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The Serum Soluble HLA-DR Antigens as a Predictive Marker of the Response to Interferon-α Treatment in Patients with Chronic Hepatitis C

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The serum concentrations of soluble HLA-DR antigens (sDR) were monitored in 40 patients with chronic hepatitis C (CHC) who received interferon treatment. The expression of HLA-class Ⅱ antigens in liver tissues was also studied by immunohistochemistry. The sDR levels in patients with chronic hepatitis C were significantly higher than those in healthy subjects (416 ± 236 [mean ± S.D.] ng/ml vs. 286 ± 163 ng/ml) (P<0.05). There was no correlation between the sDR levels and serum alanine aminotransferase levels, suggesting that sDR do not reflect the extent of liver necrosis. Although there was no difference in pretreatment sDR levels between interferon complete responders and non-responders, sDR significantly declined in complete responders, while they did not in non-responders. The hepatic expression of HLA-DR antigens was observed in dendritic cells, lymphocytes and Kupffer cells in portal area, while in Kupffer cells and endothelial cells in central acinus. These expression significantly decreased in complete responders. From these results, sDR, reflecting the hepatic expression of HLA-DR antigens, could be a predictive marker of response to inteferon treatment.

Key words : Chronic hepatitis C, HLA-class Ⅱ antigen, HLA-DR antigen, Interferon, Liver histology

INTRODUCTION

It has been known that immunological defence is deeply involved in the pathogenesis of chronic hepatitis C (CH C). There are patients whose serum alanine aminotransferase (ALT) levels are normal in spite of the existence of hepatitis C virus [1]. Furthermore we reported a case with CH C, whose serum ALT levels fell down to the normal range after he suffered from malignant lymphoma, which is known to cause immunosuppressive state [2]. These results indicate the significance of immunological surveillance in the pathogenesis of CH C.

There are several reports mentioning an importance of human leukocytes antigens (HLA) antigens in viral hepatitis. HLA-class Ⅰ [3] and DR antigens [4] are highly expressed in the liver of hepatitis C virus infection. The association of the incidence of chronic hepatitis or the severity of damage with particular types of HLA-class Ⅱ antigens has also been reported [5－11]. Then, it was considered that, while $CD8^+$ cytotoxic T lymphocytes (CTLs) restricted to HLA-class Ⅰ are mainly involved in the clearance of viral infected hepatocytes [12－14], $CD4^+$ helper T lymphocytes should have important roles for those CTLs reaction. Actually, The existence of HLA-DR restricted anti-hepatitis C virus specific $CD4^+$ T cells has recently been reported [15].

HLA-class DR antigens, similarly as class Ⅰ, are secreted as soluble forms (designated as soluble HLA-DR: sDR) [16, 17], and increases in patients with infectious disorders [18], autoimmne disorders [19], malignant disorders [20], and also in autoimmune hepatic disorders [21]. Previously, we report-

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ed that sHLA-Ⅰ, together with soluble CD8, were elevated in CHC patients and further increased after interferon (IFN) treatment [22]. It is quite reasonable as IFN itself influences the level of HLA-class Ⅰ expression both in hepatocytes and in peripheral blood lymphocytes [23, 24]. The number of HLA-DR^+ IFNα lymphocytes was also increased after IFNα-therapy, whereas it is controversial as for the HLA-class Ⅱ expression of liver cells (hepatocytes, bile duct cells, etc) after IFN-treatment [25]

Based on those backgrounds, in the present study, serum sDR were measured in patients with CH C and evaluated the relationship with the other parameters. Furthermore, we monitored the sDR levels in patients who received IFN treatment and evaluated whether the change of sDR would predict the response to the therapy. Finally, we analyzed the expression of HLA-DR antigens in liver specimen in order to clarify the relationship between sDR and HLA-DR antigens expressed in liver tissues.

MATERIALS AND METHODS

Patients and sample collection

Forty patients with histologically proven CH C were enrolled into this study. They had not received any drugs 6 months before entry. Serum was separated from peripheral venous blood taken after informed consent and stored at −80℃ until measurement.

IFN treatment

These patients received human lymphoblastoid interferon (Sumiferon, Sumitomo Pharmaceutical Co., Ltd., Osaka, Japan) treatment following the schedule of daily intramuscular injection (6 million units per an injection) for 4 weeks, then three times weekly injection for 20 weeks. The sDR levels were determined before, 2, 12 and 24 weeks after the start of IFN and 4 weeks after the end of treatment.

