Woodchuck Hepatitis Virus Infection: Serologic and Histopathologic Course and Outcome

J. Lindberg1, C. Pichoud1, O. Hantz1, L. Vitvitski1, J. A. Grimaud2, J. M. Gilbert3, L. Joubert3, D. Frommel1, C. Trepo1 *

A partial serological relationship between hepatitis B virus (HBV) and woodchuck hepatitis virus (WHV) permits the use of tests designed for HBV-markers for the detection of WHV-markers (1, 2). Taking advantage of this fact, we tried to infect woodchucks with WHV in order to study the susceptibility to infection, the course of the disease and the liver histology.

Eleven woodchucks were imported from Pennsylvania and given a period six weeks for adaption prior to the onset of the experiment. Animals were kept in separate cages, but for venipuncture every second week they were transferred to a holding cage used for all animals. Seven woodchucks, No. 5–11, were inoculated with 0.5 ml of serum from a chronic WHsAg carrier with a WHsAg titer of 1:8 as measured by immunodiffusion.

Immunodiffusion analyses were carried out using reference sera (kindly supplied by Dr. J. Summers, Philadelphia, Pennsylvania) to check the specificity of the precipitin reactions. Radioimmunoassay using the Ausria II kit (Abbott Laboratories, North Chicago, IL) was carried out according to the manufacturer's instructions. Sera which gave a sample to negative (S/N) ratio below 2.1 were considered to contain WHsAg.

The anti-WHs response was assayed by three methods:
1) Immunodiffusion as described above.
2) Radioimmunoassay taking advantage of the cross-reactivity with anti-HBs (Ausab kit, Abbott, Chicago, IL) followed by confirmation of the specificity of positive results by successful neutralization with WHsAg-containing serum to a > 50% drop in cpm.
3) A solid phase radioimmunoassay specific for anti-WHs which was developed. WHsAg was purified by isopycnic and rate zonal centrifugation and used to coat polyvinylchloride microtiter plates. The plates were also treated with a second ligand, ethylchlorofomate. Samples were inoculated at 20°C for 16 h and after washing the trapped anti-WHs was detected by its capacity to bind 125I-WHsAg read by a gamma scintillation counter.

DNA polymerase activity was determined by a modification of the method described by Kaplan et al. (3). Activity was expressed as the ratio between cpm values in sample and negative control (S/N ratio).

Liver biopsy was performed by laparatomy and wedge resection under general anaesthesia. Formalin-fixed, paraffin-embedded specimens were stained with hematoxylin-eosin, Masson's Pearls and reticulin reagents. Serum alanine and aspartic amino-transferase activity was measured by an auto-analyzer under standard conditions.

Out of 187 serial samples drawn from the eleven animals 44 were found to be positive by the homologous immunodiffusion test for WHsAg and 54 by radioimmunoassay with the cross-reacting test for HBsAg. All samples found reactive in immunodiffusion were also positive in the radioimmunoassay. In two experimentally infected woodchucks (No. 7 and 11) WHsAg could never be shown by immunodiffusion and was shown with Ausria only (Figure 1). One of these animals (No. 11) was already found to be positive for WHsAg before inoculation and had thus erroneously been classified as clear due to the insufficient sensitivity of the immunodiffusion test. Only one sample out of 187 was found positive for anti-WHs by immunodiffusion while in the heterologous anti-HBs radioimmunoassay 33 were reactive. The specific radioimmunoassay for anti-WHs was far more sensitive
Figure 1: Occurrence of WHsAg and anti-WHs in woodchucks inoculated with WHV (WC 5-11) and control animals (WC 1-4), and presence of DNA-polymerase activity before and during the acute and late phase of the infection. Symbols: WHs determined by crossreacting Ausria and homologous immunodiffusion. ; Anti-WHs determined by specific RIA and + = DNA-polymerase activity (S/N ratio > 4), (±) = weak activity (S/N ratio 2-3.9); – = no activity (S/N ratio < 2.0).

since it detected anti-WHs sooner or later in all woodchucks with transient antigenemia (Figure 1). Furthermore, in dilution experiments the homologous anti-WHs radioimmunoassay exhibited a good dose response curve not seen in the heterologous assay. The kinetics of the humoral anti-WHs response is shown in Figure 2.

The occurrence of WHs antigenemia and development of anti-WHs response are shown in Figure 1. One woodchuck (No. 11) already had WHsAg at the time of inoculation as determined by radioimmunoassay and might have been a carrier with low level antigenemia. The antigen cleared however and was followed by an anti-WHs response. Five of the six remaining inoculated woodchucks had detectable WHsAg two to 13 weeks (mean 6.2) after injection. Duration of antigenemia varied from two to 16 weeks (mean 10.5). A chronic WHsAg carrier state developed in woodchuck No. 10 two weeks after receiving WHV. Anti-WHs developed in all woodchucks which cleared WHsAg. In woodchuck No. 5 anti-WHs and WHsAg were demonstrable concomitantly for ten weeks. Finally, in one case (No. 8) only anti-WHs was found suggesting a primary antibody response.

The anti-WHs immune response curve (Figure 2) appeared biphasic with an initial moderate rise preceding a second and stronger rise in antibody titer peaking after three to four months. Three of the four control animals surprisingly developed serological evidence of infection including WHs antigenemia 22 and 23 weeks after the beginning of the observation period (Figure 1). In woodchuck No. 1 and 3 appearance of WHsAg followed a short period of hibernation. Woodchucks No. 1 and 2 had demonstrable antigenemia in only one blood sample, while in No. 3 antigenemia remained for six weeks. In none of these animals was WHsAg detectable by immunodiffusion. Furthermore, anti-WHs preceded the appearance of WHsAg in one case (No. 1) and coincided with it in another case (No. 2).

DNA polymerase activity was measured in woodchucks No. 5-11 (Figure 2). Two animals (No. 5 and 7) had slight activity (S/N ratio 2.8 and 2.7 respectively) in preinoculation samples, which might indicate the presence of non-virus specific DNA polymerase. The other animals did not exhibit activity until antigenemia had developed, but slight activity was still demonstrable after the development of anti-WHs. The animal that developed carrierhip had very high DNA polymerase activity (S/N ratio > 60).

No animals exhibited clinical signs of liver disease or elevation of aminotransferase levels. Two woodchucks (No. 9 and 10) were biopsied during the acute phase of infection. Both animals had heavy portal and moderate lobular lymphohistiocytic infiltration. Liver cell necrosis was slight and predominantly perilobular. The histopathologic changes in these cases were consistent with chronic persistent hepatitis.