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Chimeric Hepatitis B Virus Core Particles with Parts or Copies of the Hepatitis C Virus Core Protein

AKIRA YOSHIKAWA,[1] TAKESHI TANAKA,[1] YUJI HOSHI,[1] NAOMI KATO,[1] KATSUMI TACHIBANA,[1] HISAO IIZUKA,[1] ATSUHIKO MACHIDA,[2] HIROAKI OKAMOTO,[3] MAKARI YAMASAKI,[4] YUZO MIYAKAWA,[5] AND MAKOTO MAYUMI[3]*

Japanese Red Cross Saitama Blood Center, Saitama-Ken 338,[1] Yamanashi Institute for Public Health, Yamanashi-Ken 400,[2] Immunology Division, Jichi Medical School, Tochigi-Ken 329-04,[3] Department of Agricultural Chemistry, University of Tokyo, Tokyo 113,[4] and Mita Institute, Tokyo 108,[5] Japan

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Either parts or multiple copies of the core gene of hepatitis C virus (HCV) were fused to the 3′ terminus of the hepatitis B virus (HBV) core gene with 34 codons removed. As many as four copies of HCV core protein (720 amino acids) were fused to the carboxy terminus of truncated HBV core protein (149 amino acids) without preventing the assembly of HBV core particles. Chimeric core particles were sandwiched between monoclonal antibody to HBV core and that to HCV core, thereby indicating that antigenic determinants of both HBV and HCV cores were accessible on them. Proteolytic digestion deprived chimeric core particles of the antigenicity for the HCV core without affecting that of the HBV core, confirming the surface exposure of HCV core determinants. The density of HCV core determinants on chimeric core particles increased as copies of fused HCV core protein were increased. Hybrid core particles with multiple HCV core determinants would be instrumental as an antigen probe for detecting class-specific antibodies to the HCV core in patients with acute and chronic hepatitis C and for simultaneous detection of antibodies to HBV core and those to HCV core in donated blood.

The core particle of hepatitis B virus (HBV) is an ideal vehicle for the immunological expression of foreign proteins. Sequences of the other viruses can be inserted at the amino or carboxy terminus or internal sites of the HBV core protein (known as hepatitis B core antigen [HBcAg]) without affecting its ability to self-assemble into HBV core particles (5, 6, 8–10, 29, 30, 32, 33). Thus, engineered fusion proteins are particulate, and inserted epitopes are surface accessible. Combined with the potent T-cell-dependent and -independent immunogenicity of the HBV core (21, 22), chimeric core particles can be used as a general vehicle for presenting desired epitopes in subunit and hybrid vaccines.

Hepatitis C virus (HCV) is a positive-stranded RNA virus of ~9,400 nucleotides with a 5′-noncoding region of up to 341 nucleotides and a single, long open reading frame which encodes viral polyprotein and is divided into the core and envelope genes and five nonstructural regions (7, 12, 13). Individuals infected with HCV raise antibodies to various HCV proteins, which are detected by enzyme-linked immunosorbent assays (ELISAs) with recombinant proteins or synthetic oligopeptides deduced from the HCV genome (18, 20, 24–26). These assays are useful in diagnosing HCV infection in patients with acute and chronic non-A, non-B liver disease (2, 18, 20, 25, 26) and for excluding HCV-contaminated blood units from being used for transfusion (1, 2, 17).

Of the various antibodies elicited in response to HCV infection, those to the HCV core would be of the highest diagnostic value, because they develop earlier than the other antibodies and are not dependent on genotypes of HCV strains (13, 20, 24, 26). Chimeric HBV core particles with surface expression of HCV core protein will therefore provide a powerful tool in serological assays of antibodies associated with HCV infection.

Recombinant plasmids carrying either parts or multiple copies of the HCV core gene, fused to the HBV core gene with the 3′-terminal 34 codons removed, were constructed for expression in *Escherichia coli*. Chimeric core particles recovered in cell lysates, along with the hybrid HBcAg protein composing them, were tested for the qualitative and quantitative expression of HCV core epitopes.

