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Lymphoid Cells in the Spleens of Woodchuck Hepatitis Virus-Infected Woodchucks Are a Site of Active Viral Replication

BRENT E. KORBA,[1]* FRANCES WELLS,[1] BUD C. TENNANT,[2] PAUL J. COTE,[1] AND JOHN L. GERIN[1]

Division of Molecular Virology and Immunology, Georgetown University Medical Center, Rockville, Maryland 20852,[1] and College of Veterinary Medicine, Cornell University, Ithaca, New York 14853[2]

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Lymphoid cells were purified from the spleens of 15 woodchucks and examined for the presence of woodchuck hepatitis virus (WHV). Lymphoid cells from the spleens of eight of eight chronically infected animals contained high levels of WHV RNA and DNA. A 100-fold lower level of WHV DNA was found in the spleen from one of five animals that had recovered from acute WHV infections 2 years before this analysis. No WHV nucleic acids were observed in either of two uninfected animals. WHV DNA patterns in the lymphoid cells from the spleens of the chronically infected animals, which included the presence of single-stranded DNA and RNA-DNA hybrid molecules, were identical to those observed in WHV-infected liver. WHV DNA in these cells was present in intact, 27-nm core particles which also contained the endogenous DNA polymerase activity. These results indicate that the spleen is a site of active WHV DNA replication and is most likely a major source of WHV-infected cells in the circulating lymphoid cell population.

Infection of the eastern woodchuck (*Marmota monax*) by woodchuck hepatitis virus (WHV) has been shown to be an excellent model for virtually all phases of disease related to infection of humans by hepatitis B virus (HBV), including primary hepatocellular carcinoma (2, 18). Recently, a number of investigators have extended the tissue tropism of these hepadnaviruses to nonhepatic tissues, most notably peripheral blood lymphocytes (PBL) (11, 16, 19, 20; see reference 6 for a review). WHV infection of PBL is characterized by low levels of viral RNA and DNA replicative intermediates in addition to nonintegrated monomeric and multimeric genomic forms (6). Since individual cells in the PBL population are replaced relatively often, the maintenance of a persistent viral infection of these cells would appear to require continuous infection of progenitor cells coupled with expansion of the infected cell population. The most likely sources of such cells in adults would be the bone marrow and spleen. However, WHV infection of bone marrow cells is rare, and the viral genomic forms present in these cells are the same as those observed in PBL (6).

This report describes the results of an examination of lymphoid cells from the spleens of WHV-infected woodchucks. In contrast to the patterns of WHV nucleic acids observed in PBL, WHV infection of spleen lymphoid cells (SLC) was characterized by high levels of RNA and patterns of DNA replicative intermediates which were virtually identical to those found in infected liver. The presence of single-stranded viral DNA and viral RNA-DNA hybrid molecules indicated that WHV replication in SLC is similar or identical to the unique mechanism of hepadnavirus DNA replication observed in hepatocytes (15, 17).

MATERIALS AND METHODS

Source material. Experimental animals were maintained in isolation and protocols were conducted at either the animal care facilities of SEMA, Inc., Gaithersburg, Md. (National Institute of Allergy and Infectious Diseases contract NO1-AI-02651), or at the woodchuck breeding colony at the College of Veterinary Medicine, Cornell University (Ithaca, N.Y.). All animals which had recovered from acute WHV infections and animal no. 460 were experimentally infected; other animals had natural infections. Animals no. 154 and 155 had acute infections of hepatitis delta virus resulting from experimental inoculations which had fully resolved 9 and 6 months, respectively, before this analysis. Tissue preparation and lymphoid cell isolation procedures were as previously described (6). Before purification over Ficoll-Hypaque, lymphoid cells from spleens, lymph nodes, and bone marrow were released by mechanical agitation of these tissues in the presence of Dulbecco phosphate-buffered saline (lacking $MgCl_2$ and $CaCl_2$).

Routine examination of lymphoid cell preparations by light microscopy demonstrated the level of erythrocytes to be 1 to 5%. The term "lymphoid" is used to describe the general population of leukocytes isolated as described above. Estimates of specific cell type subpopulation distributions were not possible due to the lack of availability of antibodies to cell type-specific markers for woodchuck lymphocytes. However, since (i) the erythroid cell contamination of these preparations was reduced to those matching similar preparations of mononuclear cells from other members of the order *Rodentia*, (ii) the appearance of the majority of the cells by light microscopy was of distinct lymphoid structure, and (iii) the macrophage contamination of representative cell preparations, as estimated by adherence to plastic (6), ranged from 3 to 7%, we assume that the subpopulation distribution of cells described as lymphoid roughly parallels typical mononuclear cell preparations.

