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Hepatitis Delta Virus (HDV) and Woodchuck Hepatitis Virus (WHV) Nucleic Acids in Tissues of HDV-Infected Chronic WHV Carrier Woodchucks

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The molecular forms of genomic and antigenomic hepatitis delta virus (HDV) RNA and of woodchuck hepatitis virus (WHV) DNA and WHV RNA were studied in nonneoplastic liver (NL) tissues, hepatocellular carcinoma (HCC) tissues, and several extrahepatic tissues of chronic WHV carrier woodchucks acutely (two animals) and chronically (six animals) superinfected with HDV. HDV was shown to replicate in all NL and HCC tissues but not in any of the extrahepatic tissues analyzed, which included spleen, peripheral blood lymphocytes, kidney, ovary, testis, thymus, lung, and stomach. HDV RNA was present as species with molecular weights consistent with those of monomers, dimers, and trimers of both strand polarities, supporting the rolling circle model proposed for HDV RNA replication. WHV DNA levels in NL, HCC, spleens, and serum were 10- to 100-fold lower than the levels typically observed in chronic WHV carrier woodchucks not infected with HDV. WHV DNA replicative intermediates were rarely observed and only at very low levels, representing less than 10% of the total WHV DNA. By contrast, WHV RNA transcription was not significantly depressed and both primary WHV RNA transcripts, 2.3 and 3.6 kilobases, were observed in NL, HCC, spleens, and in one of the kidney tissues. In addition, a 2.6-kilobase WHV RNA transcript was found in the majority of the NL tissues.

Hepatitis delta virus (HDV) is a small RNA virus requiring the simultaneous presence of a hepadnavirus for its replication (29). The nature of the helper function(s) provided by the hepadnaviruses and required for HDV replication is unknown. The HDV virion is coated by the surface antigen of the helper virus, hepatitis B surface antigen in the case of hepatitis B virus (HBV) infection in humans or chimpanzees (2, 28) or woodchuck hepatitis surface antigen (WHsAg) in the woodchuck hepatitis virus (WHV) infection of woodchucks (24). Within the coat, there is a small circular RNA (HDV RNA) (28) and an antigen (HDAg) encoded by the antigenomic HDV RNA (35). Several HDV cDNA libraries have been constructed, and a number of partial (6, 15, 31) or complete (17, 19, 35, 36) HDV genomic sequences have been reported. The molecular forms extracted from the livers of acutely HDV-infected chimpanzees and woodchucks have been partially characterized (1, 4), and a model for HDV replication utilizing a rolling circle similar to that suggested for plant viroids (3) has been proposed (1, 4, 35).

Despite the information gathered on the HDV RNA molecular forms in infected livers, little is known about the state of the helper hepadnavirus in the same tissue. It is well established that HDV inhibits hepadnavirus replication, both in the acute phase (22, 24, 27) and in the chronic phase (10, 16, 30) of HDV superinfection. Previous studies have focused on the serum markers of HBV replication (hepatitis B e antigen and HBV DNA) or on the immunohistochemical detection of the hepatitis B core antigen in liver tissue. Few observations have been reported on the status of HBV DNA forms in the livers of individuals infected with HDV (10, 23). Liver HBV DNA is usually absent or present as nonreplicative forms. No studies on the status of HBV RNA transcription in HDV-infected liver tissues have been reported. Moreover, hepadnavirus DNA replication has been demonstrated in several extrahepatic tissues, including the spleen (11, 13, 14, 18). In contrast, no evidence has been presented that HDV replicates in extrahepatic tissues.

In this study, molecular forms and levels of genomic and antigenomic HDV RNA were examined in livers, in primary hepatocellular carcinomas (HCC), and in several extrahepatic tissues taken from chronic WHV carrier woodchucks during the acute and chronic phases of HDV infection. Similarly, the replicative status of WHV was examined in the same animals, and the molecular forms of WHV DNA and RNA were described and quantitated. The pattern and tissue distribution of the nucleic acid molecules of both HDV and WHV were then correlated to better define the complex interaction between these two agents at the molecular level.