MATERIALS AND METHODS

Construction of expression plasmids. The expression of fusion proteins by recombinant DNA was performed with *E. coli* MC1061. Plasmid pNDR260 carrying the core gene of HBV of subtype adr (27) and plasmid pCC5-J4 bearing codons 1 to 180 of the core gene of HCV of genotype II (34) have been described elsewhere. Plasmid pTRc99A (Pharmacia, Uppsala, Sweden) was used as an expression vector, which carried a *tac* promoter upstream of multicloning sites and a *laqI*^{q} gene for overproduction of the *lac* repressor. Structures of hybrid HBV and HCV core proteins, obtained in *E. coli* cells transformed with recombinant plasmids, are illustrated in Fig. 1.

The HBV core gene with the carboxyl-terminal 34 codons removed and spanning nucleotides 1901 to 2347 was amplified by polymerase chain reaction (PCR) with the sense primer carrying a restriction site for *Nco*I and the antisense primer bearing that for *Eco*RI. PCR products were digested with *Nco*I and *Eco*RI, and the restriction fragments were inserted between the *Nco*I and *Eco*RI sites of pTRc99A. The plasmid obtained (pHBC×0) coded for amino acids (aa) 1 to 149 of the HBV core protein (HBcAg) and an additional 24 aa with a sequence of EFELGTRGSSTVDLQACKLGCF GG encoded by multicloning sites (*Eco*RI-*Bam*HI-*Xba*I-*Sal*I-*Pst*I-*Hin*dIII).

* Corresponding author.

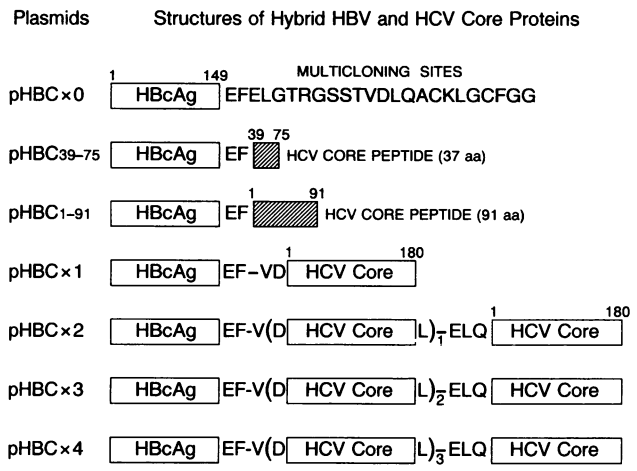

FIG. 1. Structures of hybrid HBV and HCV core proteins expressed in *E. coli* transformed with recombinant plasmids. pHBC×0 was for the expression of a carrier vector composed of HBcAg protein (with the carboxy-terminal 34 aa removed) connected to 24 aa (encoded by multicloning sites). $pHBC_{39-75}$ and $pHBC_{1-91}$ were for the expression of HBcAg protein fused with parts of the HCV core protein (shaded). pHBC×1 to pHBC×4 were for the expression of HBcAg protein fused with one to four copies of the HCV core protein, respectively, with compositions as indicated (see Materials and Methods for details).

Recombinant plasmids were obtained by inserting, into the multicloning sites, either parts of the HCV core gene or up to four copies thereof. Recombinants carrying the 3′-truncated HBV core gene (codons 1 to 149) fused to codons 39 to 75 (for 37 aa) or codons 1 to 91 (for 91 aa) of the HCV core gene were constructed by inserting, between the *Eco*RI and *Hin*dIII sites of pHBC×0, the PCR products with appropriate sense and antisense primers carrying *Eco*RI and *Hin*dIII recognition sites, respectively. They were designated $pHBC_{39-75}$ and $pHBC_{1-91}$, respectively.

The recombinant (pHBC×1) for the expression of truncated HBcAg (aa 1 to 149) and a single copy of the amino-terminal 180 aa of the HCV core protein was obtained as follows. Codons 1 to 180 of the HCV core gene were amplified on pCC5-J4 by PCR with primers incorporating desired restriction sites, 5′-AACCTGCAGATGAGCACGA ATCCTAAACC-3′ (sense, nt 1 to 20 [numbered from the 5′ terminus of a long open reading frame] with the underlined *Pst*I site) and 5′-AACAAGCTTAAGCCAAGAGGAAGAT AGAGA-3′ (antisense, nt 540 to 521 with the underlined *Hin*dIII site and overlapping termination codon [TAA]). PCR products were treated with *Pst*I and *Hin*dIII, and the digest was inserted between the *Pst*I and *Hin*dIII sites of pHBC×0 to obtain pHBC×1.

