the other two were natural elevations. In the post-IFN relapse, the elevation of HCV core antigen was observed before the elevation of ALT. HBV-DNA became positive between the HCV core antigen elevation and ALT elevation. In natural elevations, neither the HCV core antigen elevation nor the HBV-DNA elevation was observed before the ALT elevation. HBV-DNA was positive after the ALT elevation (Fig. 1a). For patient 2, a post-IFN relapse and a natural elevation were observed. In the post-IFN relapse, the elevation of HCV core antigen was observed before the elevation of ALT, and HBV-DNA was positive when the ALT reached the peak. In the natural ALT elevation, HBV-DNA was positive after the ALT elevation (Fig. 1b). For patient 3, post-IFN relapse and a natural elevation were observed. In the natural elevation HBV-DNA was positive after the ALT elevation. The elevation of HBV-DNA did not occur before ALT flares, but occurred at the same time or after the ALT flares. (Fig. 1c).

## DISCUSSION

Our RTD–PCR method indicated that HBV-DNA was amplified in eight (19.5%) HCV-related CLD patients and one (2.4%) healthy volunteer in a matched cross-sectional study. This difference was significant ($P < 0.05$). The prevalence of occult HBV infection in HCV-related CLD has been reported to be 21–87%.[4–6,9,11] For the detection of occult HBV infection, most studies applied nested PCR. In this study, an RTD–PCR method was used and the detection limit was 100 copies/mL. The detection limit was minimal, stable and reproducible for the quantitation of HBV-DNA. Because of this detection limit, the HBV-DNA detection rate in this study was slightly lower than the rates in the previous reports.[11]

All the occult HBV-positive patients in our study were positive for anti-HBc, as also found in some previous reports.[8,19] Our data showed that anti-HBc was found in 18/41 (43.9%) HCV-related CLD patients and 52/230 (22.6%) healthy volunteers. Our result was slightly higher than the results of others, who reported 32–38% anti-HBc positivity in HCV-related CLD patients.[4,5,10] There may be regional differences, however, another reason may be the difference in the sensitivity of the assays. For example, using the RIA/EIA method, the percentage inhibition is divided into three categories: true positive, true negative, and in between. In our data, true positive and in between were defined to be positive, as reported by others.[8,20] Lower prevalence data might have defined positive as true positive. The prevalence of anti-HBc in healthy volunteers who attended a periodical medical check up has not been reported, although that of anti-HBc in blood donors has been reported to be 1–4%.[21,22] The different prevalence could depend on the background of the blood donors in other studies and the healthy volunteers in our study; in particular, the mean age of the blood donors was 36 years compared with 60 years for the healthy volunteers. Thus, a comparison of these two groups is not adequate.

No correlation between occult HBV-DNA fluctuation and serum ALT fluctuation in a serial sample study has been reported. Our serial data were similar to the data in other studies,[6,23] in that occult HBV-DNA was not detected continuously. As HBV-DNA appearance after ALT elevation was found in some of these patients, this might reflect the release from the liver. More sensitive quantitative PCR assays might reveal precisely the

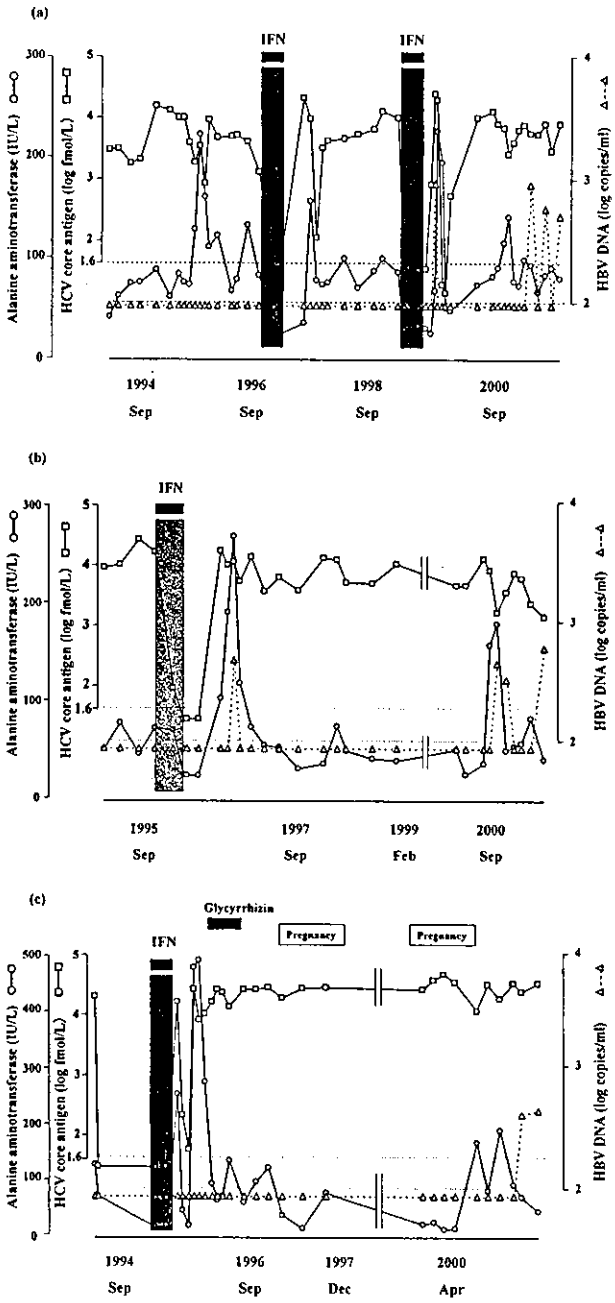


**Figure 1** Clinical course of chronic liver disease patients based on hepatitis B virus (HBV)-DNA levels, hepatitis C virus (HCV) core antigen levels, and alanine aminotransferase (ALT) levels. (△) HBV-DNA levels, (□) HCV core antigen levels, (○) ALT levels. The upper and lower dashed lines indicate the lower detection limit of HCV core antigen and HBV-DNA, respectively. (a) Patient 1, (b) patient 2, (c) patient 3. IFN, interferon.

manner in which the viral loads change. It has been reported that HCV core protein inhibits HBV-DNA replication in HCV and HBV dual infection.[24,25] The HCV core protein is thought to suppress the activity of HBV enhancer 1 and 2.[25] The HCV core antigen might have some effect on the fluctuation of HBV-DNA. However, no correlation was shown between the occult HBV-DNA fluctuation and serum HCV core antigen levels in this study.

In conclusion, the prevalence of occult HBV infection was significantly higher in HCV-related CLD patients than in age-, sex-, and anti-HBc positivity-matched-healthy volunteers, although the fluctuation of occult HBV-DNA did not directly affect the serum ALT flares. The clinical significance of occult HBV infection in HCV-related CLD is not still clear as yet, and further studies are needed.

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