Changes in Hepatitis C Virus (HCV) Antibody Status in Patients with Chronic Hepatitis C after Eradication of HCV Infection by Interferon Therapy

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Background. Changes in hepatitis C virus (HCV) antibody status were followed for 10 years after the eradication of HCV by interferon (IFN) therapy in 30 patients with chronic hepatitis C who showed a sustained virological response.

Methods. HCV core antibody titer, third-generation HCV recombinant immunoblot assay (RIBA) grade (measuring the presence of antibodies for core, NS3, NS4, and NS5 antigens), and genotype-specific antibodies to the HCV NS4 region were measured annually with commercially available kits for these antibodies.

Results. For grade of HCV antibody determined by RIBA, the most significant decrease was observed with anti-NS5 antibody, followed by anti-NS4, anti-NS3, and anti-core antibodies, in that order. Tests for anti-NS5 and anti-NS4 antibodies had negative results in almost 50% of patients 10 years after eradication of HCV. In contrast, the results of tests for anti-core antibody were still markedly positive in most patients. However, anti-core antibody titer decreased continuously during the 10-year follow-up period. Antibodies to the NS4 region specific for HCV genotypes 1 and 2 also decreased during the follow-up period. Differences in the rate at which antibody titers decreased were observed between antibodies for genotypes 1 and 2; as a consequence, the serological type of HCV changed during the follow-up period in some patients.

Conclusions. HCV antibody titer appears to continue to decrease during the 10 years after eradication of HCV by IFN therapy.

Chronic infection with hepatitis C virus (HCV) is one of the most common infections worldwide. The number of patients with chronic hepatitis due to HCV infection is estimated at 170 million worldwide, 2.7 million in the United States, and 1.2 million in Japan. HCV infection is one of the important causes of hepatocellular carcinoma, and in addition, a relationship between HCV infection and disorders other than liver disease—such as mixed cryoglobulinemia, diabetes mellitus, and lichen planus—has been suggested [1–3].

Treatment with IFN has been used to induce the normalization of the serum alanine aminotransferase (ALT) level, with a disappearance of the HCV RNA in serum in some patients with chronic HCV infection. Patients with normal ALT levels and the absence of HCV RNA in serum >6 months after the end of IFN therapy are usually described as having a sustained virological response (SVR). In such patients, HCV RNA continues to be absent, and HCV is considered to be eradicated [4].

A few studies have focused on features of patients with SVR and have specifically focused on the incidence of hepatocellular carcinoma and the resolution of liver fibrosis [5–12]. However, no studies have examined changes in HCV antibody status in patients with SVR after eradication of HCV. In the present study, we prospectively followed-up and investigated serial changes in various HCV antibodies after eradication of HCV in patients with chronic hepatitis C who achieved SVR to IFN therapy.
PATIENTS, MATERIALS, AND METHODS

Patients. A total of 751 patients with histologically and virologically proven chronic hepatitis C received IFN therapy at Ogaki Municipal Hospital (Ogaki, Japan) during 1989–2004. Of these patients, 288 showed SVR, which was defined as the continuation of normal serum ALT levels and the absence of HCV RNA in serum >1 year after the end of IFN therapy. At present, 201 of these patients with SVR continue to undergo regular follow-up and laboratory testing as outpatients every 3–6 months. Thirty patients with follow-up periods of >10 years were analyzed in the present study. At each follow-up visit, serum samples were obtained and stored at −80°C until analyzed.

Written informed consent was obtained from each patient at the time that serum samples were collected. The entire protocol was approved by the ethics committee of Ogaki Municipal Hospital and was carried out in compliance with the Helsinki Declaration.

Serum test for HCV RNA and genotyping of HCV. The presence of HCV RNA in serum obtained from each patient at 1, 3, 5, 8, and 10 years after the end of IFN therapy was determined by nested RT-PCR [13]. HCV genotype was determined by RT-PCR with genotype-specific primers [14].