Recombinants carrying the truncated HBV core gene fused with up to four copies of the HCV core gene were constructed by inserting a cassette of the HCV core gene, one after another, into pHBC×1. Codons 1 to 180 of the HCV core gene were amplified by PCR with primers 5′-AA CGTCGACATGAGCACGAATCCTAAACC-3′ (*Sal*I site underlined) and 5′-AACCTGCAGCTCGAGAGCCAAGAG GAAGATAGAG-3′ (*Xho*I and *Pst*I sites underlined). PCR products were digested with *Sal*I and *Pst*I to make the cassette, which was inserted between the *Sal*I and *Pst*I sites of the residual multicloning sites in pHBC×1. The plasmid thus constructed (pHBC×2) carried the truncated HBV core gene fused with two copies of the HCV core gene. Likewise, the cassette was inserted between *Xho*I and *Pst*I sites of pHBC×2 to produce pHBC×3. This strategy took advantage of the fact that the sequence of ligation between *Xho*I and *Sal*I sites (CTCGAC), encoding Leu-Asp, was not digestible with either *Xho*I or *Sal*I. By repeating this procedure again, pHBC×4, which carried the truncated HBV core gene fused with four copies of the HCV core gene, was obtained.

Induction and purification of fusion proteins. Fusion proteins were expressed in transformed *E. coli* by induction with isopropyl-β-D-thiogalactopyranoside by using a method described elsewhere (34). Cells were harvested and lysed by sonication, and the cellular debris were removed by centrifugation at 10,000 × *g* for 20 min. The supernatant was made 0.9 M with $(NH_4)_2SO_4$ and 1 mM with phenylmethylsulfonyl fluoride and centrifuged at 10,000 × *g* for 30 min. The precipitate was dissolved in 50 mM Tris-HCl buffer (pH 7.4) and brought to a density of 1.30 g/cm^3 with metrizamide. In a tube for a Beckman 45 Ti rotor, 30 ml of sample (at the bottom) was overlaid with 20 ml of metrizamide in buffer (1.22 g/cm^3) and 50 ml of buffer. The tube was centrifuged at 158,000 × *g* at 10°C for 16 h. The contents of the tube were fractionated, and the fractions corresponding to a density from 1.30 to 1.34 g/cm^3 were pooled. They were dialyzed against buffer and transferred to a tube in which 7 ml of 60% (wt/vol) sucrose and 10 ml of 40% sucrose had been layered. The tube was centrifuged in a Beckman SW28 rotor at 90,000 × *g* at 10°C for 16 h, and fractions with a sucrose density from 48.5 to 53.5% were pooled to obtain a purified preparation of chimeric core particles made of hybrid HBV and HCV core proteins. It was used for the analysis of constituent hybrid proteins by immunoblotting.

MAbs. Monoclonal antibody (MAb) 3120 was directed to determinant β of HBcAg, which is expressed on the surface of HBV core particles (31). MAb 905 was directed to determinant b of hepatitis B e antigen (HBeAg) (15). MAb C33 was raised, by standard methods, against a 21-mer synthetic peptide representing aa 126 to 146 of HBcAg, and MAb 9380B was raised against a 36-mer synthetic peptide (CP9) representing aa 39 to 74 of the HCV core protein (25).

ELISA. Chimeric core particles were detected by being sandwiched between two MAbs in ELISA. Wells of a plastic microtiter plate (Sumitomo Bakelite Co., Tokyo, Japan) received 50 μl of phosphate-buffered saline (PBS) containing 20 μg of MAb 3120 to HBV core per ml. The plate was left at room temperature for 4 h and then washed with PBS supplemented with 0.05% (vol/vol) Tween 20. Unsaturated binding sites were quenched with 2% (wt/vol) skim milk (Difco Laboratories, Detroit, Mich.) in PBS supplemented with 0.05% Tween 20. Samples containing chimeric core particles (50 μl) were delivered to wells, and the plate was incubated at room temperature for 1 h. The plate was washed, and wells received 50 μl of PBS supplemented with 40% (vol/vol) fetal calf serum and containing 20 ng of MAb 9380B, MAb 3210, or MAb 905 labeled with horseradish peroxidase. The binding of labeled MAb was determined by measurement of A_{492}.

