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Expression of Hepatitis B Virus Large Envelope Polypeptide Inhibits Hepatitis B Surface Antigen Secretion in Transgenic Mice†

FRANCIS V. CHISARI,[1]* PIERRE FILIPPI,[1] ALAN McLACHLAN,[1] DAVID R. MILICH,[1] MICHAEL RIGGS,[1] SUN LEE,[2] RICHARD D. PALMITER,[3] CARL A. PINKERT,[4] AND RALPH L. BRINSTER[4]

Departments of Basic and Clinical Research[1] and Immunology,[2] Research Institute of Scripps Clinic, La Jolla, California 92037; Department of Biochemistry, Howard Hughes Medical Institute, University of Washington School of Medicine, Seattle, Washington 98195[3]; and Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania,[4] Philadelphia, Pennsylvania 19104[4]

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The outer membrane of the hepatitis B virus consists of host lipid and the hepatitis B virus major (p25, gp28), middle (gp33, gp36), and large (p39, gp42) envelope polypeptides. These polypeptides are encoded by a large open reading frame that contains three in-phase translation start codons and a shared termination signal. The influence of the large envelope polypeptide on the secretion of hepatitis B surface antigen (HBsAg) subviral particles in transgenic mice was examined. The major polypeptide is the dominant structural component of the HBsAg particles, which are readily secreted into the blood. A relative increase in production of the large envelope polypeptide compared with that of the major envelope polypeptide led to profound reduction of the HBsAg concentration in serum as a result of accumulation of both envelope polypeptides in a relatively insoluble compartment within the cell. We conclude that inhibition of HBsAg secretion is related to a hitherto unknown property of the pre-S-containing domain of the large envelope polypeptide.

The major, middle, and large envelope polypeptides of hepatitis B virus (HBV) (9, 11, 16–18, 23, 24, 27–29) are encoded by a large open reading frame which has three in-phase translation start codons. The major polypeptide begins at the third ATG. It is the predominant structural constituent of the envelope of complete virions (Dane particles) and noninfectious 22-nm spherical and filamentous subviral particles. It is a hydrophobic molecule (7) that may be either nonglycosylated (p25) or glycosylated (gp28) and contains the highly conformation-dependent hepatitis B surface antigen (HBsAg). The middle polypeptide begins at the second in-phase initiation codon and contains 55 additional N-terminal amino acids [pre-S(2)]. Within The N-terminal domain is located the relatively conformation-independent (14, 16), highly immunogenic (14) pre-S(2) region, as demonstrated by the immunogenicity of the native pre-S(2) region protein (14), and a constituent synthetic peptide (16). A site in pre-S(2) is always glycosylated, resulting in two molecular forms of this polypeptide (gp33, gp36) depending on the glycosylation state of the S region of these molecules. The relative distribution of major and middle envelope polypeptides is quite similar among all three morphological forms of HBV, i.e., Dane particles, spheres, and filaments (9, 29). The large envelope polypeptide begins at the first in-phase ATG, which is located either 108, or 119 codons upstream of the middle polypeptide depending on subtype (30), and occurs in both nonglycosylated (p 39) and glycosylated (gp42) forms. The additional N-terminal amino acids contain the pre-S(1) antigen (9, 11, 23, 29). In contrast to the other two polypeptides, the large envelope polypeptide is preferentially enriched on Dane particles and 22-nm subviral filaments relative to the predominant 22-nm spheres (9, 28). While this association suggests a possible role for the large polypeptide in the assembly or structure or both of these particles, its biochemical and functional properties are currently unknown.

We recently produced transgenic mice (3) by microinjection of a recombinant construction (plasmid MT-PSX) consisting of the heavy metal-inducible mouse metallothionein I (MT) promoter located upstream of a subgenomic fragment of the HBV genome which encodes the viral envelope polypeptides. HBsAg synthesis was evident in the liver, the kidney, and other organs, in keeping with the tissue distribution of the MT promoter; and 22-nm spheres and filaments with the characteristic size, density, and morphology of authentic subviral particles were secreted into the blood at levels comparable to those found during HBV infection in man. During these studies, we attempted to maximize HBV gene expression by administration of zinc by the oral route. Although HBsAg levels in tissue increased as expected after zinc administration, the concentration of HBsAg in serum declined. Similarly, in transgenic mice in which an albumin promoter (Alb) and 5′ flanking sequence are fused to the same HBV subgenomic fragment, high and low levels of HBsAg in the liver and serum, respectively were observed. Subsequent analysis of the molecular basis for this effect disclosed a previously unknown property of the large envelope polypeptide of HBV.

MATERIALS AND METHODS

Plasmid constructions. The *Bgl*II A fragment of HBV subtype *ayw*, which spans nucleotides 2839 and 1986 (from GenBank genetic sequence data bank derived from reference 6), was cloned into the *Bgl*II site of pMT-1 and the *Bam*HI site of pAlb-hGH immediately downstream of the mouse MT and the mouse Alb promoters, respectively (Fig. 1), by conventional techniques (12). The HBV *Bgl*II A fragment contains the entire envelope region open reading frame consisting of three translation initiation codons at nucleotides 2850, 3174, and 157, which represent, respectively, the N termini of the large, middle, and major envelope

* Corresponding author.

