Follow-up of hepatitis C virus infection in chimpanzees: determination of viraemia and specific humoral immune response

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Chimpanzees were inoculated intravenously with the H strain of hepatitis C virus (HCV), and analysed for viraemia using the polymerase chain reaction and for a humoral immune response using first and second generation anti-HCV ELISAs and an immunoblot assay (4-RIBA). In all seven chimpanzees studied, viraemia occurred several weeks before a significant increase in serum alanine transferase (ALT) activity, whereas the first circulating anti-HCV antibodies became detectable at the time of significant increase in ALT levels, provided the second generation ELISA or 4-RIBA was used. On the basis of the duration of viraemia the chimpanzees studied could be assigned to two different groups: those in which viraemia disappeared in conjunction with or shortly after seroconversion, and those remaining viraemic for many weeks after the appearance of antibodies. The clearance of HCV from the circulation did not correlate with the antibody pattern determined using 4-RIBA, i.e. the HCV-specific assays currently available do not enable us to predict whether an infected chimpanzee will develop persistent viraemia. Only two of the seven chimpanzees analysed developed anti-core protein (c-22) antibodies, which appeared at the same time as the first ALT peak, whereas all animals developed antibodies to the non-structural protein, c-33, and these antibodies persisted.

Only after the hepatitis C virus (HCV) genome had been cloned by Choo et al. (1989) was it possible to develop specific assays, such as ELISA, recombinant immunoblot assay (RIBA) and the polymerase chain reaction (PCR), to diagnose HCV infection. These assays can now be used to study the development of viremia and the specific immune response in HCV-infected chimpanzees, the only experimental animals sensitive to HCV. Shimizu et al. (1990) published a study on the early stages of HCV infection in chimpanzees, focusing on the determination of viremia by PCR. Their study of the humoral immune response was limited to the first generation assay, detecting antibodies to c-100 only, a recombinant polypeptide that represents part of a non-structural HCV protein.

We have extended these studies on HCV viremia and the specific immune response in chimpanzees using the assays developed most recently. The seven animals investigated were positive (HCV-infected) controls from studies in which the inactivation of HCV by various methods included in the procedures for manufacturing human plasma proteins for therapeutic use had been studied (Mauler et al., 1987). The HCV isolate used for spiking the source plasma preparations in these studies was the human H pool plasma, now designated H strain (Ogata et al., 1991). Chimpanzees inoculated with HCV-spiked human plasma or plasma derivatives were regarded as being HCV-infected when the serum alanine transferase (ALT) activity increased significantly, and infections with hepatitis A virus (HAV), hepatitis B virus (HBV) and the human herpesviruses, cytomegalovirus (CMV) and Epstein–Barr virus (EBV), could be excluded.

We demonstrate that the chimpanzees either cleared the infection or developed persistent viremia despite the presence of serum antibodies which became detectable at approximately the same time as the first peak of the increase in ALT levels.

The inoculum for all chimpanzees infected was diluted with human plasma. A sample of the HCV H strain isolate containing $10^{6.5}$ chimpanzee 50% infectious doses (CID50)/ml of HCV (Feinstone et al., 1981) was kindly provided by Dr R. H. Purcell (National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md., U.S.A.) (Ogata et al., 1991). According to the aim of our former HCV safety studies, various amounts of this virus
sample were used to spike human plasma. Aliquots of various spiked HCV source plasmas were then inoculated intravenously into the chimpanzees, giving between $10^{3.2} \text{CID}_{50}$ and $10^{5.5} \text{CID}_{50}$ per chimpanzee. The animals used had experienced neither an HBV or HCV infection before inoculation with the HCV-spiked plasma samples. Furthermore, they had normal ALT activities, as determined weekly over a period of 3 months before HCV inoculation. Infections of these chimpanzees with HAV, CMV and EBV were excluded by assaying for specific antibodies before HCV inoculation and at the end of the study (22 to 40 weeks post-infection). Serum samples were taken weekly and assayed for ALT, HBsAg, and anti-HBc and anti-HBs antibodies using commercial assays. Liver biopsies were taken monthly, starting immediately before HCV inoculation, and routinely studied microscopically. Furthermore, biopsies taken before inoculation and after a significant increase in the level of ALT were carefully investigated by electron microscopy (EM). Before specific HCV assays were available, chimpanzees which developed a significant increase in ALT (at least twofold greater than the individual baseline during the 3 month period before HCV inoculation) and cytoplasmic tubular structures in hepatocytes detectable by EM (as described by Shimizu et al., 1990), but which did not show any parameters of HAV, HBV, CMV or EBV infection, had been designated as being HCV-infected.

