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