Isolation of viral core particles by CsCl and Cs_2SO_4 gradient centrifugation. Isolated cells or tissues were lysed and WHV core particles isolated by CsCl buoyant density gradient centrifugation as previously described (17). Fractions were analyzed for WHV core and WHV surface antigen (WHsAg) particles by electron microscopy (4) and for WHV core antigen (WHcAg) and WHsAg by radioimmune assays (1, 12). After the WHV core particle-containing CsCl fractions were cooled, WHV nucleic acids were isolated and subjected to Cs_2SO_4 buoyant density gradient centrifugation as previously described (17), except that no detergent was

* Corresponding author.

TABLE 1. WHV nucleic acids in lymphoid tissues of WHV-infected woodchucks[a]

Animal no.	WHV status[b]	WHV nucleic acids[c] in:							
		PBL		BM		LN		SLC	
		DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
162	Uninfected (30)	−	−	−	−	−	−	−	−
466	Uninfected (28)	−	−	−	−	−	−	−	−
442	Recovered (30)	−	−	−	−	ND	ND	−	−
444	Recovered (30)	+	−	−	−	ND	ND	+	−
445	Recovered (30)	−	−	−	−	ND	ND	−	−
458	Recovered (30)	−	−	−	−	ND	ND	−	−
461	Recovered (30)	−	−	−	−	ND	ND	−	−
154	Chronic infection (22)	+	−	−	−	+	+	+ + +	+
155	Chronic infection (22)	+ +	+	−	−	+	−	+ + + +	+
149	CC with HCC (18)	+	−	+	−	ND	ND	+ + +	+
152	CC with HCC (18)	−	−	−	−	ND	ND	+ + +	+
153	CC with HCC (19)	+ +	+	−	−	+ +	+	+ + + +	+
156	CC with HCC (20)	+	−	−	−	−	−	+ + +	+
161	CC with HCC (18)	+ +	+	−	−	+	−	+ + + +	+
460	CC with HCC (30)	+	+	−	−	ND	ND	+ + +	+

[a] Whole cell nucleic acids were prepared and examined by Southern and Northern blot analysis as described in Materials and Methods.

[b] Diagnosis of the clinical status of each animal was based upon serologic analysis as follows: uninfected, no history of WHV infection; recovered, seroconversion to anti-WHsAg after acute WHV infection; chronic infection, chronic WHV carrier; CC with HCC, chronic WHV carrier with primary hepatocellular carcinoma. Numbers in parentheses indicate months at indicated clinical status for experimentally infected animals or months in captivity for wild-caught animals with preexisting, natural infections.

[c] −, Absence of WHV DNA or RNA; +, less than 0.3 WHV DNA genome copies per cell; + +, 0.3 to 3 copies per cell; + + +, 3 to 30 copies per cell; + + + +, greater than 30 copies per cell; + for RNA only, presence of WHV RNA. ND, not determined. BM, Lymphoid cells from bone marrow; LN, lymphoid cells from mesenteric lymph nodes.

present during centrifugation. In some analyses, WHV core particles were isolated from cells or tissues, followed by labeling of WHV DNA by endogenous polymerase reaction (8). WHV nucleic acids were then isolated (10) and used directly for Cs_2SO_4 gradient centrifugation experiments.

Isolation of nucleic acids and Southern and Northern blot analysis. Isolated cells or tissues were lysed and nucleic acids were purified as previously described (6), except that DNA fractions were incubated for 2 h at 42 in the presence of 0.1 M Tris (pH 8.0)–0.005 M disodium EDTA–1% sodium dodecyl sulfate containing 1.0 mg of proteinase K (Boehringer Mannheim Biochemicals) per ml before extraction with phenol and chloroform. Whole cell DNA and RNA samples were subjected to agarose gel electrophoresis followed by Southern and Northern blot analysis or examined by slot-blot analysis as previously described (6) with a ^{32}P-labeled, nick-translated, purified, 3.3 kilobase (kb) *Bam*HI WHV DNA fragment (10). Estimates of the level of WHV DNA were based upon comparisons to coelectrophoresed cloned WHV DNA standards in the presence of 10 μg of sonicated salmon sperm DNA and the amounts of whole cell DNA used, assuming a yield of 1 to 3 μg of DNA from 10^6 cells.

