Lack of Polymerase Chain Reaction Amplification in the 5' Region of a Hepatitis C Virus Isolate

Colleagues—Hepatitis C virus (HCV) infection can be assessed by the detection of viral RNA by reverse transcription (RT) and nested polymerase chain reaction (PCR). The sensitivity of this technique allows early diagnosis in acute infection, evaluation of infectivity of serum positive or negative for HCV antibodies, and monitoring of antiviral therapy. Since significant variability of the HCV genome has been observed [1–3], the choice of a set of primer sets efficient for RT and PCR. Indeed, several groups have reported a correlation between detection of HCV RNA evaluation of this patient’s serum was repeated in the same batch and indeterminate data for 5-1-1, c100-3, and c33c antigens. HCV RNA evaluation of this patient’s serum was repeatedly negative with our 5' region primer set and positive with an NS5 region primer set (N92/N94/N95) [8].

Experiments were carried out on two serum samples, independently collected at a 1-week interval with each sample studied in duplicate. The amplification protocol was an RT-nested PCR done under low stringency conditions for primer hybridization; the annealing temperature was 37°C. The 5' primer set allowed the amplification of a 177-bp DNA fragment that has never been demonstrated with either ethidium bromide-stained agarose gel or hybridization in low stringency conditions with a specific oligonucleotide probe. However, with the NS5 primer set, amplified DNA fragments of the expected size were visualized directly on the agarose gel after the first (730 bp) and second (405 bp) ampliﬁcations. An internal oligonucleotide probe hybridized speciﬁcally with these two ampliﬁed fragments.

To confirm the speciﬁcity of this ampliﬁcation, the 405-bp-ampliﬁed DNA fragment was cloned in pBluescript KS (−) (Stratagene/Ozyme, Paris) and partly sequenced with the digestion and cloning method (170 bp). Sequencing was done on two strands of the 170-bp DNA fragment. The sequence obtained revealed a homology of 88.2%, 90.6%, and 77.6% with two other published

References

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HHV-6. Although there has been no direct proof of protection by maternal HHV-6 antibodies, several studies [3, 6, 7], including this one, have found that HHV-6 infection increases after the first few months of life, suggesting a protective role for the maternal antibody. In addition, our findings that all patients were seronegative before they became infected strongly supports the possibility that infection seldom occurs before maternal antibody disappears.

However, babies could not be protected long. We found that babies usually were born with a low anti-HHV-6 titer and lost the antibody within 6 months. After the protection was lost, they were easily infected by HHV-6 since other members in the home consistently excrete HHV-6 [7, 8]. The high chance (51%) of infection from 6 to 12 months of age may also be related to the frequent oral exploration of the environment by children of this age group, which allows contact with saliva or saliva-contaminated fomites.
HCV sequences [2, 3] and the HCV prototype [9], respectively (figure 1).

Finally, we examined serum from a patient who was positive for HCV antibodies by ELISA, indeterminate by RIBA, and positive by RT-nested PCR with the NS5 primer set. Partial sequence analysis of the amplified fragment showed a high homology (90.6%) with one Japanese isolate [3]. Surprisingly, serum from this patient was not amplified by a primer set located in the conserved 5' noncoding region of the HCV genome. The lack of amplification could result from a genetic variability ranging from a point mutation located at the 3' end of a primer to a large deletion in the 5' noncoding region. Thus, we suggest that HCV RNA negativity with a 5' region primer set might be confirmed with a primer set located in another conserved region in a patient positive for HCV antibodies by ELISA and indeterminate or positive by RIBA.

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References


