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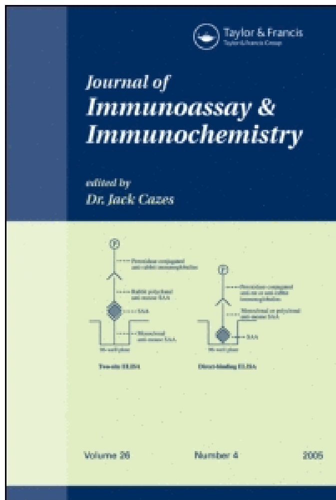

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Identification of Immunodominant Epitopes in the Core and Non-Structural Region of Hepatitis C Virus by Enzyme Immunoassay Using Synthetic Peptides

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To cite this Article Park, Hae Joon , Byun, Si Myung , Ha, Young Ju , Ahn, Jong Seong and Moon, Hong Mo(1995) 'Identification of Immunodominant Epitopes in the Core and Non-Structural Region of Hepatitis C Virus by Enzyme Immunoassay Using Synthetic Peptides', Journal of Immunoassay and Immunochemistry, 16: 2, 167 — 181
To link to this Article: DOI: 10.1080/15321819508013556
URL: http://dx.doi.org/10.1080/15321819508013556

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JOURNAL OF IMMUNOASSAY, 16(2), 167-181 (1995)

IDENTIFICATION OF IMMUNODOMINANT EPITOPES IN THE CORE AND NON-STRUCTURAL REGION OF HEPATITIS C VIRUS BY ENZYME IMMUNOASSAY USING SYNTHETIC PEPTIDES

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ABSTRACT

Thirty-two synthetic peptides, components of the core and non-structural protein of Hepatitis C virus (HCV), were tested for their reactivities against antibodies in sera of healthy, HCV antibody positive or chronic liver disease patients. Among them, 8 of the core peptides, 4 of the NS4 peptides and 3 of the NS5 peptides reacted with the HCV infected sera. In particular, C22 (core peptide) and NS4-1924 (NS4 peptide) were most reactive with the serum samples giving a positive signal with commercially available enzyme-linked immunosorbent assay (ELISA) kit. Our results indicate that the immunodominant regions of the HCV-derived proteins are located at three regions in the core protein, three regions in the NS4 protein, and one region in the NS5 protein. These results indicate that the selected peptides are useful antigens in detecting antibodies in the sera from individuals infected with HCV.

(KEY WORDS : HCV, immunodominant epitopes, ELISA)

INTRODUCTION

Hepatitis C virus is very closely associated with most of posttransfusion non-A, non-B hepatitis throught the world (1-2, 17). HCV infection can be assessed by the detection of antibodies to several gene (core, NS3, and NS4) products of HCV. It is well known that most hepatitis C patients have antibody to NS4 protein whereas, in some cases, patients have antibodies only to core

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or NS3 protein (2, 14). These results suggest that individuals infected with HCV may have manifold antibody profiles in accordance with the progress of HCV infection. Therefore, it is important to find the most useful antigenic region of HCV by analyzing the antibody profiles of individuals infected with HCV. In this study, we describe the linear immunodominant region of core and nonstructural HCV proteins identified by ELISA using synthetic peptides as antigens. Individual peptides were analyzed for serological reactivity. Sera from healthy individuals or chronic liver disease patients were also investigated for antibody prevalence to each antigen. Finally, employing the combination of immunodominant epitopes from the structural and nonstructural proteins of HCV, we confirmed that the synthetic peptides were useful antigens for the serodiagnosis of HCV infection.

MATERIALS AND METHODS

Materials

Fmoc amino acids were obtained from Novabiochem Co. (La Jolla, CA). Chemicals and solvents were purchased from Aldrich (Milwaukee, WI) and Fluka Chemie AG (Buchs, Switzerland). ELISA plates (96 well, flat bottom, high binding capacity, # 4-69914) were purchased from Nunc (DK-4000 Roskilde, Denmark). Unless otherwise mentioned, all reagents were from Sigma Chemical Co. (St. Louis, MO).