RESULTS

WHV nucleic acids are present in SLC of chronically infected woodchucks. All eight of the chronically infected woodchucks harbored high levels of both WHV DNA and RNA in the SLC (Table 1). The relative level of WHV DNA in SLC varied among the animals from approximately 10 to 100 copies per cell, with a corresponding variance in RNA levels. WHV DNA levels in SLC were approximately 10-fold lower than that routinely observed in WHV-infected liver and 10- to 100-fold greater than the levels routinely observed in the PBL of WHV-infected animals (6). The variance in DNA levels in SLC did not appear to correlate with the source of WHV infection (experimental or wild caught with preexisting WHV infection), the level of WHV nucleic acids in the serum or liver, the presence of hepatocellular carcinomas, or a previous acute hepatitis delta virus superinfection. There was, however, a general correlation between the level of WHV nucleic acid observed in the SLC and the PBL, with the exception of animal no. 152. In this animal, no WHV DNA was detected in the PBL despite the presence of a high level of viral nucleic acids in the SLC. No clinical or virologic abnormalities were observed in this animal.

Viral nucleic acids were also found in lymphoid cells isolated from the mesenteric lymph nodes of three of five chronic carriers (Table 1). The relative level of WHV DNA in the lymph nodes appeared to correlate with that observed in the PBL: approximately 0.1 to 1.0 copies per cell. A low level of WHV DNA (but no RNA) was found in the lymphoid cells isolated from the bone marrow of only one chronically infected animal (no. 149).

A low level of WHV DNA was also observed in the SLC and PBL of one of five animals (no. 444) which had recovered from acute WHV infections 2 years before this analysis. This animal had antibodies to both WHsAg and WHV core antigen and was negative for other serologic markers of viral replication (WHsAg, WHV DNA, WHV DNA polymerase). The level of WHV DNA in the SLC of this animal was approximately 1% of the level observed in the SLC of the chronic carriers. WHV nucleic acids were not found in the liver of this animal. In an earlier study, low levels of WHV DNA and RNA were observed in the PBL, and WHV DNA was observed in the bone marrow and liver, of one of four animals that had been recovered from acute WHV infections for 1 year (6). No viral nucleic acids were found in tissues from two noninfected animals (Table 1).

WHV DNA replication intermediates are present in SLC. Examples of the WHV DNA patterns found in the PBL, SLC, and liver of two of the chronically infected animals by Southern blot analysis of whole cell DNA are shown in Fig. 1. Figure 1a illustrates a typical pattern (6) of WHV DNA from the PBL of animal no. 161, which was characterized by