MATERIALS AND METHODS

Source material. Eight wild-caught woodchucks (*Marmota monax*) (Cocaleco, Inc., Reamstown, Pa.), all chronic WHV carriers, were maintained in isolation in an animal facility at the Molinette Hospital, Turin, Italy. These animals were utilized in a serial passage study of HDV of human origin, the details of which are described elsewhere (25). Tissues from woodchucks 702 (nonneoplastic liver [NL]) and 30 (NL, HCC, spleen, kidney, testis, and stomach) were obtained at the time of acute HDV infection. Six other animals, all chronically HDV infected (numbers 329, 245, 5, 10, 609, and 193), were examined an average of 49 weeks (range, 35 to 75 weeks) after inoculation with HDV. In these six animals, sera collected before inoculation with HDV and at the time of death were examined for HDV RNA, WHV DNA, and WHsAg. Tissues were taken at autopsy and

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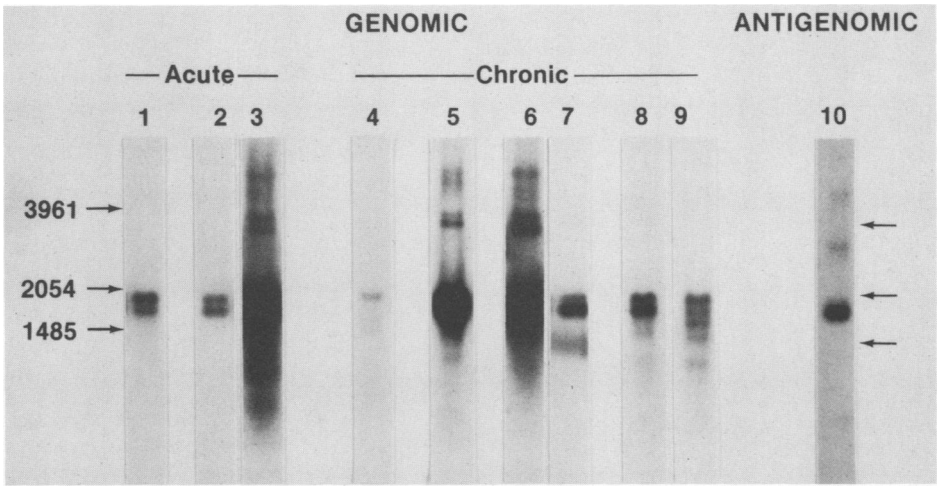

FIG. 1. Genomic and antigenomic HDV RNA in whole-cell RNA preparations from NL and HCC of woodchucks 702 (lane 1, NL), 30 (lanes 2, NL, and 3, HCC), 329 (lane 4, NL), 245 (lanes 5 and 10, NL), 5 (lanes 6, NL, and 7, HCC), and 10 (lanes 8, NL, and 9, HCC). The following amounts of RNA were loaded: lane 1, 0.5 μg; lane 2, 0.1 μg; lane 3, 50 μg; lanes 4, 5, 7, 8, and 9, 2 μg; lane 6, 10 μg; lane 10, 30 μg. Exposure times were 18 h (lanes 1, 2, 4, 5, 7, 8, and 9), 3 days (lanes 3 and 6), and 7 days (lane 10). Molecular weight markers are from coelectrophoresis of a mixture of restriction enzyme digests of the plasmids BMB-37 and BMB-104 containing two different HDV cDNA inserts (22). Sizes (in base pairs) are shown at the left.

immediately frozen in liquid nitrogen and stored at −70°C. Tissues included NL (all animals), HCC (animals 5 and 10), spleen, kidney (both tissues from animals 245 and 5), ovary (245), and lung and thymus (both from animal 5). Peripheral blood lymphocytes (PBL) were isolated as described previously (14) from woodchuck 245 during the acute phase of HDV infection (1, 2, 3, and 5 weeks after inoculation) and from woodchuck 609 10, 12, and 24 weeks after inoculation with HDV.

Serum and tissue assays. Quantitative analysis of WHsAg has been previously described (5). Tissue HDAg was detected by direct immunofluorescence with fluorescein isothiocyanate-conjugated human anti-HD immunoglobulin G (22).