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Serum samples

Sera from 985 healthy humans and 189 HCV antibody positive samples analyzed by Abbott second generation ELISA were obtained from Green Cross Reference Laboratory. Thirty-four serum samples from chronic liver disease patients were obtained from Korea Cancer Center Hospital. Mixed titer performance panel was purchased from Boston Biomedica Inc.(W. Bridgewater, MA).

Selection of immunodominant region

The amino acid sequences of core and non-structural proteins (NS3, NS4 and NS5) of HCV were used for the selection of peptides to be synthesized (3). For HCV core protein, the whole region was tested using ten sequential peptides. Epitopes in HCV nonstructural proteins were predicted by the combined analysis of the hydropathicity (4), flexibility (5), acrophilicity (6) and antigenicity parameters (7).

Peptide synthesis

Peptides corresponding to the selected regions were synthesized by solid-phase method with 431A peptide synthesizer ; Applied Biosystem (Foster City, CA). The SGSG four amino acids were added to N-terminus of each peptides as a linker arm. Biotin was conjugated to the N-terminal amino

group of each peptide after synthesis. The synthesized peptides, cleaved off from resin with trifluoroacetic acid in the presence of scavengers according to the manufacturer's instruction, were lyophilized before dissolution in 100% dimethylsulfoxide (DMSO). The peptide solution was applied to preparative reverse-phase high-performance liquid chromatography (HPLC) with a silicated octadecyl (C_{18}) column. The majority of the peptides were isolated as a single peak and were used as test antigen for ELISA.

Effect of antigen format with concentration variation

Antigenic reactivity was compared between free peptide, peptide-carrier conjugate and streptavidin-biotinylated peptide antigen. Peptide-carrier conjugation was performed with 2-iminothiolane method (8). In brief, the peptides were coupled to bovine serum albumin (BSA) as a carrier protein, followed by the addition of cysteine residue to N-terminal amino acid of each peptide. The peptide-carrier ratio was determined by amino acid analysis. Two hundred microliters of each peptide either in a free form or a BSA conjugated form was coated to 96-well microtiter plates at 4° C overnight. The concentration of each peptide ranged from 31.25 ng/ml to 80 ug/ml in 0.1 M carbonate buffer, pH8.3. In case of biotin-labelled peptides, plates were coated with 200 ul streptavidin at concentration of 2.5 ug/ml in 0.1 M carbonate buffer, pH8.3. After overnight incubation at 4°C, 200 ul/well of peptide solutions containing 31.25 ng/ml to 80 ug/ml in 0.1 M carbonate buffer, pH8.3 were added and incubated at 4° C overnight. The following steps were identical for all three variations. After suction, the wells were blocked with 250 ul of 1% bovine serum albumin (BSA) in 0.15 M phosphate buffered saline (PBS), pH 7.2, at room temperature (RT) for 2 hrs. Anti-HCV antibody positive serum was diluted to 1:1600 in PBS containing 20% normal goat serum and 0.1% Tween 20, and 200 ul of this coctail was added to the antigen-coated wells. The first incubation was carried out at 37° C for 1 hr. Plates were washed five times with PBS containing 0.1% Tween 20 (PBST), followed by incubation with horseradish peroxidase-conjugated anti-human immunoglobulin G (1/10,000 in PBS containing 20% normal goat serum and 0.1% Tween 20) at 37° C for 1 hr. After washing five times with PBST, 200 ul of 0.01% 3,3',5,5'-Tetramethylbenzidine [TMB] solution in DMSO, containing 0.01 % H_2O_2 were added and incubated for 30 min at room temperature. The reaction was stopped by the addition of 100 ul of 1.6 N sulfuric acid, and the color development was measured in a plate reader (SLT Labinstrument, Salzburg, Austria) set at 450 nm. The cutoff value was set as the mean optical density of anti-HCV antibody negative sample plus 0.3.