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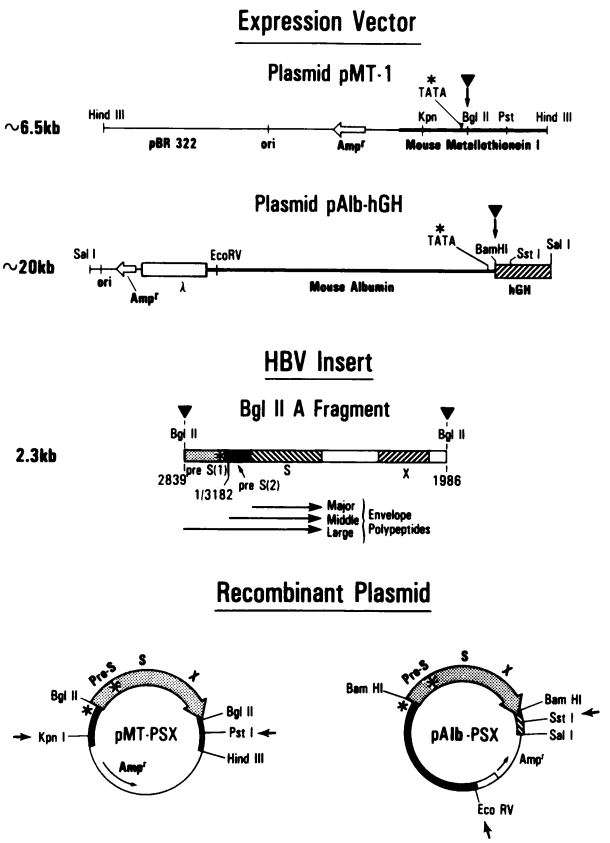

FIG. 1. Organization of expression vectors, HBV insert, and recombinant plasmids. Triangles indicate restriction sites used to generate recombinant plasmids. Arrows indicate restriction sites in these plasmids used to produce DNA fragments for microinjection. Asterisks represent promoter elements.

polypeptides, and a stop codon at nucleotide 835. Additionally, transcriptional start sites in the vicinity of nucleotide 1/3182 and mRNA polyadenylation recognition sequence starting at nucleotide 1918 define the approximate termini of the HBV transcript which encodes the major envelope polypeptide (2, 9, 19, 25). The large envelope polypeptide can be produced only from a larger transcript emanating from the transcription start sites defined by the MT or the Alb promoter.

Microinjection and production of transgenic mice. Production of transgenic mice (MT-PSX) expressing HBsAg from the MT promoter was as described previously (3). Transgenic mice (Alb-PSX) producing HBsAg from the mouse Alb promoter were generated in a similar manner by microinjection of the *Eco*RV-*Sst*I fragment of plasmid pAlb-PSX (Fig. 1). Plasmid pAlb-hGH contains the mouse Alb promoter and about 12 kilobases of flanking sequences. The offspring were analyzed for the presence of microinjected DNA by tail blot analysis (3). Six and eight transgenic animals containing MT-PSX and Alb-PSX sequences, respectively, were produced.

Analysis of gene expression. Serum was derived from blood obtained by retro-orbital plexus phlebotomy. Organ homogenates were prepared as follows. Tissue was quickly frozen in liquid nitrogen and pulverized, and the tissue powder was suspended in 0.5 ml of 0.01 M sodium phosphate buffer (pH 7.4)–0.15 M NaCl–1 mM phenylmethylsulfonyl fluoride and subjected to three cycles of freezing and thawing followed by 20 min of centrifugation (Microfuge B; Beckman Instruments, Inc., Palo Alto, Calif.). The aqueous supernatant and the insoluble pellet were analyzed as described below.

Total soluble protein was determined by Coomassie blue binding (Bio-Rad Laboratories, Richmond, Calif.). Serum or extractable tissue HBsAg was measured by a solid-phase radioimmunoassay (AUSRIA II; Abbott Laboratories, North Chicago, Ill.) and quantitative HBsAg levels were determined by endpoint serial dilution and comparison with a standard curve with the use of purified HBsAg particles of known concentration (3).

Pre-S(1) and pre-S(2) antigens were measured by an enzyme-linked immunosorbent assay (ELISA) as previously described (13a). Briefly, pre-S(1)- or pre-S(2)- specific monoclonal reagents, supplied by W. Gerlich, University of Göttingen, Göttingen, Federal Republic of Germany and M. Mayumi, Jichi Medical School, Tochigi-Ken, Japan, respectively, were used as solid-phase capture antibodies, and horseradish peroxidase-labeled monoclonal anti-S-specific antibody, provided by P. Kaplan, Ortho Laboratories, Raritan, N.J., was used as the probe.

