The Kinetics of Antibodies against Hepatitis C Virus May Predict Viral Clearance in Exposed Hemophiliacs

Philippe Lanotte, Frédéric Dubois, Sophie Le Pogam, Claude Guerois, Béatrice Finbel, Yannick Bacq, Yves Gruel, Alain Goudeau, and Francis Barin

Laboratoire de Virologie, Centre Hospitalier Universitaire Bretonneau; Centre de Traitement de l’Hémophilie and Service de Gastro-Entérologie, Centre Hospitalier Trousseau, Tours, France

Several studies have reported the spontaneous loss of hepatitis C virus (HCV) antibodies in HCV-exposed persons. However, the relationship between seroreversion and spontaneous virus clearance has yet to be precisely determined in a single homogeneous population of untreated immunocompetent patients. In this study, 32 human immunodeficiency virus–seronegative hemophiliacs who had been exposed to HCV were followed for a mean duration of 141 months; 22 remained chronic carriers (68.8%). All but 1 of the nonviremic patients (90.0%) showed partial (8 cases) or complete (2 cases) seroreversion. In contrast, all but 1 of the viremic patients (95.1%) had a stable serologic profile when analyzed by a recombinant immunoblot assay. The results indicate that any HCV antibody–positive immunocompetent patient with no detectable serum HCV RNA and normal alanine aminotransferase values and whose serial samples show a progressive decrease in the level of HCV antibodies present may be considered as having a resolved infection.

Hepatitis C virus (HCV) infection is often characterized by a chronic carriage of the virus, leading to chronic hepatitis, cirrhosis, and liver cell carcinoma [1, 2]. It is frequently assumed that at least 50% of exposed persons remain chronically infected after primary infection [1]. Initial diagnosis of HCV infection is by detection of HCV antibody by EIA. This technique has been greatly improved over the last few years [3, 4]. However, the presence of HCV antibody in a serum sample means that the patient has been exposed to the virus but does not necessarily mean that the patient harbors the virus [3].

Therefore, detection of HCV RNA in serum by reverse transcriptase–polymerase chain reaction (RT-PCR) in the serum is the standard method for characterizing the infection outcome. A positive test demonstrates the presence of the virus and is often indicative of persistent infection, whereas a negative test shows clearance of the virus, indicative of recovery. Although highly sensitive, this approach has limitations due to the methodology itself (cutoff level of detection) and the biologic properties of HCV infection, such as the intermittent viremia observed in some cases [5]. Therefore, additional markers are necessary to confirm spontaneous clearance of the virus in HCV antibody–positive patients and to correctly advise the patients. A few reports have shown that the decrease or disappearance of HCV antibodies may be predictive of self-limited infection [6–9]. We undertook a retrospective longitudinal study in a cohort of immunocompetent hemophiliacs with long-term follow-up to investigate this hypothesis.

Patients and Methods

Patients. One hundred seven hemophiliacs were followed as outpatients between July 1995 and December 1996 in our hemophilia treatment center. As the aim of this study was to analyze serologic changes in HCV antibody levels with disease progression, we retained only patients who had been followed for at least 5 years with regular visits and for whom sequential samples were available for the analysis. Patients who tested positive for antibody to the human immunodeficiency virus (HIV) were excluded due to the potential influence of immunodeficiency on the HCV serologic profile. Forty-six patients fulfilled the criteria for inclusion in this analysis, 40 suffering from hemophilia A and 6 from hemophilia B. The mean age of patients in 1995–1996 was 33 years (range, 11–82).

Methods. HCV antibody was detected by EIA using a third-generation assay (HCV 3.0 ELISA test system; Ortho, Raritan, NJ) and serologic profiles were analyzed using the third-generation recombinant immunoblot assay (RIBA HCV 3.0 SIA; Chiron, Emeryville, CA). Use of RIBA made it possible to semiquantify antibodies to the core antigen and to nonstructural proteins NS3, NS4, and NS5 (intensity 0 to 4+ when following the manufacturer’s recommendations). A decrease of at least 2+ of the intensity was necessary to reach a conclusion of seroreversion for a given antibody specificity.