Analysis of viraemia in the HCV-inoculated chimpanzees was done using nested PCR to amplify a nucleotide sequence at the 5' end of the HCV genome (Garson et al., 1990a; Fuchs et al., 1991). Briefly, reverse transcription was done using cloned Moloney murine leukaemia virus reverse transcriptase. Sequences between nucleotides (nt) were used as primers, namely nt 1 and 20 and nt 305 and 324 of the HCV J1 isolate as outer primers and those from nt 21 to 31 and nt 271 to 286 as nested primers. Nucleotides are numbered according to Okamoto et al. (1990). Single bands of 324 bp after the first round of PCR and of 265 bp after the second round were visible in the agarose gel after ethidium bromide staining. To avoid false negatives, the results of PCR on serum samples thawed not more than twice were used.

Circulating antibodies to the HCV peptide c-100 were detected by using the commercially available first generation ELISA (Ortho Diagnostic Systems) as recommended by the manufacturer. However, the conjugate included in the commercial assay was replaced with a rabbit anti-human IgG peroxidase conjugate, used at a fourfold greater concentration to achieve satisfactory cross-reactivity with chimpanzee IgG. In addition, a second generation ELISA was used; this employed the recombinant polypeptides (c-22 and c-200) which contain amino acid sequences from non-structural proteins and the core region of HCV (Ortho Diagnostic Systems). Sera producing an absorbance ($A_{450}$) $\geq$ 0.5 were regarded as being anti-HCV-positive. To identify the HCV proteins to which antibodies developed, a RIBA involving individual peptides c-33c, c-100, 5-1-1 (non-structural proteins) and c-22 (core protein) was used as described by the manufacturer (4-RIBA assay; Chiron).

The development of viraemia determined using PCR, and the immune response analysed with the various HCV antibody assays are shown for each of the seven HCV-infected chimpanzees studied (Fig. 1). Three of the seven animals (Fig. 1a to c) cleared viraemia with the appearance of the first detectable antibodies. In three cases (Fig. 1d to f) this clearance was delayed by a few weeks, and one animal (Fig. 1g) remained viremic throughout the whole 40 week observation period despite having serum antibodies. An example of each of these different groups will be discussed in more detail.

The chimpanzees analysed in Fig. 1(a), which had been inoculated with $10^{5.5} \text{CID}_{50}$ HCV, was PCR-negative 14 days after inoculation, but positive on day 28. ALT levels increased significantly 10 weeks after inoculation and specific antibodies were found 12 weeks after inoculation. When specific antibodies became detectable with the second generation ELISA, the PCR became negative, i.e. HCV and specific antibodies were not present in the circulation at the same time. According to the RIBA this animal developed antibodies to c-100, c-33 and 5-1-1, but not to c-22.

In Fig. 1(d) the results obtained for a chimpanzee inoculated with $10^{3.2} \text{CID}_{50}$ HCV show that although viraemia disappeared shortly after the peak in ALT activity, antibodies to HCV and viraemia occurred simultaneously in the serum for a period of 5 weeks. This animal developed antibodies not only to c-33, c-100 and 5-1-1, but also to c-22. The anti-c-22 antibodies were not detectable after 16 weeks post-inoculation.