Immunoblotting. Chimeric core particles were partially purified by metrizamide and sucrose density gradient centrifugations. They were broken into constituent polypeptides by being heated at 100°C for 3 min in buffer containing 1% (wt/vol) sodium dodecyl sulfate and 1% (vol/vol) 2-mercaptoethanol. Fusion proteins containing HBV and HCV core sequences were separated by electrophoresis on a linear-gradient (4 to 20%) polyacrylamide gel in the presence of

0.1% sodium dodecyl sulfate. Polypeptides on the gel were transferred onto nitrocellulose membranes and tested for binding with MAb 9380B or MAb C33 labeled with horseradish peroxidase. Bands were then visualized by the conversion of diaminobenzidine hydrochloride (Sigma Chemical Co., St. Louis, Mo.).

Proteolytic digestion. Chimeric core particles were digested with 1 mg of proteinase K (Boehringer GmbH, Mannheim, Germany) per ml in 50 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl, 0.05% 2-merecaptoethanol, and 0.5% (vol/vol) Nonidet P-40 (Sigma). The reaction was performed for 10 or 60 min at 37°C and terminated by addition of 1/10 volume of 100 mM phenylmethylsulfonyl fluoride (Boehringer).

Immunoelectron microscopy. A supernatant of sonicated *E. coli* cells (1 ml) transformed with $pHBC_{1-91}$ or pHBC×3 was loaded onto a stepwise density gradient in a Beckman SW40 tube containing CsCl at 1.2 g/cm^3 (1 ml), 1.25 g/cm^3 (3 ml), 1.35 g/cm^3 (2 ml), and 1.50 g/cm^3 (2 ml) in 50 mM Tris-HCl buffer (pH 7.4) plus 0.15 M NaCl. The tube was centrifuged at 35,000 × *g* at 20°C for 18 h and pierced at the bottom to obtain 0.5-ml fractions. The fractions were monitored for HCV core and HBV core determinants by ELISA. Fractions with high activities were incubated with either MAb 9380B or MAb 3120 at room temperature for 1 h and centrifuged at 10,000 × *g* for 5 min. The immune aggregates were washed with saline three times and dissolved with saline. They were sonicated for a few seconds, spread over a carbon-coated grid, stained with 2% (vol/vol) phosphotungstic acid, and observed in an electron microscope (model 002A; TOPCON, Tokyo, Japan).

RESULTS

Antigenic determinants on chimeric HBV and HCV core particles. *E. coli* cells were transformed with recombinant plasmids carrying the carboxy-terminally truncated HBV core gene (codons 1 to 149) fused with either parts of the HCV core gene or one to four copies of the gene. Supernatants of disrupted cells were tested for HBV core particles by sandwich ELISA with immobilized and enzyme-labeled MAb to HBV core. Core particles were detected in the supernatant of lysed *E. coli* cells that had been transformed with any recombinants carrying the hybrid HBV-HCV core gene. Lysates of *E. coli* transformed with various recombinants were standardized for HBV core particles by dilution to show an A_{492} value of 1.00 in ELISA. Chimeric core particles in them were tested for the expression of HCV core determinants and HBeAg by sandwich ELISA with immobilized MAb to HBV core and labeled MAb to an HCV core peptide or that to HBeAg (Table 1).

HCV core determinants were not available on chimeric core particles made of HBcAg fused with aa 39 to 75 of the HCV core protein. They were accessible on the surface of chimeric core particles consisting of HBcAg fused with aa 1 to 91 of the HCV core protein, however. The antigenicity for HCV core was invariably exhibited by chimeric core particles made of HBcAg fused with one to four copies of the HCV core protein. The antigenic activity for HCV on chimeric core particles increased up to three copies of HCV core protein fused to HBcAg; no additive effects were observed with four copies. The yield of chimeric core particles, however, decreased as the number of fused copies of HCV core protein was increased. The antigenic determinant of HBeAg was not detected on any chimeric core particles or core particles made of HBcAg alone.