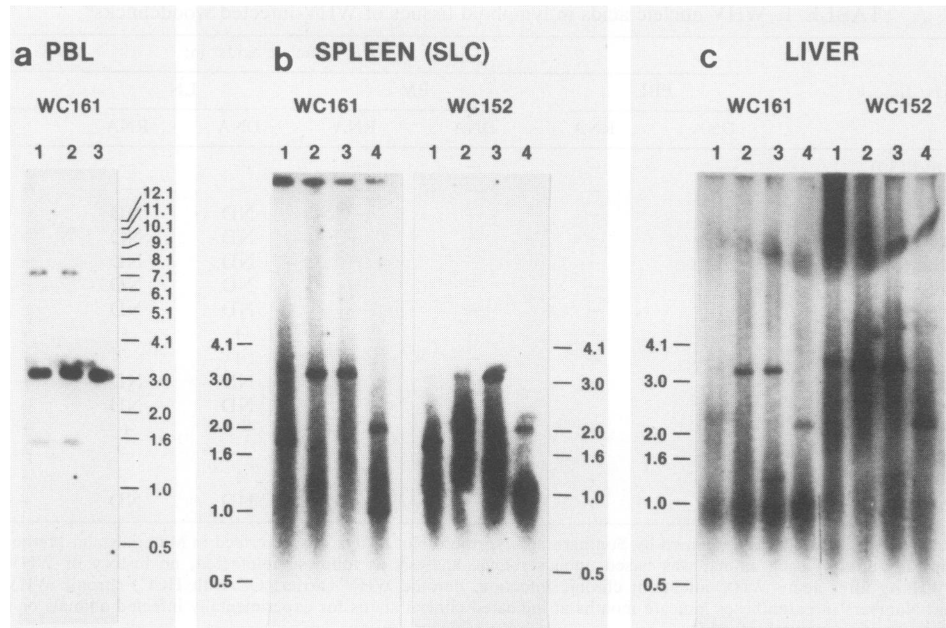

FIG. 1. WHV DNA in whole cell DNA preparations. Whole cell DNA was extracted and digested with the appropriate restriction enzymes (IBI), separated by electrophoresis in 1% agarose gels, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled WHV DNA probe as described in Materials and Methods. Exposure times were 1 (c), 3 (b), and 7 (a) days. The following amounts of whole cell DNA were used: a, 30 μg per lane; b, 5 μg per lane; c, 1 μg per lane. Restriction enzyme digests for all lanes are as follows: 1, undigested DNA; 2, *Ava*I, (no recognition site in our WHV DNA pool [5]; 3, *Bam*HI (single site in WHV DNA); 4, *Eco*RI-*Hin*dIII double digest (each has a single site in WHV DNA producing fragments of 1.0 and 2.2 kb). Molecular size markers are from coelectrophoresis of the 1-kb ladder size markers (Bethesda Research Laboratories, Inc.).

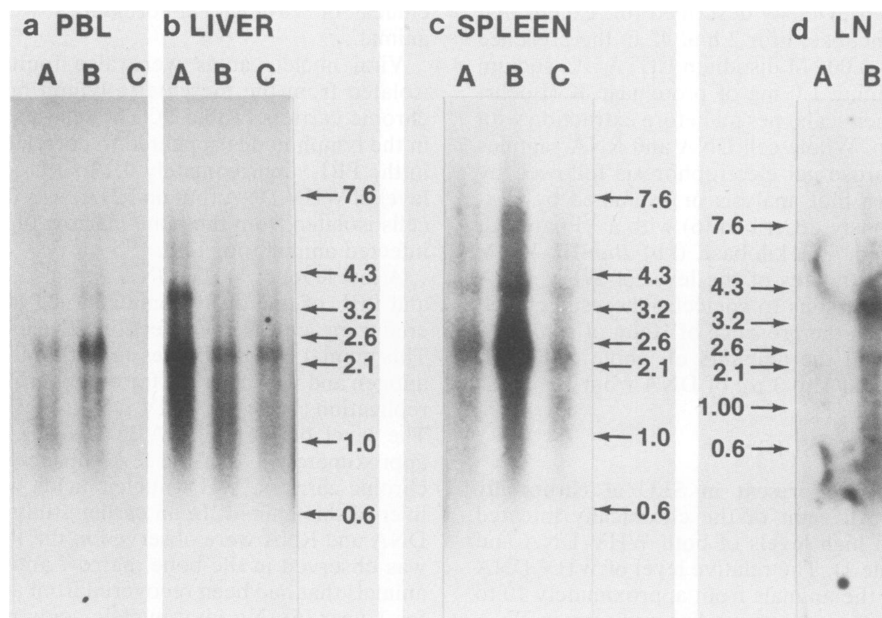

FIG. 2. WHV RNA in whole cell RNA preparations. Whole cell RNA was isolated, denatured in formamide-formaldehyde, subjected to electrophoresis in 1% agarose in 6% formaldehyde, transferred to nitrocellulose (mRNC, Schleicher Schuell Co.), and probed with ^{32}P-labeled WHV DNA as described in Materials and Methods. Exposure times were 3 (a, b, c) and 7 (d) days. The following amounts of whole cell RNA were used: a, 20 μg per lane; b, 1 μg per lane; c, 5 μg per lane; d, 20 μg per lane. The following animals used (lanes): A, no. 161; B, no. 153; C, no. 149. Molecular weight markers are from coelectrophoresis of a mixture of separate restriction enzyme digests of the WHV DNA parent plasmid (used as the source of the WHV DNA fragment used for probing) which were denatured before electrophoresis (6). LN, Lymph nodes.

