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Peptide Immunogen Mimicry of Putative E1 Glycoprotein-Specific Epitopes in Hepatitis C Virus

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Hepatitis C virus (HCV) accounts for most cases of acute and chronic non-A and non-B hepatitis with serious consequences that may lead to hepatocellular carcinoma. The putative envelope glycoproteins (E1 and E2) of HCV probably play a role in the pathophysiology of the virus. In order to map the immunodominant domains of the E1 glycoprotein, two epitopes from amino acid residues 210 to 223 (P1) and 315 to 327 (P2) were predicted from the HCV sequence. Immunization of mice with the synthetic peptides conjugated to bovine serum albumin induced an antibody response, and the antisera immunoprecipitated the E1 glycoprotein (~33 kDa) of HCV expressed by recombinant vaccinia virus. A panel of HCV-infected human sera was also tested with the synthetic peptides by enzyme-linked immunosorbent assay for epitope-specific responses. Of 38 infected serum samples, 35 (92.1%) demonstrated a spectrum of reactivity to the P2 peptide. On the other hand, only 17 of 38 (44.7%) serum samples were reactive to the P1 peptide. Strains of HCV exhibit a striking genomic diversity. The predicted P1 epitope showed localization in the sequence-variable region, and the P2 epitope localized in a highly conserved domain. Results from this study suggest that the E1 glycoprotein of HCV contains at least two potential antigenic epitopes. Synthetic peptides corresponding to these epitopes and antisera to these peptides may serve as the monospecific immunological reagents to further determine the role of E1 glycoprotein in HCV infection.

Hepatitis C virus (HCV) is the major causative agent of parenterally as well as sporadically transmitted non-A, non-B hepatitis (7). Vertical transmission of the virus from mother to newborn has also been suggested (53). HCV accounts for most cases of acute and chronic non-A, non-B liver disease leading to serious consequences, including cirrhosis and hepatocellular carcinoma (1, 7). Chronic hepatitis is the ninth leading cause of death in the United States, and recent estimates suggest that HCV contributes to over 40% of these deaths (26). Oligosaccharide moieties appear to be present on the surface of the native virus (48). The HCV genome is a linear, positive-stranded RNA molecule of ~9,500 bp encoding a polyprotein precursor of ~3,000 amino acids (7). The putative structural proteins of the polyprotein are followed by the putative nonstructural replicase components and various lengths of 3′ poly(U) or poly(A) tail. The HCV genome is heterogeneous both among infected individuals and within the same individual (3, 4, 30, 42, 50).

Processing of the putative structural proteins examined by in vitro translation suggested that the products are arranged from the amino-terminal end of the polyprotein precursor as NH_2-p22-gp35-gp70-p19 (21). Both gp35 and gp70 are transported into microsomes and are heavily glycosylated. Envelope proteins expressed in insect cells or monkey COS cells are produced by cleavage from the precursor protein, at about amino acids 190 and 380 (36). The cleavages of C, E1, and E2 are thought to result from the action of host signal peptidases, while processing of the nonstructural proteins is thought to be accomplished by virally encoded proteinases (10, 18, 19, 22, 23). Immunity in HCV infection appears to be weak, although the reasons are not clear (44). Occurrence of virus-antibody complexes in the sera of patients with chronic HCV infection has been suggested (24, 38). Up to 17% of the HCV-infected patients with chronic liver disease showed appearance of antibodies to the recombinant envelope protein (36, 37).

Synthetic peptides derived from immunodominant regions of capsid and nonstructural proteins of HCV have been previously shown to detect antibody to virus infection by enzyme immunoassay (25, 31). A similar approach was successful with a number of other important viral agents (39), including hepatitis B and human immunodeficiency viruses (13–17, 40, 41, 51). Monospecific antibodies with defined specificity against selected peptide sequences have been used for identification of native polypeptides in cellular and molecular synthesis (9, 34, 35, 43) and have been proven to be of value in immunological test systems for the detection of viral proteins synthesized during the course of infection (2). In this study, we analyzed the amino acid sequences of the putative envelope protein (E1) of HCV to define B-cell sequential epitopes. Two synthetic peptides (P1 and P2) predicted to be antigenic epitopes suggest that these regions are immunogenic in experimental animals and HCV-infected humans.