Finally, Fig. 1(g) shows the results obtained for an animal infected with $10^{3.5} \text{CID}_{50}$ HCV which developed a persistent HCV infection. Instead of an obvious ALT peak, fluctuating increased levels of ALT activity were present over a long period of time. This was accompanied by viraemia, which started 2 weeks after inoculation and lasted until the end of the 40 week observation period. Antibodies to 5-1-1 and c-100 were first detectable in week 38, whereas those to c-33 appeared in week 22. No anti-c-22 antibodies were detected.

Using PCR and various antibody detection assays we studied the course of viraemia and the humoral immune response to HCV in seven HCV-infected chimpanzees. Inflammation of the liver became detectable by the significant increase in ALT levels in the sera of these animals 8 to 16 weeks after infection, and viremia
occurred several weeks before the increase in ALT. Since serum samples taken bi-weekly starting on the day of HCV inoculation were tested, the 14 day sample was the earliest that could be positive by PCR (three animals). In two of the seven chimpanzees studied we did not detect HCV RNA in the serum before day 28, and in two other animals no HCV RNA was detected before day 42. By contrast, Shimizu et al. (1990), who analysed viraemia in chimpanzees infected with $10^6.5$ CID$_{50}$ HCV daily, found the serum samples to be PCR-positive three days after inoculation.

In our experiments the amount of infectious HCV
ALT. Prince et al. (1983) have reported that the increase in serum transaminase occurs earlier after infection with greater amounts of infectious HCV inoculated. However, this correlation varies considerably from animal to animal.

Specific serum antibodies were detected using the second generation ELISA at the time of the increase in ALT. In contrast, the first generation ELISA yielded positive results considerably later. The failure of this ELISA to detect seroconversion soon after infection has been observed by others (Weiner et al., 1990; van der Poel et al., 1991). When the chimpanzee sera were analysed with the 4-RIBA, we found positive reactions at the same time as or 2 weeks after the first positive results obtained using the second generation ELISA. However, the pattern of the antibody response differed from chimpanzee to chimpanzee. Anti-c-33 antibodies were usually the first to become detectable. Chiba et al. (1991) and Harada et al. (1991) found that anti-c-22, directed against the core protein, appeared much earlier in human serum than anti-c-100 antibodies. Nasoff et al. (1991) have reported that antibodies to an immunodominant epitope of the capsid protein develop frequently and early after infection in humans as well as in chimpanzees. However, we detected anti-c-22 antibodies in only two of seven animals. Among the HCV-infected chimpanzees studied, we found that most of them cleared viraemia concurrently with or shortly after the appearance of the first specific antibodies, and that only one animal remained viraemic during the entire observation period of 40 weeks. The pattern of antibodies detected using the 4-RIBA did not show any significant differences between the persistently infected animal and those which cleared the viraemia.

The pathogenesis of HCV infection in humans and chimpanzees is not yet fully understood. The rate of chronic infection in our chimpanzee group seems to be lower than that in humans inoculated with HCV-contaminated blood factor VIII (Garson et al., 1990b). Farci et al. (1991) have reported that two of four chimpanzees became chronically infected. More data on HCV infections in chimpanzees have to be collected to allow a conclusion to be reached as to whether the rate of chronic infection differs in chimpanzees and humans.

These data also provide some valuable information regarding the prevention of HCV transmission by exclusion of anti-HCV antibody-positive human blood or plasma donations. Esteban et al. (1990) have reported that about 90% of human blood donors with anti-c-100 antibodies have infectious virus in their blood. The authors conclude that screening for anti-c-100 antibodies using the first generation ELISA should prevent about half of the cases of transfusion-associated hepatitis. From our data it is obvious that the risk of transmission of HCV could be even further reduced by the use of the second generation ELISA. Furthermore, seropositive donations selected with the assays currently available may or may not contain infectious HCV. However, there will also be anti-HCV antibody-negative samples donated early after infection which will contain infectious HCV. Therefore a sensitive assay detecting HCV or HCV antigens would be highly desirable.

For Professor Dr Hans-Gerhard Schwick on the occasion of his 50th year at Behringwerke AG.

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References


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