HBV envelope polypeptide composition was assessed by Western blot analysis as described previously (3). Briefly, whole-tissue powder, aqueous extracts, and insoluble pellets were denatured in 2.5% sodium dodecyl sulfate–350 mM mercaptoethanol, boiled, and centrifuged, and the supernatant was subjected to electrophoresis through 15% polyacrylamide gels as described previously (3). Proteins were transferred to nitrocellulose by electroblotting (Bio-Rad), and the filters were blocked with 1% (wt/vol) bovine serum albumin and 5% (vol/vol) fetal calf serum in 50 mM Tris hydrochloride (pH 7.6)–150 mM NaCl containing Tween 20 (50 μl/liter) and incubated overnight at 4°C. Filters were developed by sequential incubation with a rabbit antiserum to a synthetic peptide fragment (p49a) of HBsAg (9), generously provided by R. Lerner, Research Institute of Scripps Clinic, and then with an ^{125}I-labeled staphylococcal protein A (10^6 cpm/ml; Sigma Chemical Co., St. Louis, Mo.) as described previously (15). In selected cases, filters were incubated with the mouse monoclonal antibodies to the pre-S(1) and pre-S(2) antigens and then with an affinity-purified goat antiserum to mouse immunoglobulin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and ^{125}I-labeled affinity-purified swine antiserum to goat immunoglobulin (2×10^5 cpm/ml; 3 μCi/μg).

The distribution of HBV envelope antigens within tissues and cells was assessed by immunofluorescence microscopy as described previously (3). Briefly, 3-μm cryostat sections of frozen tissues were fixed for 1 min in ice-cold acetone and stained with a fluorescein-conjugated antiserum to HBsAg or with the monoclonal anti-pre-S antibodies described above and then with fluorescein-conjugated, affinity-purified goat antibody to mouse immunoglobulin. The specificity of anti-HBsAg reactivity was assessed by blocking with purified HBsAg, and the specificity of anti-pre-S reactivity was assessed by simultaneous analysis of nontransgenic normal mouse tissue substrates.

HBV subviral particles were purified from serum and soluble tissue extracts by sequential isopycnic cesium chloride density gradient centrifugation followed by pelleting through 10% sucrose onto a 60% sucrose cushion as described previously (3). Purified particles were examined by immunogold electron microscopy as previously reported (3). Briefly, concentrated particles were applied to Parlodion (Mallinckrodt, Inc., St. Louis, Mo.) carbon-coated grids and

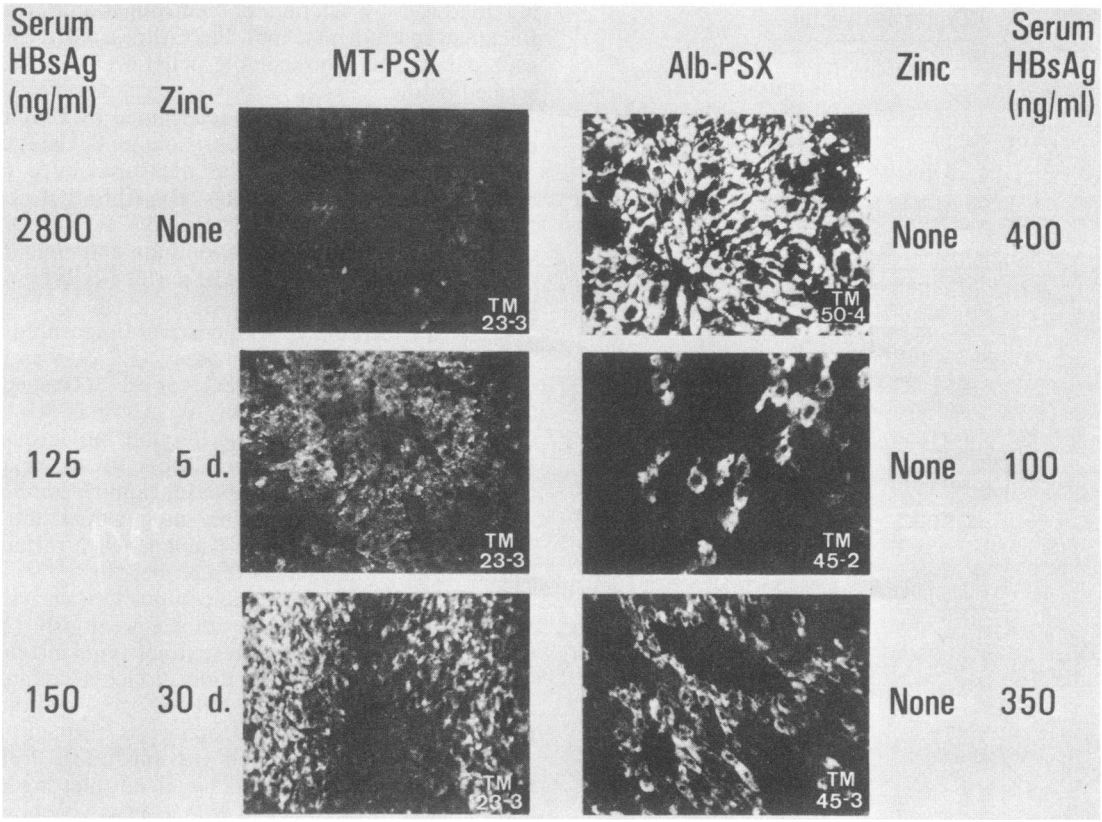

FIG. 2. Hepatic expression of HBsAg in transgenic mice. Dark-field fluorescence microscopy with a Leitz Ortholux microscope, original magnification of ×400. Left side: sequential liver biopsies obtained from an MT-PSX mouse (progeny of TM 23-3) before and during zinc administration. Right side: single liver biopsies from the progeny of three different Alb-PSX transgenic mice not provided with supplemental zinc (TM 50-4, 45-2, 45-3). HBsAg concentrations in serum were obtained at the time of biopsy.

incubated with a rabbit antiserum to HBsAg (Behring Diagnostics, La Jolla, Calif.) and then with a colloidal gold-labeled affinity-purified antibody to rabbit immunoglobulin G. Samples were negatively stained with aqueous uranyl formate. Grids were examined under a Hitachi HV12A electron microscope at ×30,000 magnification.