Comparison of antigenic reactivity

Titrations of an anti-HCV positive serum was performed using the free peptide, peptide-carrier conjugate, or streptavidin-biotinylated peptide antigen. Each antigens were coated and blocked as described above, except that the peptide concentration of 0.25 ug/ml. Anti-HCV positive serum was serially diluted from 1:100 to 1:12,800 in PBS containing 20% normal goat serum and 0.1% Tween 20. Subsequent steps were identical as described above.

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Indirect ELISA using individual peptide antigen

96 well plates (Nunc, Denmark) were coated with 200 ul/well of streptavidin at concentration of 2.5 ug/ml in 0.1M carbonate buffer, pH 8.3 at 4° C overnight. After removal of unbound streptavidin, 200 ul of biotin-labeled peptide was applied to each well at 1 ug/ml in 0.1 M carbonate buffer, pH8.3 at 4°C overnight. The subsequent steps were identical as described above, except that serum samples were diluted to 1: 20 in PBS containing 20% normal goat serum and 0.1% Tween 20.

Indirect ELISA using peptide mixture antigen

The HCV ELISA consisted of 96 well plates coated with a mixture of HCV antigenic peptides. The plates were coated with 200 ul of streptavidin at concentration of 2.5 ug/ml in 0.1 M carbonate buffer, pH8.3 at 4° C overnight. After suction, 200 ul per well of immunodominant peptide mixture containing 0.25 ug/ml of each peptide was applied at in 0.1 M carbonate buffer, pH8.3 and incubated at 4° C overnight. The subsequent steps were identical as described above, except that serum samples were diluted to 1: 20 in PBS containing 20% normal goat serum and 0.1% Tween 20.

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RESULTS AND DISCUSSION

Optimization of antigen format for indirect ELISA

The optimal antigen format with free peptides, peptide-carrier conjugates, or streptavidin-biotinylated peptides tested by ELISA was investigated. For both C22 peptide and NS4-1924 peptide, the antigen format using streptavidin-biotinylated peptide gave a stronger reactivity against HCV antibody positive serum compared to the format using free peptide (Fig. 1). These results can be assumed that, in binding to the plastic, physical constraints are imposed on the free peptide preventing it from interacting freely with the antibody. It was also suggested that the detergent used in the washing step may promote desorption of small peptide antigen (10). In case of peptide-BSA conjugate antigen, C22-BSA conjugate antigen showed low reactivity than streptavidin-biotinylated C22 peptide antigen, whereas the NS4-1924 peptide-BSA conjugate antigen showed the same reactivity with streptavidin-biotinylated NS4-1924 peptide antigen (Fig. 2). These results indicate that peptide-BSA conjugates show the variation in reactivity depending on the peptides used and that the streptavidin-biotinylated peptide antigen significantly enhanced the reactivity, not influenced by the type of antigenic peptides.

The relative sensitivity of free peptides, peptide-carrier conjugates, or streptavidin-biotinylated peptides antigens was also investigated (Fig. 2). The results demonstrate that the streptavidin-biotinylated peptide antigen is the most efficient one for detecting antibodies in HCV-infected sera and ideal setup for peptide-based identification of immunodominant epitopes. Peptide-carrier conjugate antigen shows higher sensitivity compared to free peptide, but such system has the disadvantage of the possible chemical damage on