A complete response was defined as the disappearance of serum HCV-RNA and the normalization of serum ALT levels. The other patients were rendered as a non-responder. Re-biopsy was done six months after the end of treatment. Histologic changes of the liver were classified by Inuyama Classification [26].

Determination of sDR levels

The levels of sDR were determined by sandwich ELISA according to the method described elsewhere [21]. Briefly, 500-fold diluted anti-HLA-DR antibody L243 (Beckton-Dickinson, San Jose, CA) was sorbented onto 96-well plates (Costar, Cambridge, MA). Two-fold diluted serum samples were added and incubated at 37℃ for 1h. After washed, the plates were incubated with 1000-fold diluted anti-HLA-class Ⅱ b chain antibody CR3/43 (Boehringer Mannheim GmbH, Testentwicklung Werk Tutzing, Germany) for further 1h. 1000-fold diluted horseradish-labeled anti-mouse IgG1 (Zymed, San Francisco, CA) was added followed by orthophenylendiamine (Sigma, St Louis, MO). The absorbance was measured with a microplate reader (Molecular Device Corp, Nenlo Park, CA). All experiments were undertaken in duplicate. The serum concentration of sDR was calculated from the standard curve obtained using purified HLA-class Ⅱ antigen (kindly gifted by Prof. Grosse-Wilde, Essen University) [27].

Immunohistochemistry

The expression of HLA-DR antigens was examined by immunoperoxidase method. Briefly, formalin-fixed, paraffin-embedded section were deparaffinized, hydrated, and treated with 1% aquanous hydrogen peroxide, and digested with 0.5% Saponin (MERCK, Darmstadt, Germany) for 30 minutes. The sections were incubated with anti-HLA-DR mAb, TAL.1B5 (DAKOPATTS A/S, Glostrup, Denmark), and then with biotin-conjugated anti-mouse IgG. Finally, the sections were reacted with an avidin-biotin complex (ABC) kit (Vector Lab., Inc., Burlingame, CA). The number of HLA-DR positive cells in portal tracts were evaluated using the VIDAS system (Carl Zeiss, Oberkochen, Germany) [28].

Statistical evaluation

The differences between groups were evaluated by two-tail Student's t-test. The difference of dichomatous value was analyzed by chi-square test.

RESULTS

sDR levels in patients with CH C (Fig. 1)

The mean ± S.D. of sDR in patients with

CH C was 416 ± 236 (ng/ml), which was significantly higher than healthy controls (286 ± 163) ($P<0.05$). Next, the correlation between sDR levels and other background parameters was evaluated. Regarding the liver histology, the sDR levels showed slightly increased concomitantly with the grading scores of inflammation (A factor) and fibrosis (F factor) (Fig. 2). The patients without fibrosis (F0) had relatively low levels of sDR compared with patients with progressive fibrosis, however, there were no significant differences. The serum ALT levels were not correlated with sDR levels (data not shown), suggesting that the sDR levels do not reflect the extent of liver cell necrosis. We compared sDR levels by serum HCV RNA titer or HCV serotype, however, no relationship

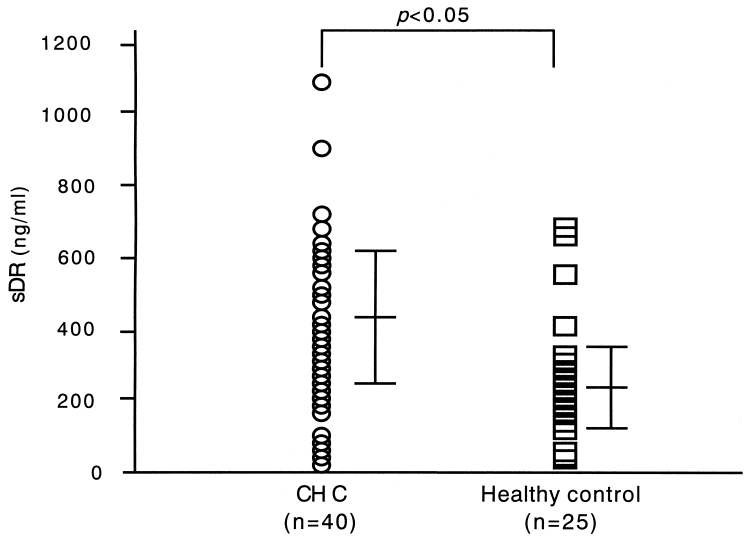

Fig. 1 sDR levels in patients with CH C and healthy controls. Bars represent the mean ± S.D. The sDR level in patients with CH C was higher than that in healthy controls ($P<0.05$).