Modulation of gene expression in vivo. Mice were usually fed a standard rodent chow diet containing approximately 50 and 25 μg of zinc and copper per g, respectively (Teklad, Madison, Wis.). Alternatively, mice received supplemental oral zinc sulfate at 7.5 g/liter in their drinking water. In separate experiments, mice were injected intraperitoneally with 1 mg of aqueous dexamethasone (Bristol Laboratories, Syracuse, N.Y.) and subcutaneously with 1 mg of long-acting dexamethasone (Decadron-LA; Merck Sharp & Dohme, Rahway, N.J.).

RESULTS

Transgenic mice were produced by microinjection of DNA fragments from plasmids pMT-PSX and pAlb-PSX (Fig. 1). Southern blot analysis of restricted genomic DNA was consistent with integration of the microinjected DNA in tandem arrays of various copy numbers at one or two random sites within the mouse genome, as previously described (3).

Total cellular HBsAg was analyzed by immunofluorescence in sequential liver biopsies derived from the same (MT-PSX) mouse before and after zinc ingestion (Fig. 2, top, middle, and bottom left panels). Note that HBsAg was present as a fine granular cytoplasmic component in a minority of peripheral lobular hepatocytes before the administration of zinc at a time when the serum HBsAg concentration was 2,800 ng/ml (Fig. 2, top left). Pre-S(1) and pre-S(2) antigens present on the large envelope polypeptide were not detectable by immunofluorescence prior to zinc ingestion (not shown). Within the first 5 days of zinc administration, the number of HBsAg-positive hepatocytes increased markedly (Fig. 2, middle left) and the pre-S antigens became detectable in the hepatocytes for the first time. Additionally, the intracellular staining pattern had changed to coarsely granular and clumped, suggesting a change in the physical characteristics of the cellular antigen. Unexpectedly, we noted a simultaneous 20-fold decrease in the HBsAg concentration in serum within this time frame. By 30 days, the cellular changes were greatly exaggerated (Fig. 2, bottom left), the pre-S antigens were easily detectable in the hepatocytes, and HBsAg levels in serum remained low. While the intracellular content of HBsAg rose in response to the zinc ingestion in MT-PSX mice, HBsAg levels in serum declined 20-fold within the first 5 days and remained at that level thereafter. These results were confirmed by using three additional MT-PSX mice (Fig. 3A) whose HBsAg levels in serum measured daily after peroral zinc administration, fell dramatically within the first 24 h and reached a basal level within 5 days. This effect was rapidly reversible upon discontinuation of zinc supplementation (Fig. 3B). Zinc

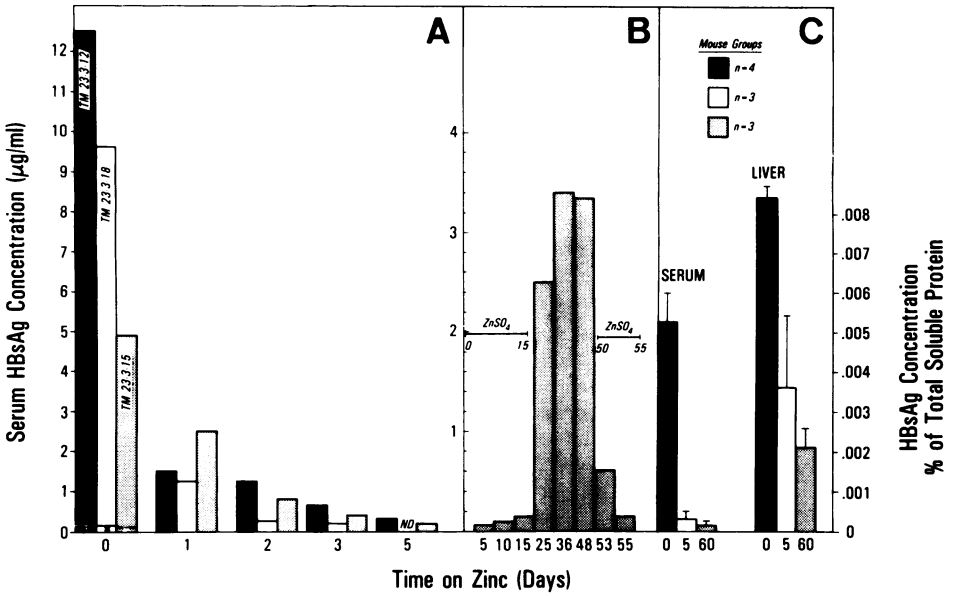

FIG. 3. Modulation of serum and extractable hepatic HBsAg levels in MT-PSX mice by zinc administration. (A) HBsAg concentration in serum in three progeny of a single MT-PSX transgenic mouse before and after zinc administration. Asterisk indicates HBsAg concentration during prior zinc administration. (B) Reversible suppression of HBsAg concentration in serum in a single MT-PSX mouse. (C) Effect of zinc administration on the concentration of HBsAg in liver homogenates from three sets of MT-PSX transgenic littermates; the homogenates were extractable by repeated freezing and thawing in aqueous solvent (phosphate-buffered saline). HBsAg levels in serum are shown for comparison. Results, representing the group mean and standard error, are expressed as the percentage of total protein in the sample.

administration also reduced the concentration of HBsAg in the soluble, aqueous-extractable compartment within the liver (Fig. 3C), in contrast with the increase in total cellular HBsAg level, after zinc administration (Fig. 2, 4, 5, and 6).