TABLE 1. Expression of the antigenic determinants of HCV core on chimeric HBV core particles[a]

Recombinant plasmids	Incorporated HCV core sequences	A_{492} of antigenic determinants[b] of:	
		HCV core[c]	HBeAg[d]
pHBC×0	None	0.03	0.02
$pHBC_{39-75}$	aa 39–75	0.03	0.01
$pHBC_{1-91}$	aa 1–91	0.39	0.03
pHBC×1	1 copy	0.44	0.02
pHBC×2	2 copies	0.97	0.03
pHBC×3	3 copies	1.40	0.03
pHBC×4	4 copies	1.49	0.03

[a] HBV core particles expressed in *E. coli* cells transformed with various recombinant plasmids, carrying the 3′-truncated HBV core gene (codons 1 to 149) and parts or copies of the HCV core gene, were tested by ELISA for antigenic determinants of HCV core and HBeAg.
[b] Determined in samples diluted, so as to have an A_{492} value of 1.00 in sandwich ELISA, with immobilized and labeled MAb 3120 to HBV core.
[c] Determined by sandwich ELISA with immobilized MAb 3120 to HBV core and labeled MAb 9380B to HCV core.
[d] Determined by sandwich ELISA with immobilized MAb 3120 to HBV core and labeled MAb 905 to HBeAg.

Chimeric core particles were digested with proteinase K (1 mg/ml) for 10 or 60 min and then tested for antigenic determinants of HCV core and HBV core by ELISA (Table 2). The antigenicity for HCV core was lost, whereas that for HBV core was preserved. Chimeric core particles maintained the particle structure, because they bound to MAb 3120, which reacts with HBV core particles but not with HBcAg protein (31). The susceptibility of HCV core determinants to proteolysis, without destroying core structure, confirmed that they were exposed on the surface of chimeric core particles.

Antigenic determinants on HBcAg fused with HCV core protein. HBV core particles assembled with HBcAg alone or chimeric core particles composed of HBcAg fused with sequences of the HCV core protein were split into constituent polypeptide under reducing conditions, and separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Polypeptide bands were tested for the expression of HBcAg and HCV core determinants by immunoblotting.

HBcAg determinants were detected in bands at positions of the expected M_r on all hybrid HBcAg polypeptides carrying parts of the HCV core protein or up to four copies of the protein (Fig. 2A). For the analysis of HBcAg fused

TABLE 2. Susceptibility to digestion with proteinase K of HCV core determinants on chimeric core particles[a]

Conditions	A_{492} of antigenic determinants of:	
	HCV core[b]	HBV core[c]
Undigested	1.005	1.649
Digested		
10 min	0.040	1.811
60 min	0.041	1.520

[a] Chimeric core particles assembled with fusion proteins consisting of carboxy-terminally truncated HBcAg and three copies of HCV core protein (obtained with pHBC×3) were digested with proteinase K (1 mg/ml) for 10 or 60 min and tested for HCV core and HBV core determinants.
[b] Determined by sandwich ELISA with immobilized MAb 3210 to HBV core and labeled MAb 9380B to HCV core.
[c] Determined by sandwich ELISA with immobilized and labeled MAb 3210 to HBV core.

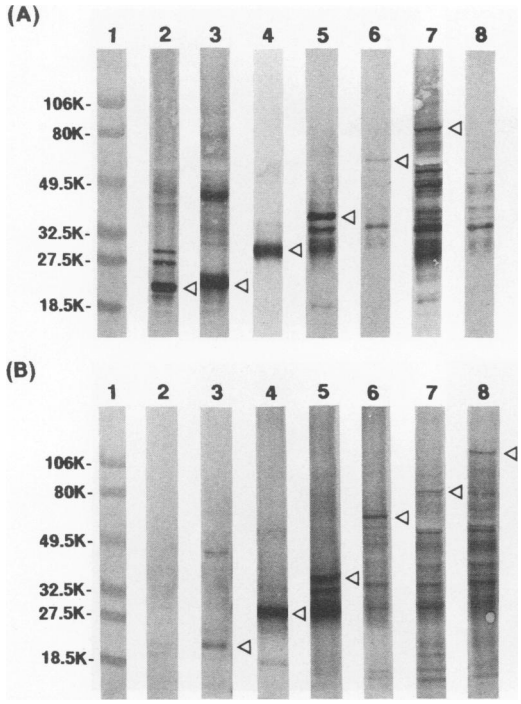

FIG. 2. Immunoblotting of hybrid HBV and HCV core proteins. Polypeptides constituting chimeric core particles, obtained with various plasmids, were separated by polyacrylamide gel electrophoresis and tested for binding with MAbs. Lanes: 1, molecular size markers; 2, pHBC×0; 3, $pHBC_{39-75}$; 4, $pHBC_{1-91}$; 5, pHBC×1; 6, pHBC×2; 7, pHBC×3; 8, pHBC×4. Polypeptide bands were stained with MAb C33 to HBcAg (A) and with MAb 9380B to HCV core (B). The open triangles indicates the positions of fusion proteins expressed at full length.

