JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1995, p. 2202–2203 Vol. 33, No. 8
0095-1137/95/$04.00+0

Tear Fluid of Hepatitis C Virus Carriers Could Be Infectious

HEINZ-HUBERT FEUCHT,[1]* BERNHARD ZÖLLNER, MATTHIAS SCHRÖTER,[1]
HANS ALTROGGE,[2] AND RAINER LAUFS[1]

Institut für Medizinische Mikrobiologie und Immunologie[1] and Kinderklinik,[2]
Universitäts-Krankenhaus Eppendorf, 20246 Hamburg, Germany

Received 1 March 1995/Returned for modification 13 April 1995/Accepted 9 May 1995

For up to 20 to 40% of patients chronically infected with hepatitis C virus (HCV), the mode of transmission is still unknown. We demonstrate that tear fluid contains HCV RNA-carrying material with the properties of infectious virus and conclude that smear infection with tear fluid may play a role in HCV transmission.

At present, intravenous drug abuse is the most important known risk factor for the transmission of hepatitis C virus (HCV), which is mainly transmitted by blood (1). Different epidemiological studies have revealed that for up to 20 to 40% of patients chronically infected with HCV, no known risk factors for HCV transmission can be demonstrated (1, 6). In order to decide whether pathways other than blood can play a role in HCV transmission, we investigated tear fluid, which contains large amounts of HCV-carrying material (3, 7).

RNAs from 50 μl of tear fluid, collected with microcapillaries, and from 50 μl of blood plasma were extracted (2) from 76 patients chronically infected with HCV who were positive by HCV antibody testing (second-generation HCV enzyme immunoassay proved by a four-antigen recombinant immunoblot assay [4]) and who had clinical evidence of hepatitis (abnormally elevated alanine aminotransferase levels). Thirty-six HCV-negative individuals served as controls. RNA was examined by reverse transcription PCR (RT-PCR) in parallel, as recently described (3). cDNA was synthesized from RNA with 200 U of Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Grand Island, N.Y.) and 75 pmol of cDNA primer. PCR was performed in the 5′ untranslated region of the HCV genome, as previously described (3). Amplification products were analyzed by electrophoresis on 2% NuSieve 3:1 agarose (Biozym, Hameln, Germany). After alkali blotting on nylon membranes (Diagen, Hilden, Germany), amplification products were hybridized under stringent conditions with a ^{32}P-labeled HCV-specific oligonucleotide and exposed to Kodak XAR-5 film overnight.

All 76 patients chronically infected with HCV were positive by RT-PCR for tear fluid and plasma. Tear fluid regularly showed a stronger RT-PCR signal, compared with that of blood plasma. All 36 controls were negative.

We have previously shown that HCV RNA is present in tear fluid (3). To decide whether the positive RT-PCR results of these 76 patients were caused by free HCV RNA or complete viral particles, we analyzed both tear fluid and blood plasma samples from six patients by sucrose density gradient (SDG) equilibrium centrifugation. Therefore, 50 μl of tear fluid, collected with microcapillaries, and 50 μl of blood plasma were laid on 4.8 ml of an 8 to 56% sucrose gradient dissolved in 20 mM Tris-HCl (pH 7.4)–150 mM NaCl. After centrifugation in a Beckman SW40 rotor at 140,000 × *g* for 40 h at 4°C, 10 fractions of 500 μl each were collected from the bottom of the tube. Extraction of RNA, amplification by PCR, and hybridization were performed as described above. For five tear fluid samples, SDG equilibrium centrifugation revealed two peaks after RT-PCR, at densities of ≤1.09 and 1.17 g/ml. The tear fluid sample of one of these six patients presented only one peak, at a density of 1.17 g/ml. The six plasma samples showed positive RT-PCR results, with peaks at ≤1.09 and 1.17 g/ml after SDG centrifugation (Fig. 1).

Genotyping, as recently described by Simmonds et al. (8), revealed that three of these six patients were infected with HCV subtype 1a; the other three patients were infected with HCV subtype 1b. There was 100% nucleotide sequence homology between isolates of tear fluid and blood plasma from each patient.