For every patient, whether positive or negative for HCV antibody in 1995–1996, the oldest available serum sample was also tested to make it possible to date the seroconversion period for those who tested positive and to detect possible serologic proof of previous infection for those who tested negative. The oldest serum sample that we tested was collected in April 1981. Changes in the serologic profile were analyzed by RIBA using at least 3 serum samples (early, intermediate, late) for every HCV antibody–positive patient. All the RIBA determinations for a given patient were performed using the same batch of reagents. The mean duration of serologic profile follow-up was 141 months (median, 151; range, 61–184).
Viremic status was analyzed by testing for HCV RNA in serum samples by RT-PCR using a commercially available assay (AmpliCor; Roche Diagnostic Systems, Branchburg, NJ). At least two samples, collected at intervals of several months between July 1995 and December 1996, were tested for each patient to determine whether the patient was viremic or nonviremic.

Genotyping was done using the InnoLIPA HCV II assay (Inno-genetics, Ghent, Belgium) for patients whose sera contained HCV RNA. The HCV infection was serotyped using the second-generation assay from Murex (HCV serotyping 1-6 assay; Murex, Dartford, UK) when patients tested negative for HCV RNA.

Alanine aminotransferase (ALT) concentrations were determined routinely for each patient (1–6 determinations/patient between July 1995 and December 1996). The upper limit of normal for ALT levels was 45 IU/L.

Fisher’s two-tailed exact test was used to assess statistical differences between compared groups.

Results

Of the 46 patients, 30 (65.2%) tested positive for HCV antibody in 1995 to 1996. All had tested positive using the oldest available serum sample, except 1, whose follow-up began in April 1985 and who seroconverted in April 1986. By studying previous sequential serum samples, we found that 2 of the 16 HCV antibody–negative hemophiliacs had previously tested positive. Only the oldest serum sample from 1 of them, collected in 1986, contained HCV antibody. At that time, RIBA detected antibodies to both NS3 and NS4 as faint bands. Subsequent serum samples always tested negative. For the other patient, the oldest serum sample tested strongly positive (maximum intensity for antibody to core, NS3, and NS4), and then a regular decrease in antibody levels was observed until they were no longer detectable in 1996 (case 2, figure 1A).

Of the 30 HCV antibody–positive patients, 22 (73.3%) tested positive for HCV RNA (viremic patients). The nonviremic patients tested negative for HCV RNA for every serum sample tested. The viremic patients tested HCV RNA–positive for every serum sample tested, except in 1 case. This particular patient tested HCV RNA–positive in 3 of 4 blood samplings. The nonviremic patients never had high ALT levels (figure 2), whereas 3 categories of viremic patients could be identified on the basis of ALT levels: those with values always below the upper limit, those with values fluctuating around the upper limit, and those with consistently high values. These data support the notion that nonviremic patients efficiently cleared the virus.

There were striking differences between the sequential serologic profiles of viremic patients and nonviremic patients. All but 1 patient positive for HCV RNA (95.1%) had stable serologic profiles, whereas a decrease in the level of antibodies was observed in 7 (87.5%) of the 8 nonviremic patients (P < .001). Representative cases are shown in figure 1. The mean time of follow-up was similar for both categories of patients: 11.3 years for viremic and 12.5 years for nonviremic patients.

The single viremic patient with decreasing antibody levels showed reduced anti-NS4 reactivity (from 4+ to 2+) but strong, stable reactivity for the other antigens. All the other viremic patients had antibodies to the core and NS3 antigens, 20 (91%) also had anti-NS4 antibodies, and 16 (73%) had antibodies against NS5. All reactivities detected were strong. All 7 nonviremic patients whose antibody levels decreased had antibodies to core, NS3, and NS4 antigens in the earliest sample, and 5 also tested positive for NS5 antibody at this early stage, with maximal reactivity only to the core and NS3 antigens. The antibodies against the core antigens remained stable for longer periods. They were still present on the last sample of all these patients. Antibodies against the NS5 protein disappeared faster and more often than those against other proteins. Only 1 of these nonviremic patients had a stable level of NS5 antibodies.