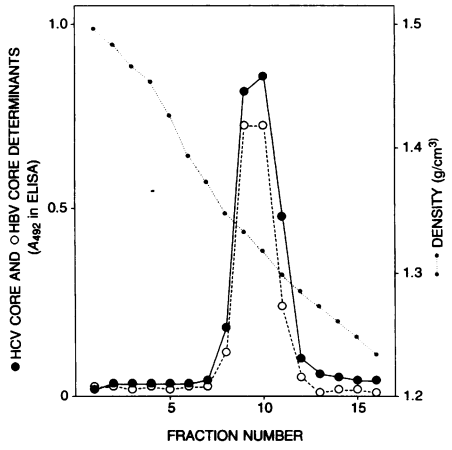

FIG. 3. Isolation of chimeric core particles by fractionation through a CsCl density gradient. Fractions of chimeric core particles expressed in *E. coli* cells transformed with pHBC×3 were tested by ELISA for HCV core and HBV core determinants.

with three copies of HCV core protein, a 10-fold more was applied than was used for the other fusion proteins. When a regular amount of HBcAg fused with four copies of the HCV core protein was applied, it hardly showed the activity for HBcAg (Fig. 2A, lane 8). When 10-fold more of it was applied to gel electrophoresis, however, HBcAg became visible at the expected position (data not shown). The activity of HBcAg decreased as the length of the incorporated heterologous sequence increased. This was probably because the proportion of HBcAg in fusion proteins would be decreased as incorporated heterologous sequences were elongated.

HCV core determinants were detected on HBcAg fused with parts of the HCV core protein or up to four copies of the protein at positions of the estimated M_r (Fig. 2B). HBcAg fused with two to four copies of HCV core protein displayed additional bands with HCV core determinants at positions of lower M_r (lanes 6 to 8). These represent degraded fusion proteins or products carrying incompletely translated sequences.

Aggregation of chimeric core particles with MAb to HCV core. Chimeric core particles in lysates of *E. coli* cells transformed with $pHBC_{1-91}$ or pHBC×3 were partially purified by fractionation through a CsCl density gradient. Each fraction was tested for antigenic determinants of HCV core and HBV core by ELISA (Fig. 3). Profiles of HCV core and HBV core determinants are shown for chimeric core particles obtained with pHBC×3 (Fig. 3). They coincided and peaked at a density of 1.31 g/cm^3.

Fractions with high activities for HCV core and HBV core determinants were pooled and subjected to immunoelectron microscopy with MAb 9380B to HCV core or MAb 3120 to HBV core (Fig. 4). Chimeric core particles obtained with $pHBC_{1-91}$ were round and smooth (A), and they aggregated by MAb to HBV core (B) or MAb to HCV core (C) with antibody haloes. Chimeric core particles obtained with pHBC×3 were deformed, and small aggregates were observed in the absence of antibodies (D). They formed large clumps after being incubated with either MAb to HBV core (E) or MAb to HCV core (F).

DISCUSSION

The amino-terminal 37 or 91 aa of the HCV core protein, as well as up to four copies of the core protein encompassing 720 aa, were fused to HBcAg with the carboxy-terminal region removed and expressed in *E. coli* by using a *tac* promoter-driven system. Six species of hybrid HBcAg fused with HCV core sequences self-assembled to make chimeric core particles, and five of them showed the surface expression of both HBV and HCV core epitopes.

The carboxy-terminal, protamine-like region of HBcAg binds with nucleic acids and is buried inside core particles (11, 19). The carboxy-terminal 34 aa (aa 150 to 183) are not required for the assembly of HBV core (11). Sequences of simian immunodeficiency virus envelope, when linked to the carboxy terminus of HBcAg (Cys-183), were not immunogenic in guinea pigs (33), probably because they were oriented inside chimeric core particles. Taken together, HBcAg with the carboxy-terminal region removed would work better than the intact HBcAg for presentation of foreign proteins on the surface of chimeric HBV core particles.