The zinc effect was specific for mice containing the MT-PSX construct. Zinc administration also did not decrease HBsAg levels in serum of HBV transgenic mice (1) microinjected with pAC_2, which contains the *Bgl*II AC fragment without exogenous promoter elements (C. Pourcel, personal communication). The pAC_2 transgenic mice have high HBsAg levels in serum (2 to 14 μg/ml) but barely detectable hepatocellular antigen by immunofluorescence (1); thus, in this regard, they resemble MT-PSX mice prior to zinc ingestion. Likewise, zinc administration to transgenic mice carrying the Alb-PSX construct had no effect on the HBsAg concentration in serum which was relatively low. In contrast to the pAC_2 mice however, the intracellular concentration of HBsAg was very high in the Alb-PSX mice (Fig. 2). Furthermore, although, the distribution of HBsAg-positive cells varied from mouse to mouse, each positive hepatocyte displayed an intense, coarsely granular clumped staining pattern resembling that for the MT-PSX mouse after zinc ingestion.

The differences between HBsAg levels in serum and tissue were examined by Western blot analysis. The HBV polypeptide composition of purified secreted HBV particles and detergent-solubilized liver from MT-PSX, Alb-PSX, and pAC_2 transgenic mice (the pAC_2 mice were generously provided by C. Pourcel, Institut Pasteur, Paris, France) are shown in Fig. 4. Total liver homogenate protein from Alb-PSX mice revealed high levels of the major and the large envelope polypeptides in tissue, while HBsAg levels in serum were low. In contrast, pAC_2 and pMT-PSX transgenic mice before zinc ingestion showed much lower levels of both polypeptides in the same amount of total liver homogenate protein, while much higher levels of HBsAg in serum were observed. After zinc ingestion, when the HBsAg concentration in serum had fallen to very low levels, MT-PSX mice showed an increase in both polypeptides intracellularly, particularly in the large envelope polypeptide. The preferential accumulation of the large envelope polypeptide relative to the major polypeptide was demonstrated by normalizing the band intensity of the major polypeptide doublet (Fig. 5). It is apparent that zinc ingestion leads to preferential accumulation of the large polypeptide in MT-PSX mice and that the large polypeptide is even more enriched in the livers of Alb-PSX mice.

Analysis of the polypeptide composition of purified secreted particles from MT-PSX mice by Western blot (Fig. 5) indicates that the major envelope polypeptide doublet is the dominant species. This was confirmed by quantitative solid-phase ELISA (Fig. 6). Solid-phase ELISAs specific for HBsAg (present on all HBV envelope polypeptides) and pre-S(1) antigen (present only on the large envelope polypeptide) were performed on whole serum, soluble liver extract (labeled cytosol), and HBsAg particles purified from both by cesium chloride density gradient ultracentrifugation. The pre-S(1)-containing large envelope polypeptide represented approximately 5% of the total HBsAg present in secreted and extractable antigen, irrespective of zinc administration and total intracellular HBsAg content (Fig. 6). The ELISA results were confirmed on a semiquantitative basis by Western blot analysis with serial dilutions of a standard control of known pre-S and HBsAg composition as determined by radioimmunoassay. This permitted us to calculate that the large envelope polypeptide represents approximately 15 and 30% of total HBsAg in the insoluble liver pellets of mice off and on zinc, respectively (Fig. 6).

These results suggest that the large envelope polypeptide is not readily secreted and that when present in excess, it can prevent secretion of the major envelope polypeptide, leading to the intracellular accumulation of HBsAg and its depletion

from serum. This interpretation is supported by an experiment illustrated in Fig. 7. In this experiment, the polypeptide composition of the soluble aqueous extract (cytosol) from the liver of a zinc-treated MT-PSX mouse was compared with the insoluble pellet and the detergent-solubilized whole-liver homogenate (lysate). It is evident that the HBV envelope polypeptides in the liver cell are preferentially sequestered in the insoluble pellet. This is consistent with the reduction in the level of soluble HBsAg detectable in aqueous extracts (Fig. 3C) and with the reduction in the HBsAg concentration in serum after zinc ingestion by MT-PSX transgenic mice (Fig. 2).