Nucleic acid analyses. Serum (22) and tissue (14) HDV RNAs were extracted and analyzed both qualitatively and quantitatively by Northern (RNA) blot hybridization. Strand-specific (genomic and antigenomic) RNA probes were transcribed from recombinants BMB-37 and BMB-104 as previously described (1, 9) with [^{32}P]CTP. Serum and tissue WHV nucleic acids were purified and analyzed by Southern and Northern blot hybridization or, when quantitative evaluation was needed, by a slot-blot technique as reported previously (14). As a probe, a nick-translated, purified, genome-length 3.3-kilobase (kb) WHV DNA fragment isolated from clone WHV7 (4a) was used. WHV nucleic acids were quantitated by using known amounts of cloned WHV DNA blotted in parallel to the test samples. HDV RNA was quantitated by Northern hybridization analysis with known amounts of cloned HDV cDNA (22). After hybridization, nitrocellulose membranes (slot and Northern blots) were scanned with an AMBIS Beta Scanning System (Automated Microbiology Systems, Inc., San Diego, Calif.). Nucleic acids were quantitated by comparison of test samples with known reference standards. Serum HDV RNA and WHV DNA were expressed as molecules per milliliter, using the conversion of 1 pg of HDV RNA and WHV DNA to 1.1×10^6 and 2.7×10^5 molecules, respectively.

In situ hybridization for tissue HDV RNA. Qualitative and quantitative analysis of HDV RNA by in situ hybridization was performed on frozen specimens as described previously (9) with the same strand-specific RNA probes as above, except that ^{125}I-labeled CTP was used (a generous gift from Dupont, NEN Research Products, Boston, Mass.) as the radiolabel.

RESULTS

Molecular forms of HDV RNA. Genomic HDV RNA was detected by gel analysis in NL and HCC from all animals. In contrast, HDV RNA was not found in any of the extrahepatic tissues studied. HDV RNA was present predominantly as two genome size species (1.7 and 2.0 kb) of approximately equal intensity (Fig. 1). In woodchuck 329, the 2.0-kb band was present in excess (about 10-fold) over the 1.7-kb band (Fig. 1, lane 4). In addition, discrete subgenomic HDV RNA species were observed in both NL and HCC from several animals (Fig. 1, lanes 3, 4, 5, 8, and 9) and ranged from 1.5 to 0.5 kb. Higher-molecular-weight HDV RNA molecules of 3.6 and 3.8 kb were seen in all animals except in woodchucks 329 and 609. The relative amounts of these two bands compared with the two smaller genomic bands were approximately the same in all liver tissues examined (5 to 10%). Assuming this ratio to be consistent, the very low amount of HDV RNA in the NL from animals 329 and 609, together with a variable yield of the RNA preparation, might explain the observed lack of higher-molecular-weight HDV RNA. A less prominent band with an approximate molecular weight of 5.4 to 5.6 kb was observed in NL from woodchucks 245 and 5 and in HCC from woodchuck 30 (Fig. 1, lanes 5, 6, and 3, respectively). The ratio of this HDV RNA band to the major 1.7- to 2.0-kb bands was about 1/20. HDV RNA of antigenomic polarity was found in NL and HCC from all animals except NL from woodchuck 609. These RNA molecules were present as three discrete bands of 1.8, 3.6, and 5.4 kb (Fig. 1, lane 10). Genomic HDV RNA levels in the liver tissues varied among the animals; these molecules represented 0.2 and 1.8% of the total cellular RNA in the two acutely HDV-infected animals, 0.01 to 0.3% in the chronically infected animals, and 0.1 to 0.2% in the HCC tissues.