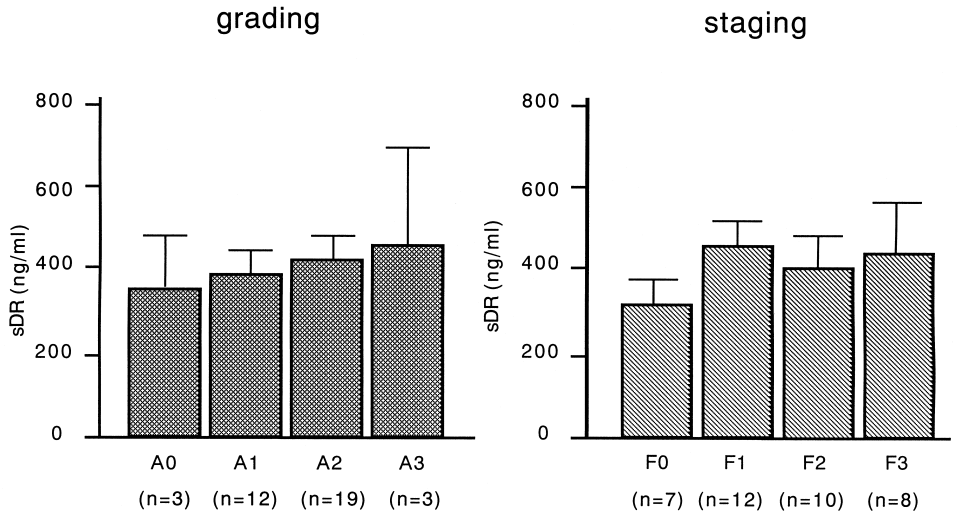

Fig. 2 Relationship between sDR levels and the scores of histological grading and staging in patients with CH C
Each value represents mean ± S.D.

was detected (data not shown).

Change in sDR levels during and after IFN treatment (Fig. 3)

Among 40 patients, 20 sustained normal serum ALT levels at 24 weeks after the end of IFN treatment without detectable serum HCV RNA. Then, they were regarded as a complete responder. The other patients were regarded as a non-responder. In patients with complete response, the sDR levels decreased by IFN treatment and sustained low, while in those with no response, sDR did not and even increased except for small decline at 2 wk. The sDR levels at 2 wk, 24wk and 28 wk (4 wk after the end of IFN

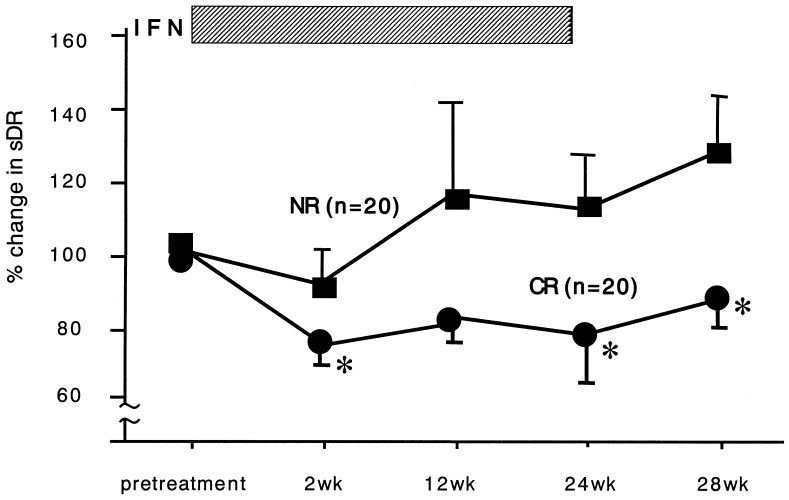

Fig. 3 Change in sDR levels during and after IFN treatment.
CR: complete responders, NR: non-responders. Each value represents mean ± S.D. *P<0.05 between CR and NR groups.