Close scrutiny of Fig. 5 reveals that the upper band of the major polypeptide doublet present in the liver has slightly greater electrophoretic mobility (27 kilodaltons [kDa]) than the equivalent band in secreted serum particles (28 kDa). These observations are confirmed in experiments shown in Fig. 8 and 9. With regard to the major polypeptide, which is represented as a 25- and 28-kDa doublet in the serum particles versus a 25- and 27-kDa doublet in the liver (Fig. 8), mixing experiments with the serum particles and crude liver pellet reveal three bands at 25, 27, and 28 kDa, confirming the size differences (Fig. 9). Since Patzer et al, (20, 21) showed that the final glycosylation event which converts the partially glycosylated major polypeptide (p27) to the fully glycosylated form (p28) occurs in the Golgi apparatus, we conclude that the large envelope polypeptide traps the major

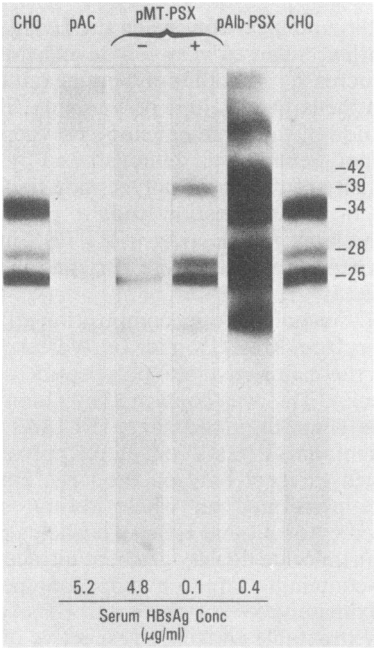

FIG. 4. Analysis of HBV envelope polypeptide composition in transgenic mice. Lanes: CHO, purified HBsAg particles (50 ng) secreted by CHO cells transfected with a plasmid encoding the middle and major envelope polypeptides of HBV (generously provided by P. Tiollais [13]): pAC liver homgenate (100 μg) derived from HBV transgenic mice produced by microinjection of plasmid pAC_2 (containing the HBV *Bgl*II AC fragment in pBR322 (generously provided by C. Pourcel [1]); pMT-PSX liver homogenate (100 μg) derived from MT-PSX transgenic mouse before (−) and after (+) zinc ingestion for 1 month; pAlb-PSX, liver homogenate (100 μg) derived from Alb-PSX transgenic mouse. Western blot. Filter probed with rabbit antiserum to synthetic peptide p49a corresponding to residues 122 to 137 of the HBsAg y subtype (8).

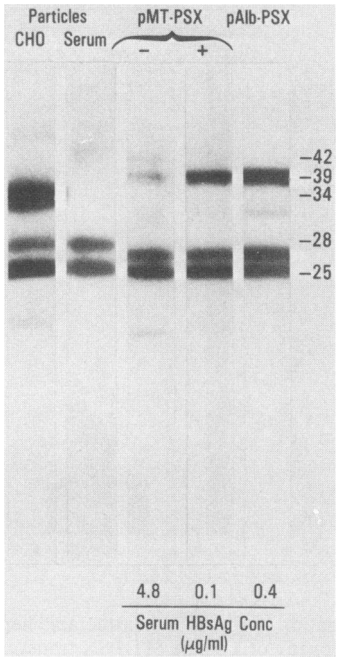

FIG. 5. Western analysis of HBV envelope polypeptides in samples normalized with respect to major polypeptide concentration. Samples are as described in the legend to Fig. 4. Lanes: CHO, 50 ng of CHO-derived HBsAg particles; Serum, 50 ng transgenic mouse serum HBsAg particles; pMT-PSX and pAlb-PSX, 120, 46, and 2.5 μg of liver protein from an MT-PSX mouse before and after zinc ingestion and from an Alb-PSX mouse, respectively, normalized with respect to the major polypeptide doublet. Filter probed with antiserum specific for HBsAg, as described in the legend to Fig. 4.

polypeptide in an early Golgi or pre-Golgi compartment in the hepatocyte (21).

The intracellular trapping effect of the large envelope polypeptide on HBsAg secretion can be overcome by parenteral dexamethasone administration. Zinc administered perorally reduces HBsAg levels in serum (Fig. 10A), while dexamethasone injection induces a transient 5- to 15-fold increase in HBsAg levels in serum in MT-PSX mice (Fig. 10B) even during a course of peroral administration of zinc (Fig. 10C). Furthermore, dexamethasone-induced enhancement of HBsAg levels in serum can be minimized by subsequent zinc ingestion (Fig. 10D). Finally, when dexamethasone injection is simultaneous with the cessation of zinc ingestion, HBsAg levels in serum rise well above the original prezinc levels (Fig. 10E) rather than returning to base-line levels as occurs merely upon withdrawl of zinc (Fig. 10F). The mechanism of this dexamethasone-enhanced HBsAg secretion is currently under investigation.

Surprisingly, we are unable to explain the influence of zinc administration on polypeptide composition and antigen secretion at the level of transcription. Analysis of total cellular RNA from MT-PSX transgenic mouse livers revealed a complex pattern of specific transcripts. However, we cannot detect quantitative or qualitative difference in these RNA species before and after zinc administration. Similar observations were made before and after dexamethasone treatment. Presumably the large polypeptide can only be produced from transcripts derived from transcription start sites defined by the MT promoter in these mice, while the major polypeptide can be produced from this transcript plus a shorter transcript emanating from the endogenous HBV