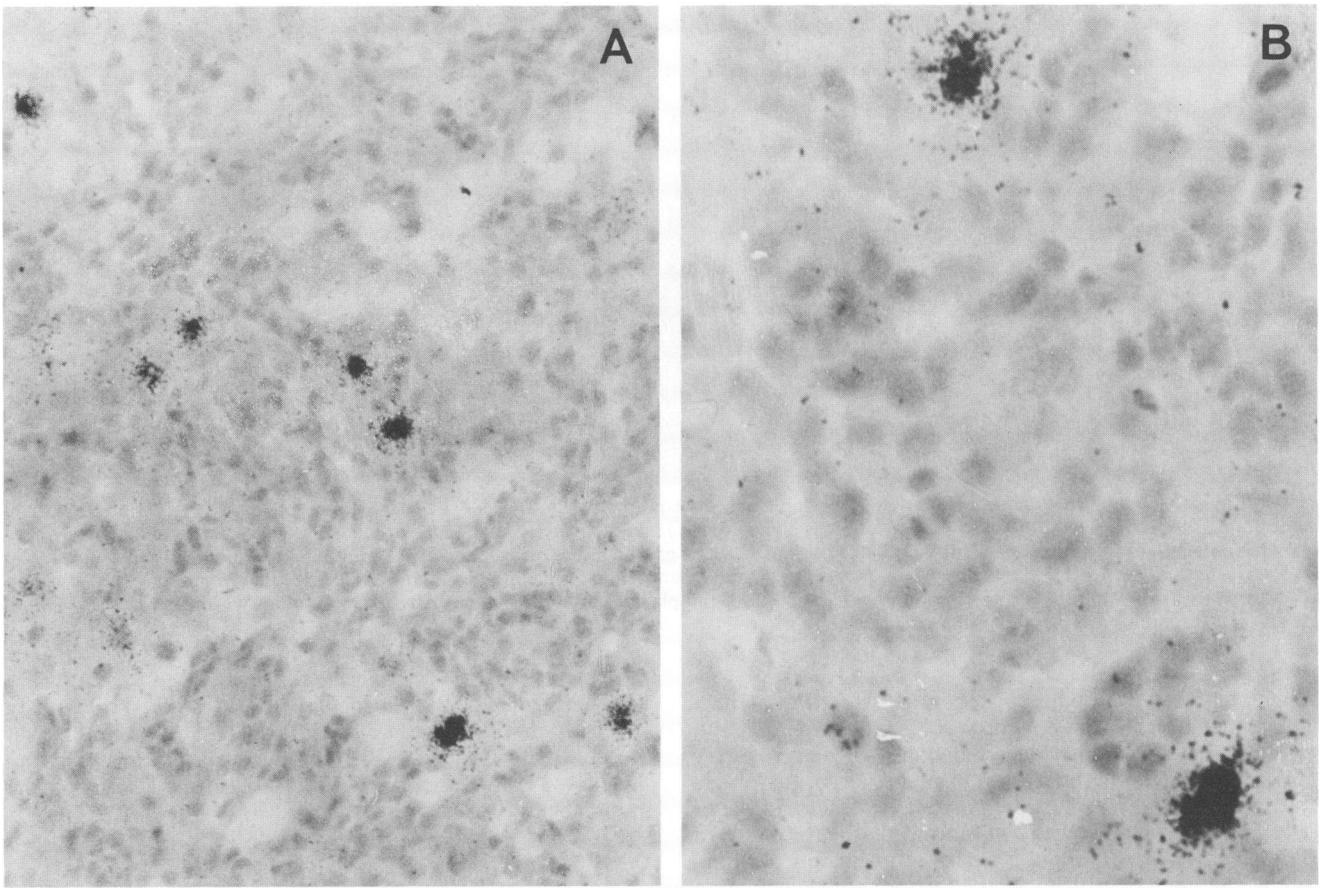

FIG. 2. Presence of genomic HDV RNA in HCC from woodchuck 5 as shown by in situ hybridization. Original magnification, ×80 (A) and ×132 (B). Counterstaining was with hematoxylin and eosin.

Analysis of HDV RNA by in situ hybridization. Cellular localization and quantitation of genomic versus antigenomic HDV RNA was achieved by in situ hybridization and performed on all NL, HCC, and spleens. Nuclear autoradiographic grains were found focally distributed within hepatocytes in all NL and also in the HCC (Fig. 2). No autoradiographic signal was detected in the spleens. The ratio of genomic to antigenomic HDV RNA in the liver tissues ranged from 5 (woodchuck 193) to 31 (woodchuck 5), with a median of 11.

Analysis of intrahepatic HDAg. All NL and HCC tissues contained variable levels of nuclear HDAg as determined by immunofluorescence (data not shown). In contrast, HDAg was not detected in any of the extrahepatic tissues examined.