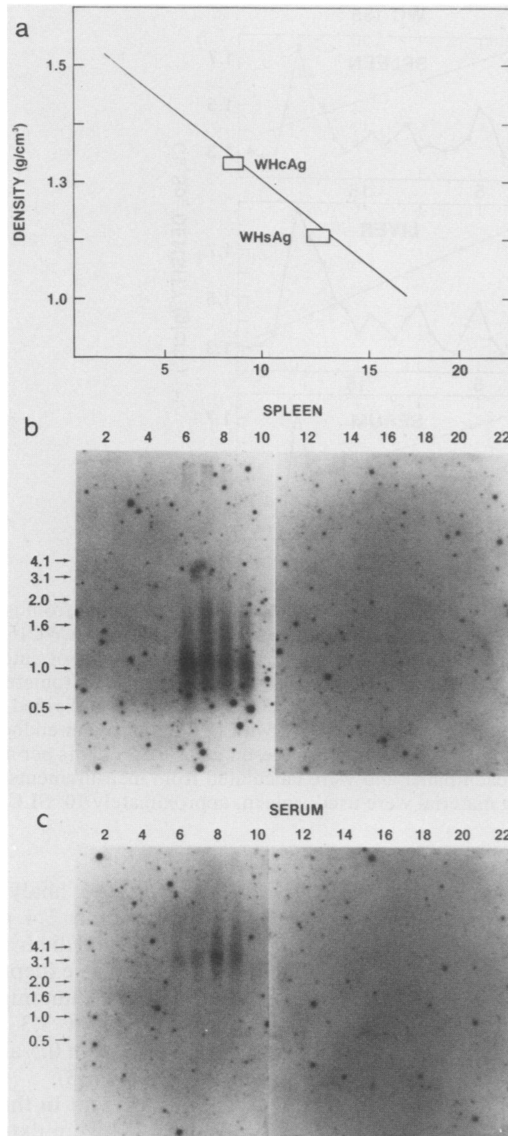

FIG. 3. Buoyant density centrifugation of WHV core particles in CsCl. WHV core particles were extracted from the serum and SLC of WC153 and subjected to buoyant density centrifugation in CsCl as described in Materials and Methods. Fractions were analyzed by electron microscopy, radioimmune assay, and Southern or slot-blot hybridization analysis as described in Materials and Methods. Open boxes (a) indicate the presence of 27-nm WHV core particles (Fig. 4) (fractions 8 and 9) or WHsAg (fractions 12 and 13). (b and c) Autoradiographic exposures (10 days) from Southern blot analysis of WHV nucleic acids in CsCl buoyant density gradients of parallel particle preparation from the spleen (approximately 10^9 SLC, total) and serum (5 ml, total) of WC153. Molecular size markers (kb) are from coelectrophoresis of the 1-kb ladder marker standards (Bethesda Research Laboratories).

(i) a low proportion of WHV DNA replicative intermediates, (ii) discrete, nonintegrated, WHV genomic forms of superhelical and open circular-linear monomers, and (iii) nonintegrated multimeric DNA molecules. Figure 1c displays the DNA patterns observed in the livers of animals no. 161 and 152, which were characterized by typical patterns (17, 18) of high levels of nonintegrated monomeric genomic forms with a high proportion of DNA replicative intermediates. No integrated WHV DNA was observed in either of these two animals.

The WHV DNA patterns in the SLC of these animals were essentially identical to those observed in the liver, indicating that WHV DNA was in a state of active replication in these cells (Fig. 1b). In some animals (no. 153, 154, and 155) nonintegrated multimeric WHV genomic forms, essentially identical to those observed in PBL, were also observed in SLC. These molecules represented approximately 10% of the total WHV DNA.

The WHV DNA patterns observed in the lymphoid cells of mesenteric lymph nodes and bone marrow from the chronic carriers were essentially identical to those observed in PBL (data not shown). WHV DNA found in the SLC of the recovered animal (no. 444), although present at a very low level, was similar to that observed in the SLC and livers of the chronically infected animals. The failure to find WHV RNA in these cells was possibly a reflection of sensitivity levels in the Northern blot analysis, since WHV RNA and DNA levels in the various tissues appeared to be well correlated in the other animals.

WHV RNA in SLC is present primarily as 2.3- and 3.6-kb transcripts. Figure 2 presents examples of Northern blot analyses of WHV RNA in whole cell RNA preparations from PBL, liver, SLC, and lymph nodes of three chronic carriers (no. 149, 153, and 161). The patterns of WHV RNA species in each of the tissues analyzed appeared to be similar or identical, although the presence and level of WHV RNA varied considerably among these tissues in individual animals. Under our electrophoretic conditions, two RNA species of approximately 2.3 and 3.6 kb were observed, consistent with earlier observations (6, 18). No obvious consistent differences were observed in the stoichometric ratios of these two RNA species in the various tissues. However, in