MATERIALS AND METHODS

Patient sera. Blood samples were collected from an ongoing investigation of asymptomatic anti-HCV-positive volunteer blood donors from the American Red Cross. Sera were separated and frozen in aliquots at −70°C until used.

Prediction of epitopes. By computer-assisted analysis, the secondary structure of the putative E1 glycoprotein was predicted (12). Potential antigenic sites were analyzed by superimposing the secondary structure with the values of hydrophi-

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TABLE 1. Mouse antibody (Ig) response following immunization with synthetic peptides determined by ELISA

Immunogen[a]	Serum dilution[b]	OD	
		Anti-P1	Anti-P2
None	1/20	0.22	0.23
P1-BSA	1/10,000	2.8	0.22
P2-BSA	1/640	0.35	2.4
P1	1/20	0.37	0.24
P2	1/20	0.25	0.85

[a] The preimmune serum pool from each group of immunized mice had similar reactivities to ELISA wells coated with P1 and P2 peptides. P1-BSA and P2-BSA designate BSA-conjugated peptides.

[b] Antipeptide sera were tested at twofold serial dilutions, and results are presented as the OD at a particular dilution for comparison.

licity, hydrophobicity, and other parameters to identify sequential epitopes localized in hydrophilic, flexible, β-turn regions with a high probability for location on the surface (8, 11, 32). All these parameters were combined in a weighted algorithm to define B-cell sequential epitopes (28). The first epitope (P1, amino acid residues 210 to 223) is located in a variable region and does not contain a conserved glycosylation site. The second epitope (P2, amino acid residues 315 to 327) is preceded by a small stretch of hydrophobic amino acids followed by strong hydrophilic domains. This region is relatively conserved among reported HCV strains and does not contain a potential glycosylation site.

Peptide synthesis. Peptides with the sequences NH_2-Ser-Ser-Ile-Val-Tyr-Glu-Ala-Ala-Asp-Met-Ile-Met-His-Thr-COOH (P1) and NH_2-Ser-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-COOH (P2) were synthesized by the solid-phase method by using an Applied Biosystems model 430A automated peptide synthesizer. After synthesis, cleavage from the resin and side-chain protection were done simultaneously by hydrogen fluoride treatment (Multiple Peptide Systems, San Diego, Calif.).

Preparation of antipeptide sera. Peptides were separately coupled to bovine serum albumin (BSA) by the glutaraldehyde conjugation method. BSA (0.5 mg) in 2 ml of physiological saline was dialyzed overnight against 0.1 M phosphate buffer, pH 6.8, at 4°C. A solution of peptide (2 mg) in 50 μl of 1% glutaraldehyde (Sigma Chemical Company, St. Louis, Mo.) was added to the BSA solution with stirring. The mixture was incubated at room temperature for 2 h, and 200 μl of 1 M lysine solution, pH 7.0, was added to quench the remaining active sites. After another 2 h of incubation, the conjugate was dialyzed against phosphate-buffered saline (PBS), pH 7.0, at 4°C overnight and clarified by high-speed centrifugation. Groups of six BALB/c mice were immunized intraperitoneally with 20 μg of the peptide-BSA conjugate or unconjugated peptide in Freund's complete adjuvant. Four weeks later, a similar quantity of the peptide-BSA conjugate or peptide alone in Freund's incomplete adjuvant was injected intraperitoneally into mice. Mice were bled 1 week after the last immunization. Sera were prepared, pooled, and stored in aliquots at −70°C until used.

Recombinant HCV proteins. Two recombinant vaccinia viruses, vv/HCV_{1-967} and v9A/C-$E1_{1-339}$, expressing the structural proteins of the HCV type 1 (HCV-1) strain were used in this study (45). When necessary, wild-type vaccinia virus (WR strain) was used as a negative control. HeLa or BSC 40 cells were grown on 35-mm-diameter petri dishes or coverslips and infected with recombinant vaccinia virus. Infected cells were labeled with ^{35}S-protein label or [^3H]mannose (DuPont, Boston, Mass.) 20 h postinfection, and cell lysates were used for immunoprecipitation of the recombinant proteins with mouse antipeptide sera and infected human sera.