Molecular forms of WHV DNA. All NL, HCC, spleens, kidneys, PBL (except those taken at the 24-week time point from animal 609), and the ovary from woodchuck 245 contained low amounts of 3.8-kb WHV DNA, the relaxed circular form of monomeric WHV DNA. No WHV DNA was detected in the remaining extrahepatic tissues. Episomal multimeric WHV DNA forms of 7 to 12 kb were also detected in the PBL samples. Both classes of episomal WHV DNA forms were converted to linear 3.3-kb DNA molecules after digestion with *Bam*HI (which recognizes a single restriction site in the WHV DNA commonly isolated from carriers living in the northeastern United States [4a, 14, 20; B. E. Korba, F. V. Wells, B. Baldwin, P. J. Cote, B. C. Tennant, H. Popper, and J. L. Gerin, Hepatology, in press]) but not with *Ava*I (no restriction sites) (Fig. 3, lanes a to c). WHV DNA replication intermediates were present at very low levels in the NL, HCC, and spleen tissues, in the kidney from woodchuck 5, and in the ovary from woodchuck 245. The WHV DNA replication intermediates represented less than 10% of the total WHV DNA present in these tissues (data not shown). Liver tissues from chronic WHV carriers not infected with HDV typically contain approximately 500 to 2,100 copies of WHV DNA per cell, with the majority (>90%) of the WHV DNA present as replication intermediates (13, 21; Korba et al., in press). Only one specimen (HCC from woodchuck 30) showed evidence of integration of WHV DNA sequences into the host genome.

Molecular forms of WHV RNA. WHV RNA transcripts were observed in all NL and HCC, as well as in spleens from woodchucks 30 and 5 and kidney from woodchuck 245. All other samples, including the PBL at all time points, were negative for WHV RNA. A major band of 2.3 kb was present in all positive samples and constituted the majority of the signal (Fig. 3, lanes d to f). All positive specimens (except NL from woodchuck 30) contained a second primary transcript of 3.6 kb. In all NL (except woodchuck 5), a WHV RNA species of approximately 2.6 kb was also observed (Fig. 3, lane e). These RNA transcripts were not detected in any of the HCC, spleens, or kidneys. Additional, minor WHV RNA species were sometimes observed: NL from woodchuck 5 contained a 1.8-kb band, NL from woodchucks 245 and 702 contained two species of 1.8 and 0.8 kb, and a WHV RNA transcript of 1.8 kb was also observed in

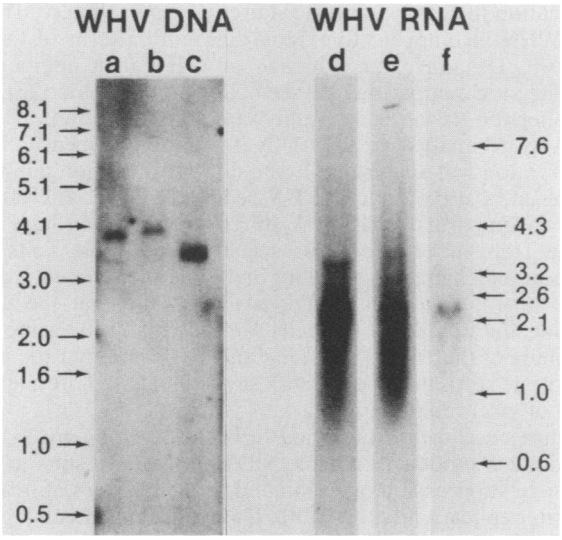

FIG. 3. WHV DNA (lanes a to c) and RNA (lanes d to f) in liver tissue preparations. Lanes a to c were loaded with 2 μg of total DNA from NL from woodchuck 30: lane a, undigested DNA; lane b, after digestion with *Ava*I (no recognition sites in WHV DNA); lane c, after digestion with *Bam*HI (single site in WHV DNA). Lane d was loaded with 10 μg and lanes e and f with 30 μg of total cell RNA from NL of woodchucks 5 (lane d) and 245 (lane e) and from HCC of woodchuck 5 (lane f). Exposure time (all lanes) was 7 days. Molecular size markers are from coelectrophoresis of 1-kb ladder size markers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) for WHV DNA (numbers on left) and of a mixture of restriction enzyme digests of the WHV DNA plasmid used for the preparation of the probe (14) for WHV RNA (sizes on right, in kilobases).

all of the HCC tissues; the origin of these species is unknown.