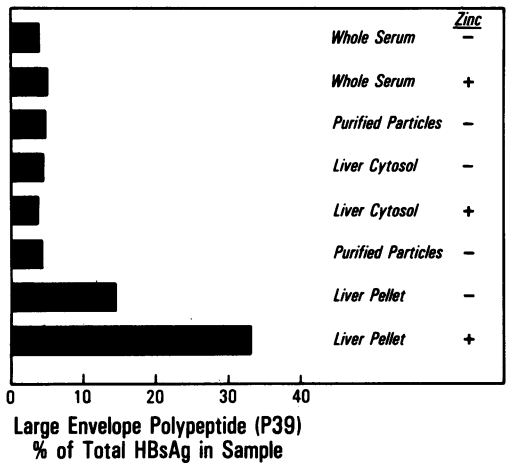

FIG. 6. Relative polypeptide composition of secreted aqueous extractable insoluble HBsAg in MT-PSX transgenic mouse liver. All samples, except the liver pellet, were analyzed by solid-phase ELISA for the presence of pre-S(1) antigen (unique to the large envelope polypeptide) and HBsAg (present on all envelope polypeptides), and the ratio was plotted. All samples plus a serum HBsAg particle standard curve were also analyzed by Western blot as for Fig. 4 and 5. Since band intensities compared favorably with quantitative ELISA results for all soluble samples, the relative band intensities of the HBsAg polypeptides present in the insoluble liver pellet were estimated by Western analysis.

promoter within the pre-S region. It therefore appears that zinc and dexamethasone may be operating at a posttranscriptional level. Studies to define the mechanism responsible for the effects of zinc and dexamethasone on polypeptide composition and secretion at the molecular level are currently under way.

DISCUSSION

The major envelope polypeptide of HBV is the dominant structural component of the outer membrane of the infectious HBV virion (Dane particle) and the noninfectious subviral filaments and spheres which represent excess viral coat produced during HBV infection (reviewed in reference 30). The large polypeptide is greatly enriched in Dane particles and subviral filaments relative to spheres and is not detectable at all on spheres in the absence of viral replication (28). While this association may suggest a role for the large polypeptide in viral and subviral filament structure and, perhaps, virion assembly, the specific properties of this polypeptide that might determine its function in the virus life cycle have not been defined.

The production of abundant quantities of the major and large envelope polypeptide in the transgenic mouse model has permitted the in vivo analysis of some of the properties of these molecules. It has been shown that the major envelope polypeptide is readily secreted from hepatocytes. This is suggested by the high HBsAg concentrations in serum and the low levels of intracellular HBsAg detected by immunofluorescence and Western blot analysis in our untreated MT-PSX transgenic mice and in the pAC_2 transgenic mice of Babinet et al. (1) in which the dominant HBV product is the major envelope polypeptide. Additionally, the production of HBsAg particles in the absence of the middle and large polypeptides confirms other reports indicating that only the major polypeptide is necessary for assembly and secretion of 22-nm HBsAg particles (5, 20–22).

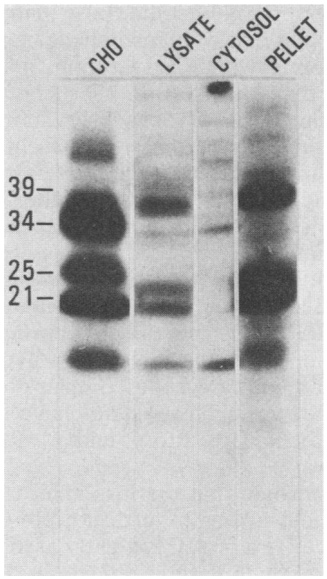

FIG. 7. Western analysis of subcellular distribution of HBsAg polypeptides in MT-PSX liver following 30 days of zinc ingestion. Lanes: CHO, CHO cell-derived HBsAg particles containing the middle and major envelope polypeptides (as for Fig. 4); LYSATE, CYTOSOL, and PELLET, 100 μg of whole-liver homogenate (lysate), aqueous liver extract (cytosol), and insoluble liver pellet (pellet), respectively, derived from the same zinc-treated MT-PSX mouse liver. Filter probed with antiserum specific for HBsAg as described in the legend to Fig. 4.

In addition, it has been demonstrated that the large envelope polypeptide is not readily secreted. Indeed, under conditions in which it constitutes approximately 30% of intracellular HBsAg, it contributes less than 5% of the envelope polypeptides in the secreted HBsAg particles present in serum. Since approximately 10% of the secreted particles in the serum of our transgenic mice are filaments (data not shown), which may be relatively rich in the large polypeptide (9, 28), it is likely that the subviral spherical particles, which constitute over 90% of the HBsAg in serum in the mice we used, contain little or no large polypeptide.

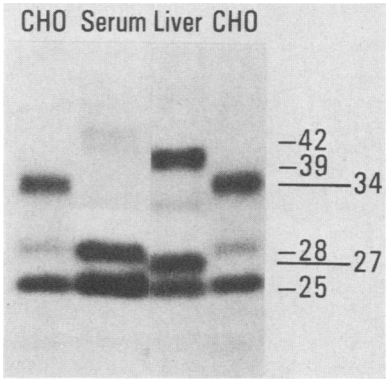

FIG. 8. Western analysis of polypeptide composition and relative molecular mass of secreted and insoluble liver HBsAg. Lanes: CHO, 50 ng of CHO particles; Serum, 50 ng of serum-derived particles from MT-PSX mouse; Liver, 100 μg of sodium dodecyl sulfate-solubilized liver pellet from MT-PSX mouse on zinc. Filter probed with antiserum specific for HBsAg, as described in the legend to Fig. 4.