Immunofluorescence. Infected cells were washed with PBS. Mouse antisera to the synthetic peptides were applied to the cells and incubated for 15 min at room temperature. After a further wash with PBS, fluorescein isothiocyanate-conjugated goat antibody to mouse immunoglobulin (Ig) (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Md.) was added and the mixture was incubated for another 15 min. After washings with PBS, the coverslips were mounted on microscope slides by use of a solution of glycerol in PBS and viewed with a fluorescence microscope (Nikon Optiphot-2) for cell surface expression of HCV proteins. A similar procedure was followed for intracellular immunofluorescence of cells previously fixed with cold 95% acetic acid–5% ethanol.

Labeling of infected cells and immunoprecipitation. Immunoprecipitation was performed by a procedure similar to one described previously (46). Briefly, recombinant vaccinia virus-infected cells were labeled 20 h postinfection with 100 μCi of ^{35}S-protein label for 1 h in methionine-free Dulbecco's minimal essential medium (DMEM). For radiolabeling with sugar precursor, cells were incubated with 200 μCi of [^3H]mannose in glucose-free DMEM for 5 h at 37°C. Cells were washed and lysed with lysis buffer (0.05 M Tris-HCl [pH 7.4], 0.15 M NaCl, 0.001 M EDTA, 1% sodium deoxycholate, 1% Triton X-100, and 0.1% sodium dodecyl sulfate [SDS]). Cell lysate was centrifuged for 10 min at 14,000 × *g*. Clear supernatant was collected and used as the source of HCV E1 glycoprotein for immunoprecipitation. Mouse antipeptide serum or HCV-infected human serum (15 μl) was mixed with the cell lysates at 4°C overnight. Protein A-Sepharose 4B (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was added to the antigen-antibody mixture and further mixed for 2 h at 4°C. The beads were pelleted by centrifugation, washed three times with lysis buffer, and suspended in sample reducing buffer for SDS-polyacrylamide gel electrophoresis (PAGE) followed by autoradiography or fluorography.

Pulse-chase. BSC 40 cells were infected at a multiplicity of infection of 1 with recombinant vaccinia virus. About 20 h postinfection, the growth medium was removed and cells were washed with cysteine- and methionine-deficient medium for 1 h at 37°C. Following starvation, cells were incubated in 200 μl of the same deficient medium supplemented with 200 μCi of the ^{35}S-protein label per 35-mm-diameter petri dish for 60 min at 37°C. The labeling medium was removed, and cells were washed and chased at 37°C in DMEM supplemented with 2% fetal bovine serum. After a chase period, the cells were lysed and used for immunoprecipitation.

ELISA. Enzyme-linked immunosorbent assay (ELISA) was performed by a procedure similar to one described previously (47). Immulon 1 flat-bottom plates (Dynatech Laboratories Inc., Chantilly, Va.) were coated with 0.5 μg of the peptide per well in 100 μl of PBS, pH 7.2, by incubation overnight at 4°C. After four washes with PBS, the wells were filled with 1% BSA in PBS for 2 h at 37°C to block the remaining active sites. Next, 100 μl of diluted experimental sera in PBS containing 1% BSA was added to the peptide-coated wells, and plates were incubated overnight at 4°C. The plates were washed six times with PBS containing 0.1% Tween 20 by using an automated washer (Dynatech). A predetermined dilution of the second antibody-enzyme conjugate (tested by checkerboard titration) was used. For detection of human Ig, goat anti-human IgG plus IgA plus IgM (heavy plus light chains) coupled to alkaline phosphatase (Zymed Laboratories Inc., San Francisco, Calif.) was used. For detection of mouse Ig, goat anti-mouse IgG plus IgA plus IgM