Serological tests for anti-HCV core antibody titer, recombinant immunoblot assay (RIBA) grade, and genotype-specific antibodies. Anti-HCV core antibody specific for the c22-3 antigen was measured by radioimmunoassay with a commercially available kit (Ortho HCV Cire-Ab Irma Test; Mitsubishi Kagaku Iatron) according to the standard. Semiquantitative titer of antibody against HCV was measured by a third-generation RIBA [15] with use of the Chiron RIBA HCV Test 3.0 (Chiron) according to the manufacturer’s instructions. This assay detects antibodies directed to both structural antigens (core antigen, c22 synthetic peptide) and nonstructural antigens (NS3 antigen, c33c recombinant protein; NS4 antigen, mixed 5.1.1 and c100 peptides; NS5 antigen, recombinant protein). In the assay, the intensities of colored bands on the nitrocellulose strip are proportional to amounts of bound antibody and are graded as negative, 1+, 2+, 3+, and 4+, according to the manufacturer’s instructions. Sample reactivity to superoxide dismutase, to which all the HCV antigens were fused, was also assessed.

Genotype-specific antibodies against the HCV NS-4 region were measured by ELISA [16] with an Immucheck F-HCVGr assay (International Reagents) according to the manufacturer’s instructions. Titers of genotype-specific antibodies C14-1 and C14-2 were measured. Samples with a cut-off index (COI) of >1.0 were judged as positive. Serological type 1 included samples with a C14-1/C14-2 antibody COI ratio >2 or samples positive for C14-1 antibody and negative for C14-2 antibody. Serological type 2 included samples with a C14-2/C14-1 antibody COI ratio >2 or samples negative for C14-1 antibody and positive for C14-2 antibody. The serological type was classified as 1 and 2 when the sample was positive for both C14-1 and C14-2 antibodies and the COI ratio of C14-1 to C14-2 was <2. The serological type was classified as undetermined when tests for both C14-1 and C14-2 antibodies had negative results.

RESULTS

Patient characteristics. The presence of HCV RNA in serum was confirmed before initiation of IFN therapy by nested RT-PCR in all 30 patients. The study group included 16 men and 14 women, and the mean age (±SD) was 50.5 ± 10.5 years at the start of IFN therapy. HCV genotypes, determined on the basis of Simmonds’ nomenclature [17], were 1b (10 patients), 2a (12 patients), and 2b (3 patients). HCV genotype could not be determined or was mixed in the remaining 5 patients. Histological study of the liver biopsy specimens obtained within 3 months before the start of the IFN therapy revealed activity grades to be A1 (21 patients), A2 (7 patients), and A3 (2 patients). Grades of fibrosis were F0 (5 patients), F1 (16 patients), F2 (4 patients), and F3 (5 patients), determined on the basis of the classification by Desmet et al. [18]. Twenty-three patients received IFN-α, and the remaining 7 received IFN-β.

In all 30 patients, HCV RNA was not detected in serum samples obtained at 1, 3, 5, 8, and 10 years after the end of IFN therapy, and ALT levels in serum samples were less than the normal limit throughout the follow-up period. No patients showed immunosuppression before or during IFN therapy or during the follow-up period.

Changes in annual HCV RIBA grade and HCV core antibody titer after the eradication of HCV by IFN therapy. Annual changes in semiquantitative antibody titers for HCV core protein (c22) are shown in figure 1A. In most patients, the antibody titer for c22 was maintained at 4+ during the 10 years of follow-up. However, when we analyzed HCV core antibody (c22-3) annually, the titer decreased over the 10-year follow-up period (figure 2). In contrast to the titer of HCV core antibody as determined with use of RIBA, titers for HCV NS3 (c33c), NS4 (5.1.1 and c100), and NS5 antibodies decreased serially after eradication of HCV by IFN therapy. The decrease in antibody was most marked for antibodies specific for NS5, NS4, and NS3, in that order (figures 1B, 1C, and 1D).