Levels of WHV nucleic acids. Tissue WHV DNA and RNA were quantitated by slot-blot analysis (Table 1). Assuming a total DNA content of 2 pg per cell, WHV DNA levels varied from 30 to 60 genomes per cell in the acutely HDV-infected NL to 12 to 126 copies in the NL from the chronic HDV carriers. HCC tissue contained 18 genomes per cell in the acutely HDV-infected woodchuck (number 30) and 30 and 54 genomes per cell in the chronically HDV-infected animals (numbers 10 and 5, respectively). WHV DNA levels in spleens, PBL, kidneys, and ovary were below one copy per cell. WHV RNA content in the NL ranged from 70 to 105 pg/μg of total cellular RNA (acutely HDV-infected woodchucks) to 8 to 200 pg/μg (chronically HDV-infected animals). HCC tissues contained 7 and 2 to 8 pg of WHV RNA per μg of total cell RNA, respectively. Levels of WHV RNA in spleens and kidney from woodchuck 245 were less than 0.1 pg/μg of total cell RNA. Liver tissues and spleens from WHV chronic carriers not infected with HDV typically contain an average of 40 to 160 pg and 3 to 12 pg of WHV RNA per μg of total cell RNA, respectively (15; Korba et al., in press).

Serum HDV RNA, WHV DNA, and WHsAg. Serum HDV RNA and WHV DNA, respectively, are measures of the infectious HDV and WHV contents of serum and markers of active viral replication. In the woodchucks that were chronic HDV carriers, the concentrations of HDV and WHV ranged from 5.5×10^9 to 4.6×10^{11} (range, 80-fold) and 3×10^8 to 5.5×10^9 (range, 20-fold) molecules per ml, respectively (Table 2). Comparison of the serum WHV concentrations of

TABLE 1. Levels of WHV nucleic acids in liver tissues of HDV-infected woodchucks

Animal no.	Tissue	WHV DNA (genomes/cell)[a]	WHV RNA (pg/μg of total cell RNA)
Acute HDV infection			
702	NL	60	70
30	NL	30	105
	HCC	18	7
Chronic HDV infection			
329	NL	12	34
245	NL	12	200
5	NL	42	50
	HCC	54	2
10	NL	126	18
	HCC	30	8
609	NL	90	8
193	NL	42	34

[a] The number of WHV genomes per cell was calculated assuming a total DNA content of 2 pg per cell.

HDV carrier woodchucks with preinoculation values (serum pairs) indicated a partial suppression (range, 6- to 240-fold) in four of six animals and no substantial change in two animals. In chronic HDV carriers, the absolute concentration of HDV was equal to or greater than that of the WHV (range, 2- to 1,700-fold), based on quantitative hybridization analysis of a single serum specimen from five animals. Comparison of the serum pairs for WHsAg content indicated a suppression of WHsAg in five of the six carriers (range, 5- to 45-fold), but only three of these had a corresponding drop in WHV DNA.

DISCUSSION

HDV is a defective human pathogen that causes severe liver disease (delta hepatitis). HDV consists of a single-stranded RNA genome and, accordingly, replicates by mechanisms quite unlike those of its helper DNA virus, HBV. Another closely related hepadnavirus, WHV, can also provide the requisite helper functions for HDV infection-replication, and experimental HDV infection of WHV carrier woodchucks is a highly applicable model for studies on the mechanisms of HDV replication and the nature of the HDV-hepadnavirus interaction at the molecular level of analysis.

Published studies (1, 4) of HDV nucleic acids in infected livers in the woodchuck and chimpanzee models have sup-

TABLE 2. Serum levels of HDV RNA, WHV DNA, and WHsAg in chronically HDV-infected woodchucks

Animal no.	HDV RNA (10^8 molecules/ml)	WHV DNA (10^8 molecules/ml)		WHsAg (μg/ml)	
		Pre-I[a]	Post-I[b]	Pre-I	Post-I
329	120	60	55	242	38.7
245	4,600	660	3	12	8.4
5	440	95	6	290	15.0
10	NT[c]	97	3	212	4.7
609	880	84	52	76	15.0
193	55	360	4	91	4.7

[a] Pre-I, Preinoculation with HDV.
[b] Post-I, Postinoculation with HDV.
[c] NT, Not tested.