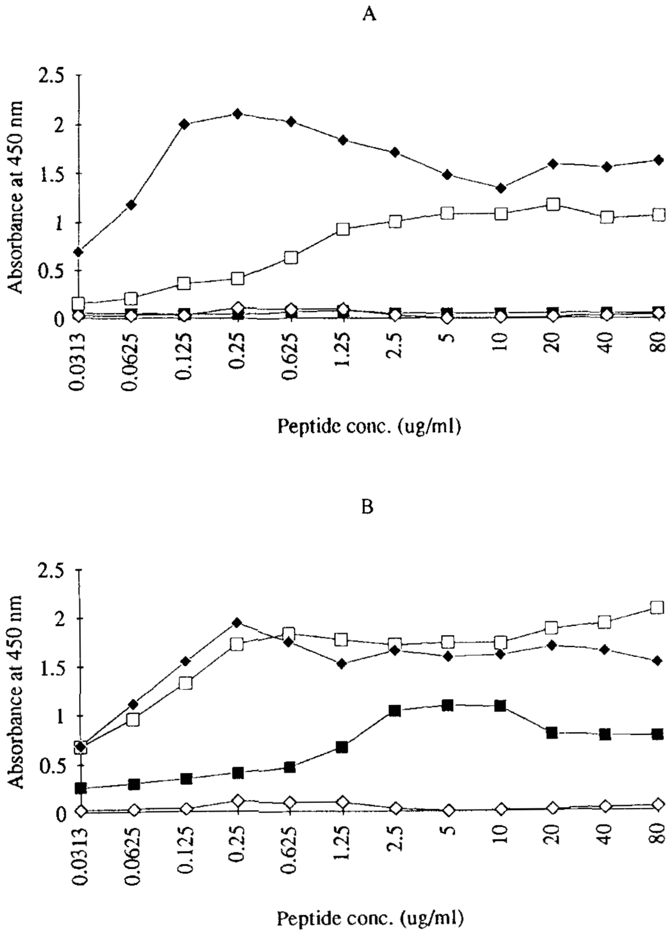

Figure 1. Reactivity in indirect ELISA with two peptide antigens. Antisera diluted 1:1600 was tested against C22 peptide (in A) and NS4-1924 peptide (in B) employing three different formats. ■ :C22 or NS4-1924 free peptide; □ :C22 or NS4-1924 peptide coupled to BSA. Cysteine residue was added to N-terminal amino acid of each peptide. ◇ :Biotinylated SGSG tetrapeptide bound to streptavidin; ◆ :Biotinylated C22 or NS4-1924 peptide bound to streptavidin. The ELISA procedure was as described in materials and method.

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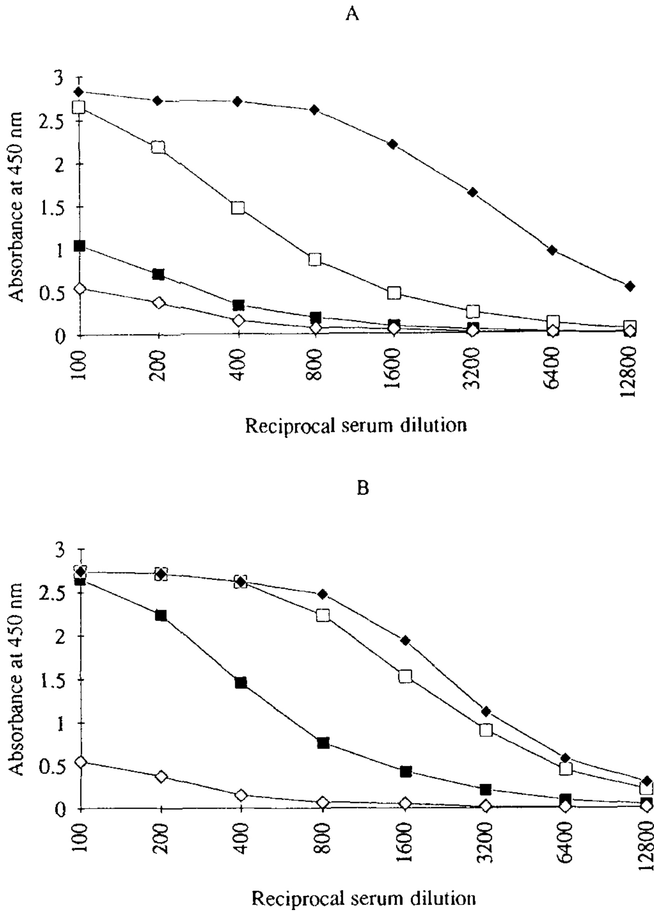

Figure 2. Titration curves of anti-HCV positive serum against two peptide antigens in indirect ELISA. Antiserum serially diluted was tested against C22 peptide (in A) or NS4-1924 peptide (in B) using three different formats. ■ :C22 or NS4-1924 free peptide; □ :C22 or NS4-1924 peptide coupled to BSA. Cysteine residue was added to N-terminal amino acid of each peptide. ◇ :Biotinylated GSGS tetrapeptide bound to streptavidin; ◆ :Biotinylated C22 or NS4-1924 peptide bound to streptavidin. The ELISA procedure was as described in materials and method.