Next we compared the clinical characteristics of patients who had a rapid decrease of HCV antibodies after eradication of HCV with those of patients who did not have a rapid decrease. We found no difference in patient characteristics, including age, sex, pretreatment HCV RNA concentration, HCV genotype, ALT level, and liver histological findings.

Changes in HCV genotype-specific antibodies in NS4 region after the eradication of HCV by IFN therapy. Decreases in the titers of 2 kinds of HCV genotype-specific antibodies—
anti-HCV genotype 1 (1a or 1b) and anti-HCV genotype 2 (2a or 2b)—to HCV NS4 region were measured annually (figure 3). Titers of both antibodies decreased continuously after the eradication of HCV, and the rate of decrease was similar between the 2 antibodies. However, the rate of decrease of these 2 antibodies was sometimes different between genotype 1– and genotype 2–specific antibodies. This difference caused a discrepancy between the original HCV genotype and the serological data, and it resulted in the incorrect determination of the genotype of the eradicated HCV on the basis of serological typing in 2 of 30 patients (table 1).

**DISCUSSION**

There are several reports of changes in HCV antibody titers or RIBA grade in patients with acute hepatitis C after spontaneous eradication of HCV during the acute phase of the illness in a case series with a few patients [19–21]. Other reports have documented changes in HCV antibody status during and after the end of IFN therapy [22–26] and changes after spontaneous clearance of HCV in patients with chronic hepatitis C [27]. However, there have been very few reports of long-term follow-up of HCV antibody status after eradication of HCV by IFN therapy. Only Lefrere et al. [28, 29] reported the long-term changes of HCV antibody status of patients with chronic hepatitis C after eradication of HCV. They observed decreases in various HCV antibody titers in patients in whom HCV had been eradicated, including in 1 patient who experienced the eradication of HCV by IFN therapy [28, 29].

Patients with SVR are more frequently lost to follow-up after the end of IFN therapy than are other patients [12], because the eradication of HCV is often considered to be a complete cure of chronic hepatitis. Therefore, regular and long-term follow-up of patients with SVR is sometimes difficult, and that is why there are few studies of changes in laboratory data for patients with chronic hepatitis C after eradication of HCV.

In the present study, we prospectively observed HCV anti-HCV antibody titers to HCV core protein (c22p), HCV NS3 protein (c33c), HCV NS4 protein (c100p), and HCV NS5 protein (NS5). The antibody titers were graded as negative, 1+, 2+, 3+, and 4+, according to the manufacturer’s instructions. The antibody titers were measured at the end of IFN therapy and then annually. The results showed that the antibody titers decreased continuously after the eradication of HCV, and the rate of decrease was similar between the 2 antibodies. However, the rate of decrease of these 2 antibodies was sometimes different between genotype 1– and genotype 2–specific antibodies. This difference caused a discrepancy between the original HCV genotype and the serological data, and it resulted in the incorrect determination of the genotype of the eradicated HCV on the basis of serological typing in 2 of 30 patients (table 1).

**Figure 1.** Annual changes in semiquantitative titer of antibody against hepatitis C virus (HCV) after eradication of HCV, as measured by third-generation recombinant immunoblot assay (Chiron RIBA HCV Test 3.0; Chiron). Antibody titers were graded as negative, 1+, 2+, 3+, and 4+, according to the manufacturer’s instructions. A, Antibody against HCV core protein (c22p). B, Antibody against HCV NS3 protein (c33c). C, Antibody against HCV NS4 protein (c100p). D, Antibody against HCV NS5 protein (NS5). Neg, negative; Year, year after the end of IFN treatment.
body status for antigens specific to HCV core protein and to nonstructural proteins 3–5. On the basis of the results of RIBA, antibody against HCV core protein remained strongly positive (4+, according to semiquantitation) in most patients even 10 years after eradication of HCV, whereas antibodies against HCV nonstructural proteins (NS3, NS4, and NS5) weakened consecutively. Antibodies against NS4 and NS5 were absent in approximately one-half of patients 10 years after the eradication of HCV. The titer of HCV core antibody (c22-3), however, showed an annual decrease. Therefore, titers of HCV antibodies decrease regardless of their specific targets. In a more recent study, Wiegand et al. [27] reported the lack of a decrease in HCV antibody titer after eradication of HCV by IFN therapy in patients with chronic hepatitis C—in contrast to a marked decrease in patients with acute hepatitis C treated with IFN therapy—in a study involving patients who were observed for up to 80 weeks after completion of IFN therapy. In contrast, Lefrere et al. [28] observed a disappearance of HCV antibodies, except for antibody to HCV core protein. Although the antibody discussed in the study by Wiegand et al. [27] is different from that reported in ours, our study provided evidence of a decrease in HCV antibody following eradication of HCV even in patients with chronic HCV infection.