Heterologous peptides thus far incorporated into chimeric HBV core particles are rather short, ranging to 100 aa at most (5, 6, 8–10, 29, 30, 32, 33). HBV core particles appear to have the capacity to accommodate much longer heterologous sequences, however. Hybrid HBcAg fused with four copies of the HCV core protein spanning 720 amino acids, plus 21 amino acids for linkers, was still capable of self-assembling to make chimeric core particles. Antigenic determinants of HCV core were surface available on chimeric

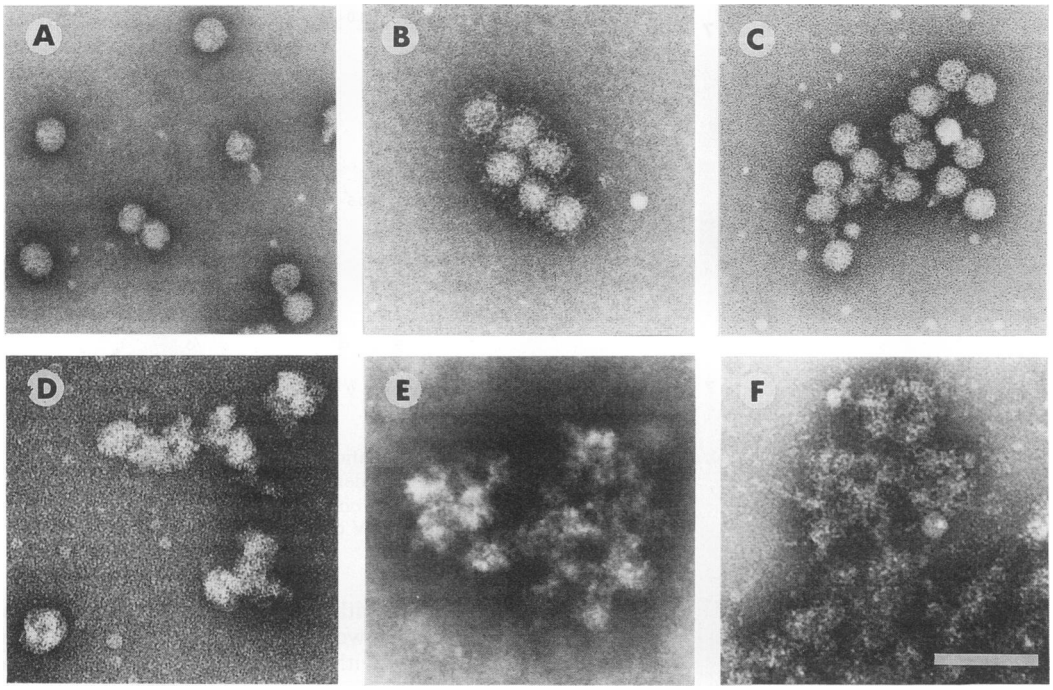

FIG. 4. Immunoelectron microscopy of chimeric core particles. Chimeric core particles expressed in *E. coli* cells transformed with $pHBC_{1-91}$ (A to C) or pHBC×3 (D to F) were observed without antibodies (panels A and D) or after being incubated with MAb 3120 to HBV core (panels B and E) or MAb 9380B to HCV core (panels C and F). Bar, 100 nm.

core particles and were susceptible to proteolytic digestion. Taken together, these lines of evidence indicate that a good part of the HCV core sequence would protrude outside core particles and would thereby not interfere with the particle assembly.

Ulrich et al. (32) reported that fusion of 90 aa, representing a sequence of the Gag protein of human immunodeficiency virus type 1, to Pro-144 of HBcAg still allowed the formation of HBV cores with the surface expression of the foreign protein. Fusion of 317, 189, or 100 aa of the Gag protein to HBcAg (aa 1 to 144), however, prevented self-assembly of chimeric core particles. They used HBcAg truncated of the carboxy-terminal 39 aa, which are not required for particle assembly (4). Their observation is at variance with the present results indicating that HBcAg (aa 1 to 149), with the carboxy-terminal 34 aa missing, can carry much longer sequences without inhibiting particle assembly. It is not known whether this discrepancy is due to distinct foreign sequences to be incorporated or HBcAg polypeptides with different extents of carboxy-terminal truncation in their study and ours.