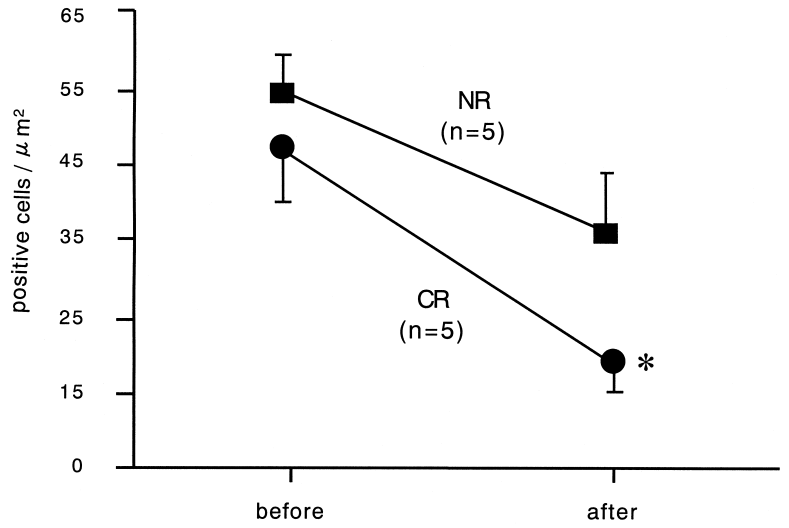

Fig. 4 Change in the number of HLA-DR antigen expressing cells in liver by IFN treatment.
CR: complete responders, NR: non-responders. Each value represents mean ± S.D. *P<0.05 between before and after treatment in CR group.

treatment) were significantly lower in complete responders than those in non-responders ($P<0.05$).

The pretreatment sDR levels were not different between complete responders (419.9 ± 212.4 [mean ± S.D.]) and non-responders (413.2 ± 263.2) (data not shown). Thus, it is difficult to predict the response to IFN by sDR before treatment.

Immunohistochemistry

The HLA-DR antigen was stained in liver biopsy specimen to analyze whether sDR would reflect the expression of HLA-DR antigen in liver. Before IFN treatment, HLA-DR antigen was stained in dendritic cells, lymphocytes and Kupffer cells in portal area, while it was stained in Kupffer cells and endothelial cells in central acinus [4].

Next, we quantitated the number of positively stained cells using con-focal laser scan microscopy. The number of positively stained cells in liver specimen before IFN treatment was not different between complete responders and non-responders (data not shown). Fig. 4 shows the effect of IFN on the number of positively stained cells. In complete responders, positive stained cells remarkably decreased below the half (from 48.6 ± 30.4 [mean ± S.D.] cells/μm^2 to 19.7 ± 13.4), while in non-responders the decline was not significant, although a similar tendency was observed (from 54.8 ± 19.8 to 36.7 ± 28.5). These results indicate that the decrease of sDR by IFN in complete responders reflects the decrease of the expression of HLA-DR antigen in liver.

DISCUSSION

The present study, for the first time, shows that the sDR levels in patients with CH C are significantly higher than normal controls. It has been reported that the sDR levels are elevated in post-transplantation [29], some kinds of malignancy [20], GVHD [29], infection [18] or collagen diseases [19]. We also reported previously the remarkable elevation in autoimmune hepatitis [21]. To know the significance of sDR as a parameter of disease activity, the correlation between sDR and various parameters, such as serum ALT or AST, was evaluated. However, we could not find any correlation and also between sDR levels and grading or staging of liver histology, supposing that sDR does not reflect the damage or necrosis of hepatic tissue. Immunohistochemistry using anti-HLA-DR mAb revealed that various types of cells, inflammatory cells such as lymphocytes or antigen presenting cells such as dendritic cells or Kupper cells, strongly expressed HLA-DR antigen. These cells are thought to be activated because such high amount of expression was not seen in the liver of normal subjects. Both sDR and HLA-DR levels in the tissue paralelly decreased not in non-responder but in complete-responders by IFN treatment. They significantly dropped in responders in as early as 2 wk after the start of treatment. On the other hand, in the non-responders, they did not decline both during and after IFN treatment except for small decline at 2 wk. In contrast, they were maintained in low levels in responders. There will be two explanations for the decrease of sDR in responders. First, IFN might rapidly decrease the amount of HCV and consequently improve inflammation in liver. The interval of 2 weeks may not be long enough for sDR to reflect the extinguishment of inflammation in liver. Second, IFN might directly affect the cells originating sDR. To clarify this hypothesis, the in vitro effect of IFN on cultured cells should be investigated. We reported that sHLA-Ⅰ, contrary to sDR, increased at 2–4 wk both in responders and non-responders, and then declined to pretreatment level only in responders [30]. IFN upregurates HLA-class Ⅰ expression of infected hepatocytes or activated lymphocytes [23, 24]. As for HLA-class Ⅱ antigens, Thiel et al. [25] reported that the expression on bile duct or hepatocytes was increased during IFN treatment and returned to the baseline after the withdrawal with no difference between responders and non-responders. While, Banner et al. [31] mentioned that no change of HLA-DR was found after IFN in CHC patients. In our cases, the effect of IFN might have appeared in responders as early as 2 weeks, reflecting subsided inflammation of the affected liver tissues. Anyway, it can be concluded that sDR could be more reliable and predictive marker of response to IFN compared with sHLA-Ⅰ.