ported the hypothesis that HDV replicates by a rolling circle model, as proposed for plant viroids (1, 3, 4, 35). Cleavage and ligation of the new transcript to form monomeric circles are essential features of the model, and the capacity of the antigenomic and genomic strands of HDV RNA to undergo an autocatalytic self-cleavage has recently been reported (32; H. D. Robertson, personal communication; H. N. Wu and M. M. C. Lai, personal communication). The molecular species of HDV RNA described here are consistent with those reported previously and, possibly, with the rolling circle model of replication. In fact, discrete forms of both genomic and antigenomic polarity were observed which migrated in agarose gels to the positions expected for dimeric and trimeric molecules. Such molecules could arise by transcription from circular forms of both polarities followed by an autocatalytic cleavage process that eventually yields monomeric forms through a cascade of larger discrete replicative intermediates. Double bands corresponding to the dimeric and trimeric species of the genomic polarity were observed in liver tissues from six of the eight woodchucks that were examined in the present study; no such doublets of the antigenomic polarity were detected. The structure of these species requires further analyses to examine the possibility that they represent dimeric and trimeric circular molecules; if so, the lack of such antigenomic doublets could indicate differences in the efficiencies of self-cleavage of genomic and antigenomic molecules. The significance of subgenomic species of genomic polarity in liver tissues is unknown, but such forms have been observed in other studies; no such forms of antigenomic polarity were detected. Accordingly, while the various species of HDV RNA observed in woodchuck liver are consistent with a complex rolling circle model, precise structural analyses of the individual species will be necessary before such a model of HDV replication can be stated with certainty. The observations made in the woodchuck model, however, indicate that it represents an important source of relevant material for such studies.

No differences were noted in the genomic HDV RNA content or ratio of genomic to antigenomic species between animals undergoing acute or chronic HDV superinfection. In all animals, the content of greater-than-genome-length RNA represented about 5 to 10% that of the monomeric form for both the genomic and antigenomic polarities. The serum of HDV-infected woodchucks contained only monomeric forms (1.7 and 2.0 kb) of genomic polarity, confirming that multimeric and subgenomic HDV RNA species were not packaged into mature virions at detectable levels despite their presence in the corresponding liver tissues.

Although the role of hepadnavirus in HDV infection-replication is unknown, in vitro studies have shown that hepatocytes from normal woodchucks can be infected with HDV in the absence of detectable WHV infection (34). These data indicate that the helper function of WHV might be limited to the contribution of the WHsAg envelope to the HD virion and that HDV gains entry to the hepatocyte by the same mechanism as HBV or WHV. HDV of human origin, however, can be readily transmitted to woodchucks and, even after multiple serial passage in woodchucks, readily reinfects chimpanzees (22, 25). This is in sharp contrast to the rather restricted host range that is exhibited by members of the family *Hepadnaviridae* and considered to be due to species-specific differences in the hepatocyte receptor and its interaction with the virus envelope. Transfection studies with heterologous virus-host cell systems indicated that there is no restriction at the level of the intracellular viral replication (8, 12, 26). Furthermore, as reported here, HDV and WHV also appear to exhibit different patterns of tissue tropism. Despite clear evidence of WHV in a number of extrahepatic compartments (see below and reference 13), no extrahepatic tissue in this study contained detectable HDV RNA by gel analysis or HDAg by immunofluorescence. This observation was confirmed by in situ hybridization analysis of spleen, a site of active WHV replication (13). Therefore, the hepadnaviruses and HDV differ in both host range and tissue tropism. These viruses may infect host cells by separate mechanisms, possibly involving unique cell receptors. On the other hand, it is also possible that the viral binding and uptake by the cells are essentially the same for members of the *Hepadnaviridae* and HDV and that the host and tissue restriction occurs at a subsequent step, i.e., at the level of viral replication.