The large envelope polypeptide also entraps the major polypeptide within a relatively insoluble compartment in the cell, thus interfering with the secretion, and possibly the transport and assembly, of HBsAg particles. We draw this conclusion on the basis of our observations that (i) the HBsAg concentration in serum shows an inverse relationship with the intracellular large envelope polypeptide concent; (ii) zinc-induced increase in the large envelope polypeptide relative to the major polypeptide is accompanied by a rapid and reversible reduction in HBsAg concentration in serum; and (iii) zinc treatment also reduces the amount of soluble HBsAg present in aqueous liver cell extracts in MT-PSX transgenic mice, causing the preferential partitioning of all of the envelope polypeptides in the insoluble cellular membrane pellet. Similar observations in a variety of other experimental systems have been made (D. Ganem, A. McLachlan, B. Moss, and W. Rutter, personal communication).

Finally, we conclude that the insoluble, nonsecreted envelope polypeptides are entrapped in a pre-Golgi compartment and that they are probably tightly associated with the membranes of the endoplasmic reticulum. This interpretation is based on the comparative sizes of the higher-molecular-weight forms of the major polypeptide in the intracellular and secreted forms of HBsAg (Fig. 8 and 9). The results are compatible with those of Patzer et al. (21), who showed with transfected Chinese hamster ovary (CHO) cells, that cell-associated HBsAg contains only high-mannose residues and is therefore smaller than secreted HBsAg, which contains complex oligosaccharides, and that no Golgi-mediated oligosaccharide processing of the high-mannose chains was detectable inside the cells. If HBsAg processing in transgenic mouse hepatocytes parallels that in CHO cells, these results suggest that most intracellular HBsAg resides in a pre-Golgi or early Golgi compartment and that the major envelope polypeptide undergoes efficient processing of its high-mannose chains as it rapidly passes through the Golgi during secretion. It therefore appears that the large polypeptide inhibits secretion of HBsAg by blocking a processing step which occurs prior to transport to the Golgi apparatus.

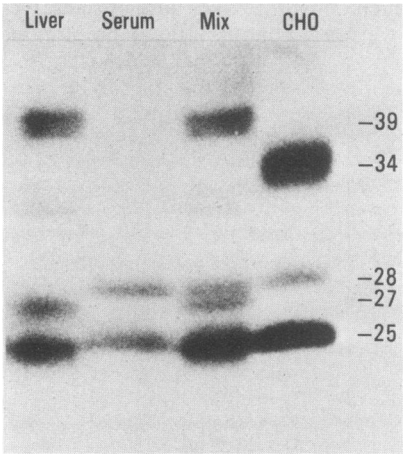

FIG. 9. Western analysis illustrating differential electrophoretic migration of the major envelope polypeptide in secreted and insoluble liver HBsAg. Lanes: Liver, 75 μg of sodium dodecyl sulfate-solubilized liver pellet from MT-PSX mouse on zinc; Serum, 25 ng of serum-derived secreted particles from MT-PSX mouse; Mix, mixture of samples applied to lanes 1 and 2; CHO, 50 ng of CHO particles. Filter probed with antiserum specific for HBsAg as described in the legend to Fig. 4.

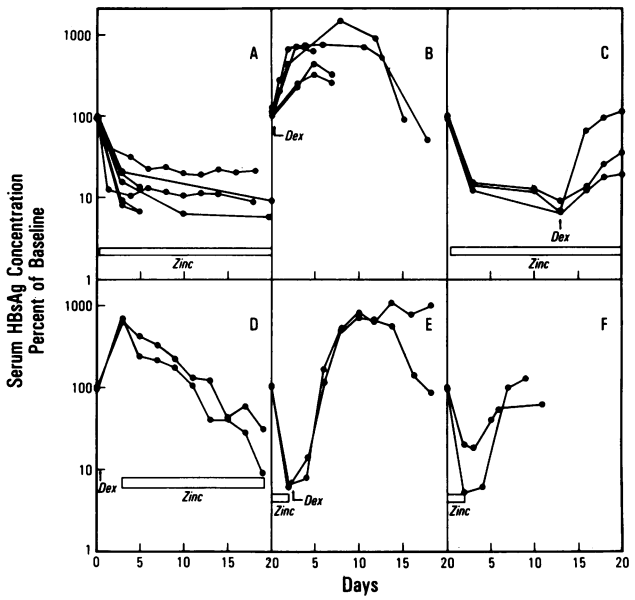

FIG. 10. Modulation of the HBsAg concentration in serum by zinc and dexamethasone. Results are expressed as a percentage of the HBsAg concentration in serum prior to treatment. Zinc was administered in drinking water at 7.5 g/liter for the duration indicated by horizontal bar. Dexamethasone, was injected intraperitoneally and subcutaneously, as described in text, at the time indicated by the arrow.

Since the pre-S containing, large envelope polypeptide prevents the major HBsAg polypeptide from being secreted, it seems likely that it interacts with the major polypeptide at an early stage in the particle assembly pathway, trapping it in an insoluble cellular compartment. Considering that the pre-S region of the large envelope polypeptide has no known function, the properties described here may be relevant to the role it plays in the assembly, transport, and secretion of viral and subviral particles during HBV infection in man.

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