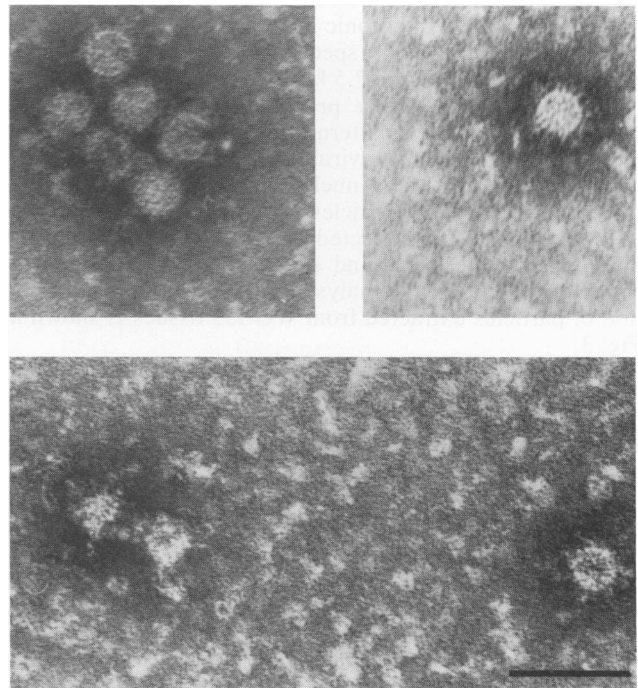

FIG. 4. WHV core particles isolated from the SLC of WC153, subjected to buoyant density centrifugation in CsCl (Fig. 3), and examined by electron microscopy as described in Materials and Methods. Bar, 0.1 μm.

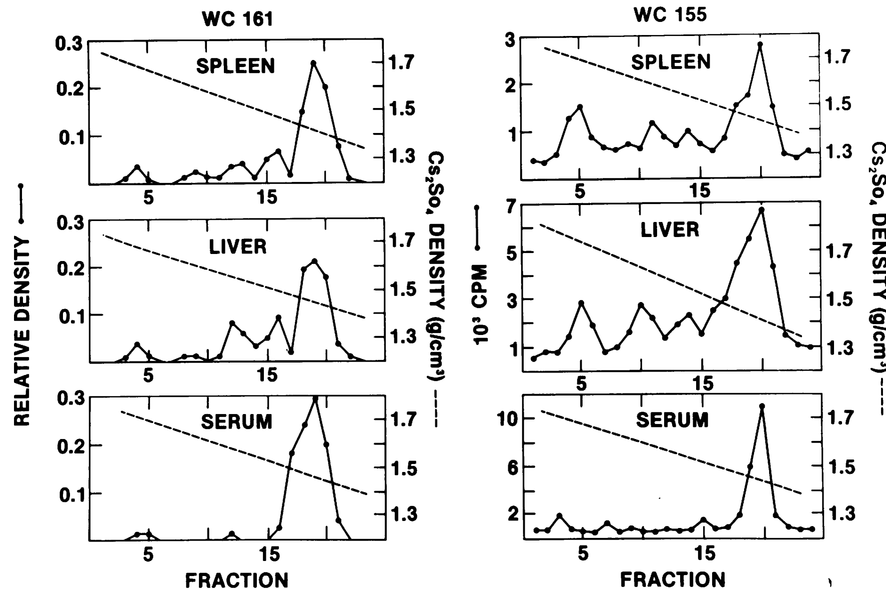

FIG. 5. Profiles of WHV nucleic acids after buoyant density centrifugation in Cs_2SO_4. WHV nucleic acids were extracted from isolated WHV viral core particles and subjected to centrifugation as described in Materials and Methods. Since WHV nucleic acids from WC161 were not radioactively labeled, fractions were examined by slot-blot hybridization analysis (6) followed by densitometric analysis of autoradiographic exposures with a spectrophotometer (DU8; Beckman Instruments, Inc.) equipped with a scanning-integrating densitometer. The amounts of WHV nucleic acids in each fraction are represented as the measured exposure density in each gradient fraction divided by the sum of the densitometric measurements for all fractions. WHV nucleic acids from WC155 were labeled with [^{32}P]dCTP by an endogenous polymerase reaction before centrifugation (8). The amounts of WHV nucleic acids in each fraction are represented as total counts per minute. Profiles of the Cs_2SO_4 density in each gradient are indicated by dashed lines in each panel and were calculated from measurements of the refractive index of individual fractions (8). The following total amounts of starting material were used: spleen, approximately 10^9 SLC; liver, approximately 1.5 g; serum, 5 ml.

some animals, the SLC appeared to contain a higher proportion of RNA species greater than 3.6 kb, especially molecules of approximately 7.2 kb in size (Fig. 2b and C, lanes A and B). In the PBL of chronic HBV carrier chimpanzees the predominant HBV RNA species are molecules migrating approximately at 3.6 and 7.5 kb (6).