We determined a density of between 1.06 and 1.17 g/ml for HCV RNA-carrying material in blood plasma specimens as well as in tear fluid specimens. Recent studies (5, 9) have demonstrated that HCV RNA-carrying material, which bands at these densities, correlates with the density of the complete infectious virus. In particular, HCV RNA-carrying material that bands at a low density (≤1.09 g/ml), as observed for five of our six patients, has been described as highly infectious (5). In tear fluid from one of these six patients, we found only one peak, at a density of 1.17 g/ml. This higher density of HCV RNA-carrying material may have been due to the fact that HCV was either bound to immunoglobulins or had lost its envelope (5).

Our results demonstrate that tear fluid is a suitable diagnos-

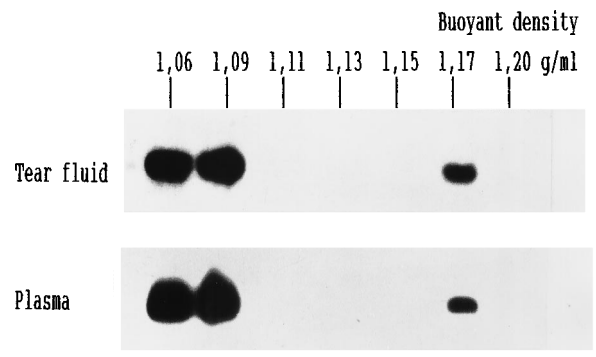

FIG. 1. Density distribution of HCV genomes in tear fluid and blood plasma samples after SDG equilibrium centrifugation. HCV genomes were detected by RT-PCR and subsequent hybridization with ^{32}P-labeled HCV-specific oligonucleotides. The buoyant density of each fraction is shown at the top. HCV RNA peaks were found at densities of ≤1.09 and 1.17 g/ml. In particular, particles with densities of ≤1.09 g/ml have been shown to correspond with infectious HCV (5).

* Corresponding author. Mailing address: Institut für Medizinische Mikrobiologie und Immunologie, Universitäts-Krankenhaus Eppendorf (UKE), Martinistr. 52, 20246 Hamburg, Germany.

tic material for detection of HCV RNA. Tear fluid of a person infected with HCV most likely contains infectious HCV particles and may therefore play a role in smear infection. Final proof may be achieved by inoculation of chimpanzees.

REFERENCES

1. **Alter, M. J., H. S. Margolis, K. Krawczynski, F. N. Judson, A. Mares, W. J. Alexander, P. Hu, J. K. Miller, M. A. Gerber, R. E. Sampliner, E. L. Meeks, and M. J. Beach.** 1992. The natural history of community-acquired hepatitis C in the United States. N. Engl. J. Med. **327:**1899–1905.
2. **Chomczynski, P., and N. Sacchi.** 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
3. **Feucht, H. H., S. Polywka, B. Zöllner, and R. Laufs.** 1994. Greater amount of HCV-RNA in tears compared to blood. Microbiol. Immunol. **38(2):**157–158.
4. **Feucht, H.-H., B. Zöllner, S. Polywka, and R. Laufs.** 1995. Study on reliability of commercially available hepatitis C virus antibody tests. J. Clin. Microbiol. **33:**620–624.
5. **Hijikata, M., Y. K. Shimizu, H. Kato, A. Iwamoto, J. W. Shih, H. J. Alter, R. H. Purcell, and H. Yoshikura.** 1993. Equilibrium centrifugation studies of hepatitis C virus: evidence for circulating immune complexes. J. Virol. **67:** 1953–1958.
6. **Laufs, R., S. Polywka, H. H. Feucht, M. Ebeling, L. Iske, K. Friedrich, G. Oehler, M. Keitel, H. Nolte, and B. Thiele.** 1994. Was bedeutet der Befund "HCV-Antikörper positiv"? Dtsch. Ärzteblatt **91(5):**285–287.
7. **Shimazako, J., K. Tsubota, Y. Fukushima, and M. Honda.** 1994. Detection of hepatitis C virus RNA in tears and aqueous humor. Am. J. Ophthalmol. **118(4):**524–525.
8. **Simmonds, P., E. C. Holmes, T. A. Cha, S. W. Chan, F. McOmish, B. Irvine, E. Beall, P. Yap, J. Kolberg, and M. Urdea.** 1993. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J. Gen. Virol. **74:**2391–2399.
9. **Thomssen, R., S. Bonk, and A. Thiele.** 1993. Density heterogeneities of hepatitis C virus in human sera due to the binding of β-lipoproteins and immunoglobulins. Med. Microbiol. Immunol. **182:**329–334.