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some peptides during coupling step (12). The reason for the higher reactivity of streptavidin-biotinylated peptides compared to the free or peptide-carrier conjugate is presumed as follows ; streptavidin-biotinylated antigen probably avoids the physical constraints and the chemical damage during antigen preparation. In relation to this observation, similar finding has been reported in that the application of the streptavidin-biotinylated antigen enhanced the absorbance values of HIV-1 positive sera, whereas HIV-1 negative sera exhibited similar absorbance values when compared with free peptide coated antigen (11).

Identification of immunodominant epitopes

To localize the antigenic regions of the core and nonstructural proteins of HCV, we synthesized 32 biotinylated peptides (8 to 30 amino acid long, Table 1) covering the core, NS3, NS4, and NS5 proteins based on the sequences reported by Kato et al. (3). Using indirect ELISA, we tested the reactivities of each peptides against 189 anti-HCV positive sera. Table 1 shows the incidence of positive sera with antibody responses to the individual peptides of the 32 synthetic peptides. The fifteen peptides (8 derived from the core protein, 4 derived from the NS4 protein, and 3 derived from the NS5 protein) reacted with antibodies in sera from HCV-positive patients.

For core protein, the whole region was analyzed because of reported discrepancies in the identification of immunodominant epitopes in this region (9, 13, 15, 16). Except for C132 and C169, the peptides C2, C22, C42, C50, C75, C100, C116 and C143 displayed antigenicity with varying extent ; ranging from 1.6 % to 93.1 % against the 189 sera. Of these, three peptides (C2, C22, C75) which showed more than 40 % positive ratio were considered to be the major immunogenic epitopes. In particular, the peptide C22 showed the highest reactivity (93.1%) to HCV patient sera irrespective of antigen format, which was in good agreement with the results of other reports (9, 13). In our study, the peptide C75 was determined to be immunodominant, although the results of the two other reports were controversial for this epitope (13, 15). However, this region was not the most immunodominant epitope as ascertained by Ching et al. (15). The discrepancies between different reports colud be attributed to the different methods of antigen preparation. From these studies, we conclude that there are at least three major immunodominant antigenic epitopes at the N-terminal 115 amino acids.

To determine the immunodominant epitopes within the NS3 protein, we selected 9 peptides. Unfortunately, none of the peptides reacted to HCV-infected human sera. There are two possible explanations for the negative results. One is that the NS3 protein elicits the antibodies to conformational epitopes which is not detectable in our system. However, it can not be excluded the possibility of missing the linear epitope region due to the limitation of prediction methods using computer algorithm.

Of 7 peptides derived from the NS4 protein, four (NS4-1694, NS4- 1719, NS4-1738, and NS4-1924) reacted with HCV-infected sera (Table 1). Of these peptides, three (NS4-1694, NS4-1719, and NS4-1924) reacted with antibodies in 99 (52.4%), 76 (40.2%), and 159 (84.1%) of the 189 serum samples, respectively, and were determined to be immunodominant. Antibody to NS4 protein develops in nearly all cases of posttransfusion

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TABLE 1 Reactivity of the peptides to antibodies from 189 HCV antibody-positive sera.