It has been well established in both the chimpanzee and woodchuck models that acute HDV infection results in the transient suppression of serum and tissue markers of hepadnavirus replication (22, 24, 28). The underlying mechanisms for interference are unknown, and no studies to date have examined the state of hepadnaviral transcription in coinfected tissues. In the evolution to chronic HDV infection, no permanent suppression of HBV replication was observed in chimpanzees (22) and no studies to date have been reported in the woodchuck model. Consistent with the chimpanzee data, the analysis of paired sera from the six chronic HDV carrier woodchucks, taken prior to HDV inoculation and during the period of HDV chronicity (serum pairs), revealed a depression of serum markers of WHV replication in the majority of animals but not total suppression. Interestingly, in two animals, serum WHV DNA was not significantly affected, despite the concomitant suppression of serum WHsAg level and liver WHV DNA replication. The absolute concentration of HDV (genomes per milliliter) was higher than that of the helper hepadnavirus in the chronic HDV carrier woodchuck; this relationship has not been extensively investigated in the HBV system, but limited studies in both chimpanzees (22) and humans (33) suggest that a much wider range of HDV concentrations occurs. Although in some patients the concentration of HBV might exceed that of HDV, the data in the WHV-woodchuck model predict that the transmission risk of HDV may be greater than that of HBV for most chronic HDV carriers. The serum concentration of WHsAg was depressed in five of the six HDV carrier animals in comparison with the preinoculation values, but the degree of depression did not directly correlate with the WHV DNA or HDV RNA values; for WHV, this may reflect the discrepancy in the analysis of transcription and replication observed. There was no simple quantitative relationship between the degree of depression of serum WHV and the serum or tissue levels of HDV RNA. Experimental HDV superinfection resulted in a marked decrease (10- to 100-fold) in the WHV DNA content of liver (NL and HCC) and splenic tissues when compared with that of chronic WHV carriers without HDV infection from other studies (13; Korba et al., in press). It is interesting that this inhibition preferentially affected the WHV DNA replication intermediates; the underlying level of full-length WHV genomes was unaffected. The general patterns and relative concentrations of tissue WHV DNA did not correlate with the phase (acute or chronic) of HDV infection or tissue level of HDV RNA.

Since the overall level of WHV genomic template and 3.7-kb RNA transcript appear to be unaffected, the inhibitory effect of HDV on WHV replication seems to be exerted

primarily at the level of reverse transcription of the WHV RNA pregenome. Nontumorous liver tissue from chronic WHV carrier woodchucks acutely or chronically infected with HDV contained 8 to 200 pg of WHV RNA per μg of total cell RNA (Table 1), values not different from those (40 to 160 pg/μg) reported for WHV carrier animals without HDV infection (13; Korba et al., in press). The HDV-associated suppression of WHV replication in liver tissues could be secondary to a loss of transcriptional activity of the pregenome RNA or to a direct effect on the viral reverse transcriptase. A naturally occurring heterogeneity has been observed at the 5′ end of hepadnavirus RNA transcripts (7, 37). In the ground squirrel hepatitis virus model system, this heterogeneity has been linked to a targeting of the 3.7-kb RNA transcript for use as a pregenome template or the translation of the viral core antigen (7). HDV could affect the transcription of the helper virus pregenome RNA so that it could not serve as a suitable substrate for the reverse transcriptase. Alternatively, HDV RNA might encode a protein capable of directly inhibiting the hepadnavirus reverse transcriptase by using one of several uncharacterized open reading frames (17, 19, 35). Either of these potential mechanisms may be mediated by a host factor induced by HDV infection.

The major molecular WHV RNA species observed in the tissues examined in this study were similar in size to those described in the livers of woodchucks not infected with HDV (14, 21). An additional RNA species of 2.6 kb was frequently detected in NL tissues. The origin and significance of this and of other transcripts of smaller size is unclear. The 2.6-kb RNA transcript has never been reported for in vivo infection of WHV in the absence of HDV (13, 14, 21). Therefore, its presence might be related to the interaction between WHV and HDV and deserves further study.

WHV DNA replication and RNA transcription in the spleens of HDV-infected woodchucks were almost totally abolished. Since no HDV was found in the spleen, the HDV-associated inhibition of WHV DNA replication and RNA transcription in spleen tissue appears to be indirect. It is possible that the extrahepatic WHV pools need continuous replenishment of WHV virions from the liver. Alternatively, HDV may code for a soluble factor or induce a cytokine in the host cell which acts systemically to interfere with WHV DNA replication.

The results presented here confirm and extend the analysis of HDV infection to the woodchuck animal model infected with HDV. The similarities of the HDV molecular species to those observed in chimpanzees indicate that the woodchuck model of hepadnavirus infection is suitable for studies of human HDV infection and of diverse features of the complex interaction between HDV and WHV at the molecular level.

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