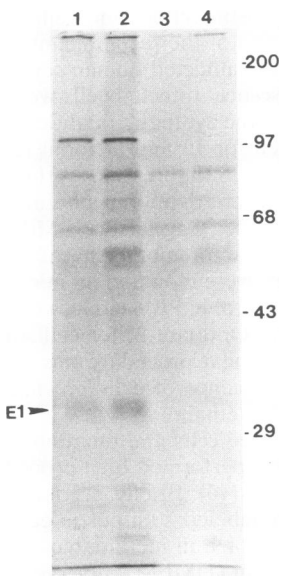

FIG. 1. Immunoprecipitation of HCV polypeptides by a mouse antipeptide serum pool. BSC 40 cells were infected with vv/HCV_{1-967}. Cells were labeled at 24 h postinfection with ^{35}S-protein label for 1 h. Cell lysates were immunoprecipitated with mouse antisera to P2 (lane 1) and P1 (lane 2) peptides and analyzed by SDS–10% PAGE under reducing conditions. Similar analyses of wild-type vaccinia virus-infected cell lysate with antisera to P2 (lane 3) and P1 (lane 4) peptides as negative controls are also shown. Numbers on the right represent the positions of molecular size markers (in kilodaltons).

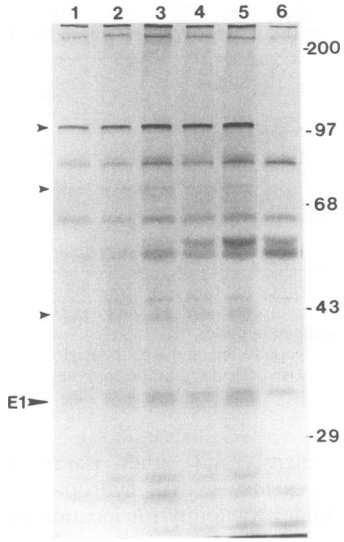

FIG. 2. Processing of E1 glycoprotein and recognition of recombinant HCV polypeptides by an anti-P1 serum pool. BSC 40 cells were infected with vv/HCV_{1-967}, labeled for 1 h with ^{35}S-protein label, and chased for 2.5 h. Following the chase, cells were lysed, processed for immunoprecipitation, and analyzed by SDS–10% PAGE under reducing conditions. Arrows indicate the positions of immunoprecipitated HCV polypeptides at 0, 0.5, 1.0, 1.5, and 2.5 h into the chase period (lanes 1 through 5, respectively). Reactivity of the antipeptide serum pool with the wild-type vaccinia virus-infected cell lysate after a chase for 2 h is also shown as a negative control (lane 6). Numbers on the right represent the positions of molecular size markers (in kilodaltons).

(heavy plus light chains) coupled to alkaline phosphatase (Kirkegaard & Perry Laboratories Inc.) was used. To each well, 100 μg of the antibody-enzyme conjugate diluted in PBS containing 1% BSA was added, and plates were incubated at 37°C for 2 h. The plates were washed, and 100 μl of *p*-nitrophenyl phosphate (Sigma Chemical Company) in 10% diethanol amine buffer containing 0.5 M $MgCl_2$ was added to each well. After incubation for 30 min, the color reaction was stopped by the addition of 100 μl of 3 N sodium hydroxide and optical density (OD) was read at a wavelength of 405 nm with a spectrophotometer (Tecan Corp., Research Triangle Park, N.C.). An OD of >0.52 was considered the cutoff for human sera.

Peptide-based neutralization ELISA. A competitive-binding ELISA was performed by following a procedure similar to the one described by Hosein et al. (25) for determining specificity of the positive samples reactive in the ELISA. Sera diluted 1:20 in sample diluent containing P1 or P2 peptide at 100 μg/ml were preincubated for 60 min at 37°C. The preincubated sera were added to the wells of microtiter plates coated with the peptide and were assayed by following the similar ELISA procedure described earlier (47). The specimen was confirmed as positive when its response in the ELISA was inhibited >50% or was below the cutoff limit in the neutralization assay.

RESULTS

Characterization of HCV-infected sera. Human sera were tested for hepatitis B virus and HCV infection by using commercially available serodiagnostic assays. Subjects were asymptomatic American Red Cross donors whose sera screened repeatedly positive by second-generation anti-HCV ELISA (Ortho Diagnostics, Raritan, N.J.) and were repeatedly positive for HCV viremia as defined by reverse transcription-PCR amplification of the 5′ noncoding region (27). Thirty-eight HCV-positive human serum samples were included in this study.