Protein and location[a]	Amino acid sequence[b]	Peptide code	% Positive samples
Core			
2-21	Biotin-SGSG STNPKPQRKTKRNTNRRPQD	C2	74.6
22-41	Biotin-SGSG VKFPGGGQIVGGVYLLPRRG	C22	92.6
42-49	Biotin-SGSG PRLGVRAT	C42	18.6
50-74	Biotin-SGSG RKTSERSQPRGRRQPIPKARRPEGR	C50	28.0
75-99	Biotin-SGSG TWAQPGYPWPLYGNEGMGWAGWLLS	C75	52.4
100-115	Biotin-SGSG PRGSRPSWGPTDPRRR	C100	26.5
116-131	Biotin-SGSG SRNLGKVIDTLTCGFA	C116	5.3
132-142	Biotin-SGSG DLMGYIPLVGA	C132	0.0
143-168	Biotin-SGSG PLGGAARALAHGVRVLEDGVNYATGN	C143	1.6
169-191	Biotin-SGSG LPGCS FSIFLLALLSCLTIPASA	C169	0.0
NS3			
1046-1061	Biotin-SGSG SLTGRDKNQVDGEVQV	NS3-1046	0.0
1086-1105	Biotin-SGSG GSKTLAGPKGPITQMYTNVD	NS3-1086	0.0
1134-1153	Biotin-SGSG TRHADVVPVRRRGDSRGSLL	NS3-1134	0.0
1197-1214	Biotin-SGSG PVESMETTMRSPVFTDNS	NS3-1197	0.0
1223-1241	Biotin-SGSG FQVAHLHAPTGSGKSTKVP	NS3-1223	0.0
1269-1289	Biotin-SGSG SKAHGIEPNIRTGVRTITTGG	NS3-1269	0.0
1316-1334	Biotin-SGSG DECHSTDSTTILGIGTVLD	NS3-1316	0.0
1386-1406	Biotin-SGSG KGGRHLIFCHSKKKCDELAAK	NS3-1386	0.0
1479-1497	Biotin-SGSG QDAVSRAQRRGRTGRGRSG	NS3-1479	0.0
NS4			
1529-1551	Biotin-SGSG ELTPAETSVRLRAYLNT PGLPV	NS4-1529	0.0
1569-1581	Biotin-SGSG DAHFLSQTKQAGD	NS4-1569	0.0
1694-1713	Biotin-SGSG VIPDREVLYQEFDEMEECAS	NS4-1694	52.4
1719-1735	Biotin-SGSG EQGMQLAEQFKQKALGL	NS4-1719	40.2
1738-1756	Biotin-SGSG TATKQAEAAAPVVESKWRA	NS4-1738	16.3
1903-1918	Biotin-SGSG RRHVGPGEGAVQWMNR	NS4-1903	0.0
1924-1943	Biotin-SGSG SRGNHVSPTHYVPESDAAAR	NS4-1924	84.1
NS5			
2184-2212	Biotin-SGSG ETAKRRLARGSPPSLASSSASQLSAPSLK	NS5-2184	0.0
2282-2305	Biotin-SGSG PPALPIWARPDYNPPLLESWKDPD	NS5-2282	46.6
2363-2383	Biotin-SGSG GTATGPPDQASDDGDKDSDVE	NS5-2363	8.5
2469-2491	Biotin-SGSG KKVTFDRLQVLDDHYRDVLKEMK	NS5-2469	6.3
2628-2657	Biotin-SGSG KSKKCPMGFSYDTRCFDSTVTENDIRTEES	NS5-2628	0.0
2666-2690	Biotin-SGSG PEARQAIRSLTERLYVGGPLTNSKG	NS5-2666	0.0

[a] The numbers represent the amino acid positions counting from the N-terminal end of the putative structural polyprotein encoded in the HCV genome.

[b] The amino acids are represented by the single-letter code.

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non-A, non-B hepatitis (1) ; it is a good marker of the presence of infectious virus (18). In particular, NS4-1924 peptide was located outside the C-terminus of C100-3 protein which was used in early serological assays.

Finally, six epitopes of NS5 protein were predicted and tested. Of those NS5 peptides, three (NS5-2282, NS5-2363, and NS5-2469) reacted with HCV-infected sera. Among them, the NS5-2282 peptide detected 88 (46.6%) of 189 serum samples (Table 1). Interestingly, antibody to this peptide was never found alone, but was always with antibodies to core or NS4 peptides.