To quantify the serologic profile, we calculated for each patient an RIBA ratio, defined as the sum of the reactivities to the 4 antigens (value 0–4 for each antigen) divided by the number of reactive antigens detected with the RIBA performed at the last visit. This ratio was at least 3.5 for all the viremic patients (100%) but was at least 3.5 in only 2 nonviremic patients (25%), showing strong association of this simple serologic analysis with the RT-PCR result. There was no difference in the genotype or serotype distribution of viremic and nonviremic patients.

Discussion

Several studies have reported the spontaneous loss of HCV antibodies in HCV-exposed persons [6–9]. This has been called HCV seroreversion and can be full (complete disappearance of HCV antibody) or partial (disappearance or decreased level of antibodies to several but not all HCV antigens). It has been observed in persons coinfected with HIV [9–12]. However, in these cases, immunodeficiency associated with HIV infection makes interpretation of the HCV serologic data difficult. It has been also observed in immunocompetent persons who seroreverted after complete response to interferon-α (IFN-α) therapy [9, 13]. The impact of some of these studies was reduced by the inclusion of small numbers of cases and short-term follow-up. Moreover, most used the less sensitive first- or second-generation assays.

We conducted this retrospective study to investigate the frequency and biologic characteristics of spontaneous HCV seroreversion in a homogeneous population of immunocompetent persons not treated with IFN-α, with long-term follow-up. Therefore, we believe that this work provides informative and relevant data, contributing to our understanding of the natural history of HCV infection and to the better interpretation of the virologic markers associated with it.

We found an almost complete correlation between seroreversion and resolved infection, on the basis of nonviremic status in HCV-exposed hemophiliacs. These full or partial serorever-
sions may be due to the loss of antigenic stimulations resulting from elimination of the virus, whereas a stable serologic profile with a strong reactivity is probably due to continuous antigenic stimulation associated with chronic replication of the virus. This observation has direct practical consequences for counseling. Indeed, one of the key questions when a patient is identified as HCV antibody–positive is how to distinguish between a chronic infection, with all its consequences for the future health of the patient, and more favorable self-limited infection. Chronic infection is easily diagnosed on the basis of persistent viremia detected by RT-PCR and/or consistently high ALT values. The firm diagnosis of self-limited infection is more difficult, mainly because chronic infection can be associated with intermittent viremia (false-negative RT-PCR) and normal ALT values despite liver injury [1, 3, 5, 14]. Therefore, in addition to the absence of HCV RNA by RT-PCR and normal ALT levels, surrogate markers are necessary.

The kinetics of HCV antibody, analyzed by an immunoblot assay such as RIBA, can be used to improve reliability of diagnosis. Other assays facilitating dissection of the antibody response to HCV could be used to provide the same information [15]. On the basis of our data and previous reports, any HCV antibody–positive immunocompetent patient with no detectable serum HCV RNA and normal ALT values and whose serial samples show a progressive decrease in antibody levels may be considered to have a resolved infection. Further evaluations of a larger number of cases should demonstrate the usefulness of the RIBA ratio, the sum of reactivities to the 4 antigens (value 0–4 for each antigen) divided by the number of reactive antigens, in predicting self-limited infection on the basis of a single serum sample.

This study also provides information on the frequency of self-limited HCV infections. Of 32 persons exposed to the virus, 30 were still HCV antibody–positive many years after infection and 2 had lost their HCV antibody. Twenty-two were HCV RNA–positive. Crude analysis suggests that chronic infection occurred in 68.8% of these patients and resolution of infection in 31.2%. However, this estimate must be treated with caution. Due to the iterative nature of their treatment, hemophiliacs may have been exposed to a number of infectious batches. Therefore, the frequency of chronic carriage may be overestimated in this population compared with patients ex-

Figure 1. Representative cases of sequential serologic profiles analyzed by RIBA. A, HCV RNA–negative patients showing seroreversion. B, HCV RNA–positive patients with stable profile. Positions of various antigens on strips are indicated on positive serum control (top left of each panel); C = core.
posed to the virus only once. It is also possible that some HCV antibody–negative hemophiliacs were HCV antibody–positive before inclusion in the cohort, also resulting in overestimation of the frequency of chronic carriage. On the basis of our data, it seems probable that chronic HCV infection does not occur in >70% of exposed persons. Prospective cohort studies are necessary to investigate this issue further.

References
1. Alter HJ. To C or not to C: these are the questions. Blood 1995;85:1681–95.