WHV core particles are present in SLC. To determine whether the observed patterns of WHV DNA in SLC, in fact, represented hepadnavirus replication intermediates, a detailed analysis of these nucleic acids was performed. As the initial step, WHV particles were extracted from serum, SLC, and liver tissue collected from animals no. 153 and 161 by standard procedures and subjected to buoyant density centrifugation in CsCl. Analysis of CsCl density centrifugation of particles extracted from WC 153 tissues is shown in Fig. 3.

WHV core particles, 27 nm in diameter (Fig. 4), extracted from the SLC of both animals were found by electron microscopic analysis of gradient fractions at densities of 1.31 to 1.33 g/cm^3 (Fig. 3), consistent with the expected density (1.35 g/cm^3) for hepadnavirus core particles containing nucleic acids (4). The slightly lighter buoyant density of these particles was explained by the abundance of anti-WHc in the chronic carriers which was complexed with the core particles (Fig. 4). These intrinsic levels of antibody inhibited accurate analysis of the gradient fractions for WHcAg by radioimmune assay. A low level of WHsAg particles was detected by both radioimmune assay and electron microscopy at the appropriate density of 1.21 g/cm^3 (Fig. 3).

WHV DNA was detected by Southern blot analysis after prolonged exposure times (7 to 10 days) only in the fractions containing core particles and in adjacent fractions with densities of 1.35 to 1.37 g/cm^3 (Fig. 3). No free WHV DNA was evident in these cells, based upon the analysis of gradient fractions with densities greater than 1.4 g/cm^3. Analysis of parallel gradients with serum and liver tissue produced essentially identical observations, except that WHV DNA in core particles extracted from serum migrated as a diffuse band migrating approximately at 3.3 kb in agarose gels rather than as a smear ranging from 0.5 and 3.3 kb from the SLC and liver core particles (Fig. 3).

WHV RNA-DNA hybrid molecules are present in the viral particles found in SLC. WHV core particles were extracted from the liver, SLC, and serum of four animals (no. 153, 154, 155, and 161). WHV particle DNAs from animals no. 154 and 155 were labeled by using an endogenous polymerase reaction followed by analysis of the ^{32}P-labeled WHV DNA by Cs_2SO_4 buoyant density centrifugation (8, 17). WHV particle nucleic acids from animals no. 153 and 161 were not radiolabeled, and Cs_2SO_4 gradient fractions were analyzed by Southern and slot-blot hybridization. Examples of the buoyant density patterns of WHV particle DNAs are shown for animals no. 155 and 161 in Fig. 5.

The distribution in Cs_2SO_4 of WHV particle nucleic acids isolated from the SLC of these animals clearly indicated that these subviral particles contained a high proportion of RNA-DNA hybrid molecules (17). These patterns were essentially identical to the patterns observed for WHV particle DNA in the liver of these animals and were distinctly different from the WHV DNA buoyant density patterns for the serum viral particles. Hepadnavirus particles in serum, in contrast to those present in the liver, contain only minute proportions of RNA-DNA hybrid molecules (9). In subsequent experiments, RNase A digestion of the particle nucleic acids from WC161 SLC, after the polymerase reaction and before density centrifugation (17), caused a shift in the nucleic acid

profile from the denser to the lighter portions of the gradients (data not shown). Gel electrophoresis of the WHV particle nucleic acids from the Cs_2SO_4 gradient fractions produced the expected patterns (17) of a progressive increase in the size of WHV DNA from less than 0.5 kb in the fractions with a higher densities of Cs_2SO_4 to full genomic size (3.3 kb) in the less dense fractions (data not shown).