Antibody response to synthetic peptides. Both the peptides coupled to BSA were found to be immunogenic as judged by the ability of the mouse antisera to react with the peptides applied to an ELISA plate (Table 1). P1 peptide appeared to be a better immunogen than P2, as evident from the differences (16-fold) in ELISA titers. Antisera, when similarly tested with BSA-coated ELISA wells, showed a minor reactivity (1/40 to 1/160 dilutions). Mouse antisera reactive by ELISA were further confirmed by peptide neutralization assay for specificity of the antibodies to P1 and P2 peptides. Immunization of mice with unconjugated P1 peptide did not induce a significant antibody response. P2 induced a relatively poor antibody response in comparison with the peptide-BSA conjugate. Subsequent experiments were conducted with serum pooled from mice immunized with peptides coupled to BSA.

Reactivity of antipeptide sera with recombinant HCV proteins. To determine the specificity of mouse antipeptide antibodies, we analyzed their ability to immunoprecipitate ^{35}S-protein-labeled polypeptides from recombinant vaccinia virus (vv/HCV_{1-967})-infected BSC 40 cells. Antiserum to the P1 peptide immunoprecipitated a diffuse polypeptide band of ~33 kDa representing the putative HCV E1 protein (Fig. 1, lane 1). Other polypeptides immunoprecipitated by this antiserum included 99- and 73-kDa polypeptides and a diffuse 42-kDa band. Antiserum to the P2 peptide immunoprecipitated diffuse polypeptide bands of 33 and 42 kDa and a 99-kDa band (Fig. 1, lane 2).

Expression patterns of the recombinant HCV proteins were further analyzed by a pulse-chase experiment (Fig. 2), and

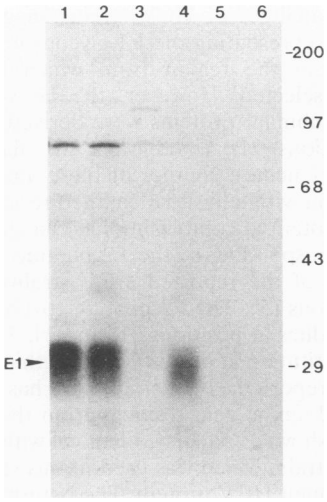

FIG. 3. Immunoprecipitation of HCV E1 glycoprotein by infected human sera and a mouse antipeptide serum pool. BSC 40 cells were infected with v9A/C-E1$_{1-339}$. Cells were labeled at 24 h postinfection with [^{3}H]mannose for 5 h. Cell lysates were immunoprecipitated with HCV-infected human sera (lanes 1 and 2) and mouse anti-P2 (lane 3) and anti-P1 (lane 4) sera and analyzed by SDS–10% PAGE under reducing conditions. Similar analyses with wild-type vaccinia virus-infected cells with anti-P1 (lane 5) and anti-P2 (lane 6) sera as negative controls are also shown. Numbers on the right represent the positions of molecular size markers (in kilodaltons).