Pawlotsky et al. [30, 31] reported a difference in the reactivity of antibodies between HCV genotypes with use of a second-generation RIBA kit but not with a third-generation RIBA kit. In keeping with their findings, we observed no difference in reactivity between genotypes and no difference in the rate of decrease of antibody titers between patients with HCV genotypes 1 (1b) and 2 (2a or 2b) in an assessment with a third-generation RIBA kit. In addition, we found no patient pre-

Table 1. Changes in hepatitis C virus (HCV) genotype-specific antibody titers and determination of serotype for 2 patients, by year after IFN treatment.

<table>
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<th>Variable</th>
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<th>Year after IFN treatment</th>
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<sup>a</sup> Serological type 1 included samples with a C14-1/C14-2 antibody cut-off index (COI) ratio >2 or samples with that were positive for C14-1 antibody and negative for C14-2 antibody. Serological type 2 included samples with a C14-2/C14-1 antibody COI ratio >2 or samples that were negative for C14-1 antibody and positive for C14-2 antibody. The serological type was classified as 1 and 2 when the sample was positive for both C14-1 and C14-2 antibodies and the COI ratio of C14-1 antibody to C14-2 antibody was <2.
treatment characteristics that influenced the rate of decrease in HCV antibody titer. Additional studies are needed to elucidate the factors that affect the rate of decrease in the HCV antibody titer after eradication of HCV.

Previous studies have revealed that the antigenicities of HCV polypeptides differ according to genotype in some particular protein sequences, such as the core region or NS4 [32–34]. Typing techniques that detect genotype-specific antibodies have been developed as a means of serotyping to [16] and are clinically useful for determination of HCV type [35, 36]. Serological type can be used for HCV typing when HCV is absent from serum [37] (for example, in patients with acute-phase self-limiting hepatitis C or patients with chronic hepatitis C in whom HCV was eradicated by antiviral therapy).

Maertens et al. [38] reported that antibodies to NS4 are usually cleared after resolution of HCV infection. In the present study, genotype-specific antibodies to NS4 were detected in many patients during follow-up but continued to decrease annually. In addition, a difference in the rate of decrease in titer between genotype-specific antibodies 1 and 2 was sometimes observed, and this caused a discrepancy between the serotype and genotype of the eradicated HCV in 2 patients. Thus, we found that serotype does not always correspond to eradicated HCV genotype in patients with SVR, and one should be careful to take this into account when genotype of eradicated HCV is analyzed.

In conclusion, HCV antibody titers decrease during the 10 years after eradication of HCV by IFN therapy. Because a decrease in HCV antibody represents, in part, the changes in immune status associated with HCV infection, this decrease in HCV antibody titer may be associated with changes caused by diseases related to HCV infection. These include, not only liver disease, but also extrahepatic disorders, such as mixed cryoglobulinemia, diabetes mellitus, lichen planus, and thyroid disease. Additional studies are needed to clarify the mechanisms of persistence and clearance of HCV antibody after the eradication of HCV and to investigate the association between the decrease in HCV antibody titers and patient immune status in patients with SVR. In addition, further studies are needed to examine the association between the decrease in HCV antibody titers and changes in extrahepatic manifestations associated with chronic HCV infection in patients with SVR.

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References