DISCUSSION

This report demonstrates that lymphoid cells in the spleens of chronically infected woodchucks are a site of active viral DNA replication. Furthermore, WHV replication in these cells appears to progress by mechanisms similar or identical to those observed in hepadnavirus-infected hepatocytes (15, 17). Evidence to support this conclusion includes the presence in SLC of (i) WHV DNA replicative intermediates, (ii) WHV RNA, and (iii) intact WHV core particles containing RNA-DNA hybrid molecules and an active endogenous DNA polymerase. However, it is not known whether these cells produce complete, infectious virions. Previously, viral DNA patterns apparently representing DNA replicative intermediates and virus-specific RNA transcripts were observed in the pancreas, kidney, and spleen of duck HBV-infected Pekin ducks (3, 19). Hepadnavirus genomic DNA has been shown to be present in the PBL of chronically infected humans, chimpanzees, and woodchucks in a number of published reports (11, 16, 19, 20; see reference 6 for a review).

The virus pool which serves as the source of infection for PBL has not as yet been determined. Transfer of virus to the PBL may occur through an expansion of a pool of infected progenitor cells. The observation of active WHV replication in SLC is most consistent with this latter mechanism, although other mechanisms, such as the direct infection of PBL by intact viral particles, are not excluded. WHV may be carried and transferred among cells during active cell division in the spleen, resulting in movement of virus into the PBL population as the infected cells migrate out of the spleen. Such a scheme would not require the production of complete viral particles and predicts that WHV infection of PBL should reflect the level of viral activity in the spleen. We observed a general correlation between the relative levels of WHV nucleic acids in PBL and SLC in individual animals among the different chronic carriers. The apparent absence of WHV DNA in the PBL of animal no. 152, despite the presence of relatively high levels of WHV DNA in the SLC, may be a reflection of the periodic loss of hepadnavirus nucleic acids from the PBL population observed during analysis of serial PBL samples from individual, chronically infected chimpanzees and woodchucks (6). These clearing periods are followed by the reappearance of virus in the PBL (6).

Significant differences are evident between the SLC and PBL with regard to the state and level of WHV nucleic acids. Although it has not been shown directly, hepadnaviruses most likely require a number of host cell replication factors to complete their own reproductive cycle. The change in the apparent replication status of WHV from active to relatively quiescent forms coincides with the relative replication status of lymphoid cells in the spleen and PBL. Thus, the state of WHV DNA in lymphoid cells may be a consequence of the activity of the host cell.

The data included in this and a previous report (6) do not support an active involvement of lymphoid cells in the bone marrow as a source of virus for the infection of PBL in chronically infected woodchucks. WHV DNA was found in the bone marrow of only 1 of 14 chronically infected animals and 1 of 7 recovered animals. No WHV RNA was observed in the bone marrow cells from either of these animals. However, it is of interest to note that several of the initial reports of HBV infection of human lymphoid cells involved bone marrow cells (14). Our observations do not necessarily exclude the involvement of the bone marrow at stages of hepadnavirus infection that are earlier than those studied here.

The presence of WHV in the lymphoid cells from lymph nodes of chronically infected animals was predictable since circulating lymphocytes migrate through these tissues. This is consistent with the observations that the WHV nucleic acid patterns in the lymphnodes match those found in PBL.

To date, there is still no definitive assessment of the distribution of hepadnavirus among the various cell subpopulations in the lymphoid system. A previous report indicated that the presence of WHV and HBV in experimental animals was not merely a consequence of direct uptake by phagocytic cells (6). The present study reaffirms this position. HBV DNA and RNA have been shown to be distributed primarily, if not exclusively, in the B and T cells of chronically infected chimpanzees (6) and in human T cells after long-term culture (7).

WHV DNA can persist in lymphoid cells in a low proportion (two of nine) woodchucks (6) for at least 2 years after the resolution of acute WHV infections. The relevance of these observations is unclear at the present time. However, it should be noted that the WHV genomes present in these animals are unlikely to be completely latent, since individual cells in PBL are replaced relatively often and WHV DNA replicative intermediates were observed in the SLC of a long-term recovered animal, although at a very low level.

The presence of an extrahepatic replicating pool for WHV more firmly extends the tissue of this virus. The ability of WHV to persist in lymphoid cells long after markers of viral replication have disappeared from the primary target organ, the liver, adds to the complexity of the pathogenic mechanisms of hepadnaviruses. Currently, insufficient information has been accumulated to accurately determine how viral infection of lymphoid cells relates to the overall pathology of hepadnavirus infection and disease. More specific information is needed regarding the lymphoid cell subpopulations involved and the kinetics of infection for the different compartments of the lymphoid system during various stages of viral infection. When such information becomes available, we will be able to better determine the role of viral infection of lymphoid cells in the natural course of hepadnavirus-induced disease.

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