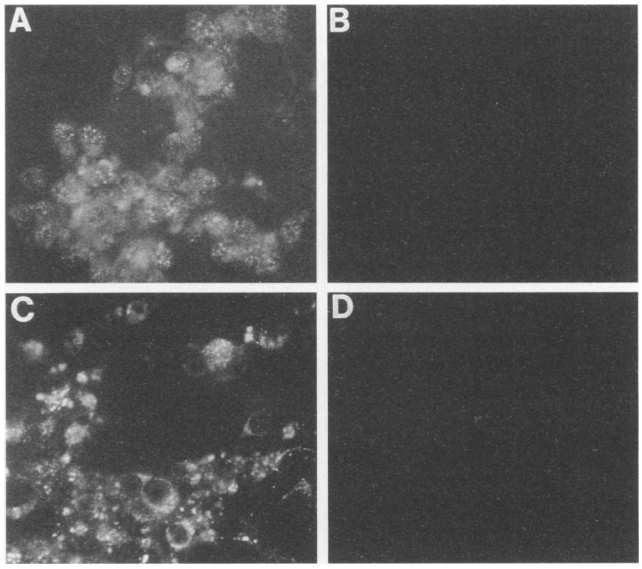

FIG. 4. Immunofluorescence staining of HCV E1 protein by an antipeptide serum pool. BSC 40 cells were infected with vv/HCV$_{1-967}$, and fixed cells were incubated with antisera to P1 (A) and P2 (C) and fluorescein-isothiocyanate-conjugated second antibody. Reactivity of antisera to P1 (B) and P2 (D) with wild-type vaccinia virus-infected cells as a negative control of immunofluorescence is also shown.

proteins showed polypeptide profiles similar to those presented in Fig. 1. Mouse anti-P1 serum immunoprecipitated polypeptides of ~99, 72, 42, and 33 kDa. Increases in the intensities of the other three polypeptides correlated with the appearance of a larger quantity of the ~99-kDa protein produced by the recombinant vaccinia virus following a 60-min chase period. Results indicate that the antiserum to P1 recognizes the putative ~33-kDa E1 glycoprotein product. The 99-kDa polypeptide band may represent the precursor of E1. The other two polypeptides, 72 and 42 kDa, may represent completely or partially glycosylated forms of E2 being coprecipitated with E1 by mouse antiserum (45).

To confirm the reactivity of antipeptide serum with the HCV E1 glycoprotein, recombinant vaccinia virus (v9A/C-E1$_{1-339}$)-infected HeLa cells were labeled with [^{3}H]mannose and immunoprecipitated with mouse sera and HCV-infected human sera (Fig. 3). Patient sera immunoprecipitated diffuse ~33-kDa and sharp 99-kDa glycoprotein bands (Fig. 3, lanes 1 and 2). Mouse antipeptide sera also precipitated glycoprotein bands of similar sizes (Fig. 3, lane 4). Mouse antiserum to the P2 peptide showed an extremely weak E1 glycoprotein band with [^{3}H]mannose-labeled cell lysates. This is probably due to weak reactivity of the anti-P2 serum, as observed with ^{35}S-protein-labeled recombinant proteins. Test sera reactive with the E1 glycoprotein did not immunoprecipitate polypeptide bands of similar sizes from the wild-type vaccinia virus-infected cell lysate (Fig. 3, lanes 5 and 6) used as a negative control.

Examination of recombinant vaccinia virus (vv/HCV$_{1-967}$)-infected cells by immunofluorescence with mouse antipeptide sera showed punctate and slightly diffuse intracellular staining (Fig. 4A and C), while wild-type vaccinia virus-infected cells were not reactive (B and D). Immunofluorescence was not observed on the surface of unfixed cells, as previously noted (49).

Antibody response to the E1 glycoprotein in infected human sera. Eleven HCV-infected human serum samples were tested for reactivity to the recombinant E1 glycoprotein by immunoprecipitation. Human sera showed variable reactivity with the recombinant 33-kDa E1 polypeptide (Fig. 5). None of these sera showed immunoprecipitation of a similar-size polypeptide band with wild-type vaccinia virus-infected cells used as a negative control. Although core protein was expressed by the recombinant vaccinia virus, as evidenced by immunofluores-