Chimeric core particles, made of carboxy-terminally truncated HBcAg fused with 37-mer HCV core peptide (aa 39 to 75) via a linker of 2 aa, did not expose HCV core epitopes on their surface. The fusion protein itself did show these epitopes on immunoblotting analysis, however. Since the carboxy terminus of truncated HBcAg (Val-149) is positioned inside core particles (3), a longer spacer might have been necessary to present this HCV core sequence through the shell of the nucleocapsid onto the surface of chimeric core particles. HCV core epitopes became available on the surface of chimeric core particles made of carboxy-terminally truncated HBcAg fused with a longer, 91-mer, HCV core peptide representing aa 1 to 91.

The surface expression of HCV core determinants was dose dependent, increasing pari passu with copies of HCV core protein fused to HBcAg. The additive effect was not observed beyond three copies of HCV core protein, however. Steric hindrance would have inhibited an unlimited presentation of HCV core determinants on chimeric core particles. Longer heterologous sequences appeared to affect the efficiency of core assembly, since the yield of chimeric cores decreased with increasing copies of HCV core proteins fused to HBcAg. Fusion proteins carrying many copies of HCV core protein revealed bands at positions smaller than the expected M_r on immunoblotting. This would have been due to degradation of chimeric cores, particularly of HCV sequences most of which were surface accessible, despite the use of phenylmethylsulfonyl fluoride in their isolation.

The cassette used for the expression of HCV core protein included aa 1 to 180 of the product of the core gene, short of 11 carboxy-terminal amino acids allocated to the core protein by in vitro processing analysis (12). Inasmuch as immunodominant regions cluster within the amino-terminal two-thirds of the HCV core protein (24, 28), the expression of aa 1 to 180 would be sufficient for immunological purposes.

Chimeric core particles with the surface expression of HCV core epitopes have potential applications. First, they would offer an excellent antigen probe for the detection of class-specific antibodies to HCV core in sera from hosts infected with HCV. Immunoglobulin M (IgM) or IgA antibodies in serum may be captured by anti-IgM/μ or anti-IgA/α immobilized on the solid support. Then hybrid core particles with multivalent HCV determinants would be added, whose binding can be detected later by antibodies to HCV core labeled with horseradish peroxidase. Recombinant HCV core protein or synthetic oligopeptides presently used in ELISA are invariably univalent (18, 20, 25, 26), and such an

antibody capture assay for class-specific antibodies is therefore not feasible with them.

Immunoassays with chimeric HBV core particles bearing HCV core determinants inevitably detect antibodies to HBV core as well. For clinical and epidemiological purposes, therefore, the assay for antibody to HCV core must be restricted to serum samples without antibodies to HBV core. Chimeric core particles would be useful, however, for detecting blood units from donors infected with either HBV or HCV, or both. HBV DNA is detected in blood units with antibodies to HBV core in high titers in which hepatitis B surface antigen is not detectable (14). Titers of antibodies to synthetic HCV core peptides correlate with HCV viremia (25, 26). Therefore, immunoassays with chimeric core particles, possibly tailored to a hemaggutination method for easier determination of antibody, titers, would enable a simultaneous detection of HBV and HCV viremia for the exclusion of blood units contaminated with either or both of these viruses.

Finally, there is a theoretical possibility that chimeric core particles would be used as a vaccine for the immunoprophylaxis of HCV infection. This deserves considerations in the interim, while the envelope protein of HCV is not characterized for the development of vaccines. It is believed that antibodies to HCV core protein would have no prophylactic value, because they occur in the circulation of persistently infected hosts. Likewise, antibodies to HBV core, found in high titers in carriers, would not be prophylactic against HBV infection. Chimpanzees inoculated with a vaccine incorporating HBV core particles, however, are partially protected from challenge with HBV (16, 23). Hepatocytes with the surface expression of core epitopes might be eliminated by immune T lymphocytes inducible by HBV core vaccines (21).

Only linear determinants of HCV core were presented on chimeric HBV core particles obtained with the present expression system. More sophisticated methods are required for the expression of conformational epitopes, which are expected to be borne by the authentic HCV core particle and stimulate strong B-cell-dependent immune responses in hosts. Such epitopes might be mimicked by inserting HCV core sequences into the e1 loop of HBcAg (3), as shown for conformationally restricted epitopes of human rhinovirus (6), simian immunodeficiency virus (33), and the product of the HBV pre-S1 region (29).

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