Taken together, when the individual patient is considered, sDR reflects the number of cells expressing HLA-DR antigen in liver, which are associated with the activity

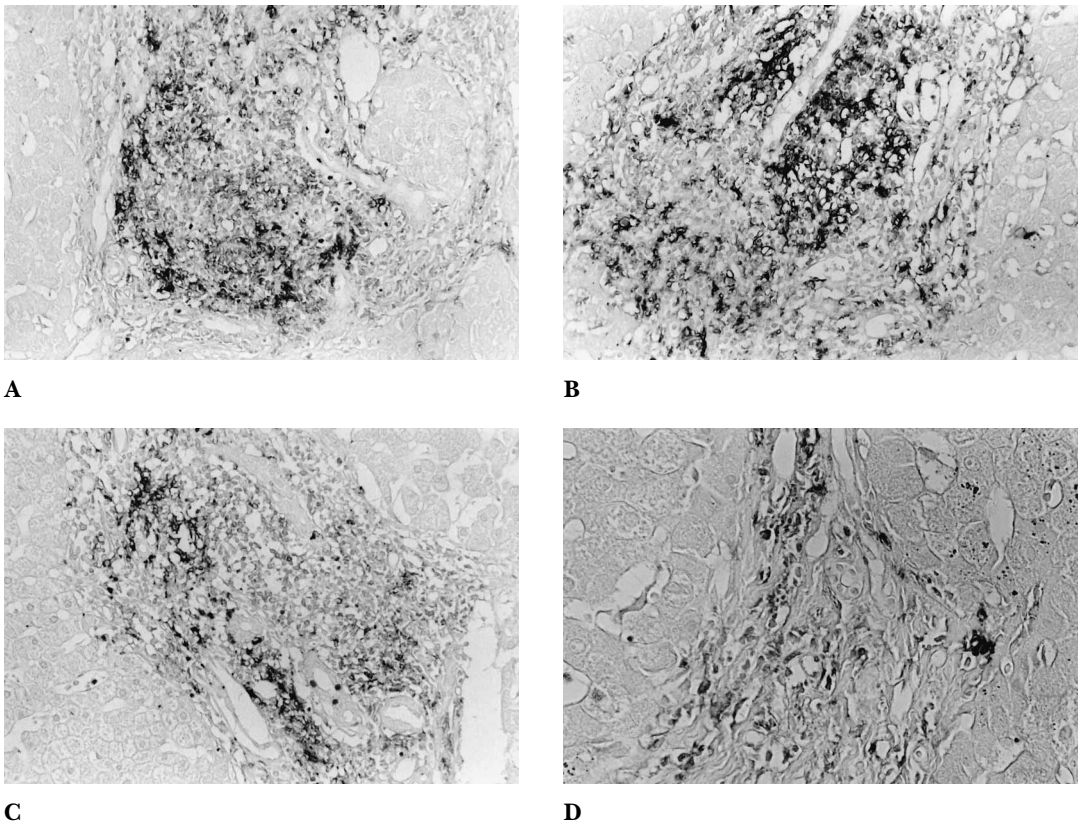

Fig. 5 Expression of HLA-DR antigen in liver before (A, B) and after (C, D) IFN treatment.
Before IFN treatment, strong reactions of HLA-DR antigen were recognized in dendritic cells, lymphocytes and Kupffer cells in a portal tract.
HLA-DR antigens-positive cells significantly decreased in number after IFN therapy in the CR group (B, D), where as they did not in NR group (A, C).

of hepatitis. Unexpectedly, there was also no correlation between sDR and levels of surface HLA in the affected liver (data not shown) at the pre-treatment point. The productivity of sHLA is sometimes influenced by the HLA allotypes [32], which might be one reason why soluble and surface HLA levels were not simply correlated. Another possibility is that sDR was originated much more from peripherally circulating cells, such as activating T lymphocytes, than from the focal tissue. In case of the HLA class I antigens, its soluble form has been demonstrated to be secreted by activated lymphocytes and possess immunomodulatory functions [33]. Thus, sDR also might be secreted to play some immunological roles.

We also evaluated the relationship between pre-treatment levels of sDR, and HCV serotype or serum viral load in order to know whether these factors would be associated with the severity of inflammation. But we could not find any relationship between them in accordance with some reports describing that disease activity is independent of HCV genotype and viral load [34, 35]. Whereas, an early (assayed at week 2) virological response was found to be a significant predictor of a complete response to IFN [36]. In the near future, the change of viral load during IFN treatment would be examined together with that of sDR values.

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