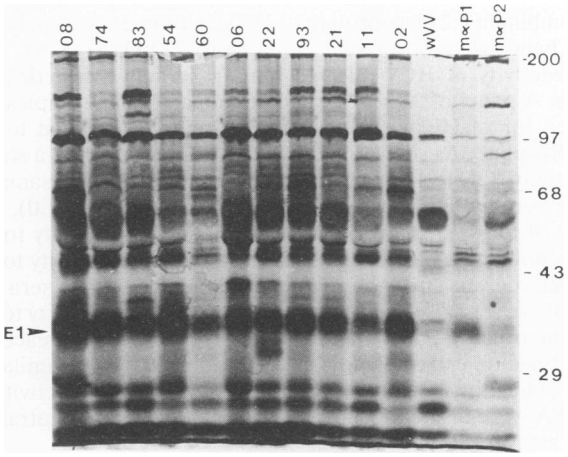

FIG. 5. Immunoprecipitation of ^{35}S-protein-labeled HCV proteins from vv/HCV$_{1-967}$-infected BSC 40 cell lysates with HCV-infected human sera (samples are identified above the lanes by the last two digits of the sample numbers listed in Table 2) and a mouse antipeptide serum pool (mαP1 and mαP2). Immunoprecipitates were analyzed by SDS–10% PAGE under reducing conditions. Immunoprecipitates appearing with wild-type vaccinia virus (wVV)-infected cell lysates and pooled HCV-infected human sera are also shown. Numbers on the right represent the positions of molecular size markers (in kilodaltons).

TABLE 2. Reactivity of HCV-infected human sera with synthetic peptides by ELISA

Test serum sample[a]	OD		Test serum sample[a]	OD	
	Anti-P1	Anti-P2		Anti-P1	Anti-P2
9002	0.72	0.78	9064	0.55	0.61
9008	1.22	0.78	9074	1.50	1.05
9011	0.73	0.92	9075	0.48	0.68
9014	0.63	0.75	9078	0.43	1.10
9017	0.39	0.71	9083	0.98	0.75
9018	0.34	0.62	9084	0.41	0.69
9020	0.50	1.25	9085	0.36	0.76
9021	1.77	0.75	9093	0.82	0.63
9022	1.34	0.95	9095	0.45	0.67
9027	0.37	1.13	9101	0.42	0.65
9032	0.37	1.40	9105	0.46	0.82
9035	0.37	0.83	9106	1.17	0.88
9036	0.39	0.78	9115	0.30	0.98
9038	0.30	1.00	9116	0.34	0.75
9045	0.64	0.81	AP001	0.36	0.34
9052	0.51	0.72	AP002	1.50	1.54
9054	0.46	1.06	AP003	1.62	1.50
9060	2.27	0.94	AP006	0.40	0.35
9062	0.41	0.50	PMW	0.52	0.70

[a] Human sera were tested for Ig reactivity at a 1:20 dilution. Sera from 14 apparently healthy individuals were used as negative controls with mean ODs of 0.187 (range, 0.1 to 0.28) and 0.198 (range, 0 to 0.25) to P1 peptide and P2 peptide, respectively. The ELISA cutoffs were 0.52 and 0.45 OD units (mean for the negative controls plus five times the standard deviation) for P1 and P2, respectively.

cence, it was not immunoprecipitated by the infected human sera. This suggests that although the recombinant vaccinia virus expressed the capsid protein of HCV, our reaction conditions for immunoprecipitation did not allow recognition of the capsid protein by infected human sera. A similar observation was noted earlier, although the core protein was detected by Western blot (immunoblot) analysis (45). Formation of an E1 and E2 complex of HCV has been previously suggested (20, 45). Mouse antipeptide sera or human sera included in this study did not convincingly coprecipitate the recombinant E2 glycoprotein of HCV, although weak polypeptide bands were observed around 68 to 72 kDa (Fig. 5).

Reactivity of HCV-infected human sera to synthetic peptides. A panel of 38 HCV-infected human serum samples was tested for reactivity to the synthetic peptides applied to the ELISA plate. As shown in Table 2, the assay detected a strong reactivity (OD $\geq$ 1.0) to the P1 peptide in eight serum samples. Nine serum samples had reacted strongly (OD $\geq$ 1.0), and most of the sera showed a weak-to-moderate reactivity to the P2 peptide by ELISA. It is interesting that the reactivity to the P1 peptide was strong in a limited number of human sera and that these sera also showed a moderate-to-high reactivity to P2. On the other hand, some of the sera showing strong reactivity to P2 had an extremely low level of reactivity to P1, similar to that of healthy individuals. Sera showing repeated reactivity by ELISA were also confirmed as positive by peptide neutralization assay.

DISCUSSION

In this report, we have demonstrated the immunogenicity of two peptides from the HCV E1 glycoprotein. Mouse antisera to the peptides reacted specifically with the HCV E1 glycoprotein expressed by recombinant vaccinia virus, and a number of confirmed HCV-infected human sera showed antibody reactivity to these peptides. Nucleotide and predicted amino acid sequence analyses of HCV strains suggest that the P1 peptide region representing the E1 glycoprotein shows extensive variations in the region from which the P1 peptide sequence was selected. However, the HCV-1 strain, from which the recombinant proteins were derived, showed substitution at positions 219 (methionine to alanine) and 221 (methionine to leucine). Neither of these amino acids represents substitution with a basic or charged residue, a contributing factor for potential conformational change of this region. The region corresponding to the P2 peptide was highly conserved in most of the reported HCV strains from different geographic regions (5). The P2 peptide covered 11 conserved amino acid residues at positions 316 to 324, 326, and 327. An amino acid substitution at position 325 (methionine to leucine) in four of the reported HCV strains (5) has been reported. Additionally, 12 amino acid residues from the N terminus of the P2 peptide showed sequence identical with the E1 protein of the HCV-1 strain, which was used in this study as a source for the recombinant HCV protein. The N-terminal amino acid serine was substituted with threonine in the HCV-1 strain. However, this change did not hinder the reactivity of anti-P2 peptide sera with the recombinant E1 protein from the HCV-1 strain. It will be interesting to determine the relationship between the reactivity with the peptides and the genotype of the infecting HCV strain in a future study.

HCV-infected human sera demonstrated an antibody response to the synthetic peptides by ELISA. Amino acid variations in the P1 region may account for absence of antibody reactivity in some of the HCV-infected human sera. Heterogeneity in this region may alter the HCV epitope in an infected host. The putative E2 glycoprotein of HCV has been shown to possess an N-terminal hypervariable domain from amino acids 384 to 414 comparable to the human immunodeficiency virus type 1 gp120 V3 domain (52). Five isolate-specific linear epitopes have been suggested to be located in the E2 hypervariable region. These variations probably result from immune selection. A study of the hypervariable region (amino acids 384 to 410) using dihydrofolate reductase fusion protein synthesized by in vitro transcription and translation showed similar results (29). Antibodies to this region have been suggested to be involved in the genetic drift of the hypervariable region by immunoselection. The region represented by the P1 peptide used in this study may also function as a variable epitope responsible for inducing antibody responses. The biological importance of this region is not known at this time. It is thus likely that the P1 domain contains a distinct linear epitope with a hypervariable region, but it is beyond the scope of this study to detect antibody activity by using sequential isolate-specific peptides as an antigen source. In contrast, the P2 peptide domain showed reactivity with most of the HCV-infected human sera, and this region represents a conserved immunodominant epitope in an infected host.

Processing of the putative structural proteins of HCV has been examined by in vitro translation. Both 35- and 70-kDa glycoproteins have been shown to be transported into microsomes and are heavily glycosylated (21). A similar observation was also noted in an in vivo study (45). Our results from pulse-chase experiments indicate that the ~99-kDa protein may be a precursor of the ~33-kDa E1 glycoprotein of HCV. Expression of the putative envelope protein by recombinant baculovirus resulted in a series of 24- to 35-kDa polypeptides in insect cells, probably due to different degrees of glycosylation (33, 36), while a single species of 35-kDa glycoprotein was observed on expression in monkey COS cells (36).

A recent study indicates that 95 to 100% of patients with chronic liver disease have detectable antibodies to the recom-

binant envelope proteins (6). A previous immunofluorescence study using a baculovirus expression system demonstrated a greater reactivity to E2 than E1 glycoprotein in infected human sera (33). However, our immunoprecipitation and ELISA results suggested a significant antibody response to the E1 glycoprotein in HCV-infected human sera (20, 45). Independent and sequential isolates of HCV show striking genomic diversity. Variations in the putative envelope glycoproteins may reflect the effect of immune selection and evasion of antibody responses in an infected host. Operating mechanisms of HCV for immune evasion and establishment of persistent infection in an infected host appear to be complex. Further characterization of the role of antibodies to the variable regions by in vitro neutralization assay should help in understanding the important immunodominant regions responsible for pathophysiology and immune evasion.

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