Antibody spectra

Sera from 189 HCV infected patients were analyzed to examine the antibody specificity against individual peptide antigen. The characteristic two patterns of antibody specificity to each peptide antigens were noticed (Table 2). The first was characterized by the presence of the anti-HCV antibody positive exclusively to the core peptides (12.7%) of the tested 189 sera. Six different cases were observed, which were summarized in Table 2. The three samples were detected to be exclusively reactive to C2 antigen and ten samples (5.3% of the 189 tested sera) showed exclusive reactivity with C22 antigen. In the other four cases, more than two of four peptide antigens (C2, C22, C50, C75) showed positivity in 11 samples.

The second important aspect is related to the NS4 peptide antigens. The five typical cases showed HCV antibodies reactive only to NS4 peptide antigen (4.2%). Of these, one serum sample each was positive exclusively to NS4-1719 and NS4-1924. The remaining three cases showed the NS4 antibody positivity in 3.2%. Although antibody specificity to each peptide antigens can not be correlated with disease progress, these results are good agreement with the previous report that some patients may produce only antibodies to core, NS3 or NS4 antigens (2). These results indicate that C2, C22, NS4-1719 and NS4-1924 peptide antigens are essential antigens for serological assay system.

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Prevalence of HCV antibodies in blood donors and chronic liver disease patients

The detection rate of HCV antibodies in blood donors varied between 0.1% and 1.2% depending on the synthesized peptides used, with the highest detection rate observed with the C22 peptide, followed by NS4-1924, and then C2 peptide (Table 3). The other peptides showed the detection rate lower than 0.8 %. The combined prevalence of HCV antibodies detected with any of the HCV peptides was 1.5%.

Assays were also performed on serum samples obtained from 34 Koreans clinically diagnosed as chronic liver disease patients. The detection rate of HCV antibodies varied from 0% to 100%, with the highest rate observed for the C22 peptide in liver cancer and liver cirrhosis patients and the NS4-1694 peptide in chronic hepatitis patients (Table 3). The combined detection rate of HCV antibodies for any of the synthetic peptides was 100%, when Abbott second generation ELISA kit was used as a control. For the blood donors and chronic liver disease patients, most common

TABLE 2 Antibody spectra of the anti-HCV antibody assays compared with the result of an second generation ELISA.

Pattern and number tested	Abbott 2nd ELISA	Results with the following peptide antigen : C2	C22	C50	C75	C100	NS4-1694	NS4-1719	NS4-1924	NS5-2282
Core-positive pattern										
3	+	+	-	-	-	-	-	-	-	-
10	+	-	+	-	-	-	-	-	-	-
8	+	+	+	-	-	-	-	-	-	-
1	+	+	-	-	+	-	-	-	-	-
1	+	+	+	+	-	-	-	-	-	-
1	+	+	+	+	+	-	-	-	-	-
NS4-positive pattern										
1	+	-	-	-	-	-	-	+	-	-
1	+	-	-	-	-	-	-	-	+	-
1	+	-	-	-	-	-	-	+	+	-
1	+	-	-	-	-	-	-	+	+	-
4	+	-	-	-	-	-	+	+	+	-

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TABLE 3 Prevalence of HCV antibodies in blood donors and chronic liver disease patients in Korea.

Group	Number tested	Abbott 2nd EIA	Number (%) anti-HCV positive									Any
			C2	C22	C50	C75	C100	NS4-1694	NS4-1719	NS4-1924	NS5-2282	
Blood donors	985	15(1.5)	9(0.9)	12(1.2)	3(0.3)	1(0.1)	6(0.6)	7(0.7)	7(0.7)	11(1.1)	6(0.6)	15(1.5)
Liver cancer	19	19(100)	16(84.2)	19(100)	3(15.8)	5(26.3)	3(15.8)	13(68.4)	7(36.8)	15(78.9)	8(42.1)	19(100)
Liver cirrhosis	9	9(100)	4(44.4)	9(100)	0(0.0)	8(88.9)	0(0.0)	4(44.4)	2(22.2)	6(66.6)	6(66.6)	9(100)
Chronic hepatitis	6	6(100)	2(33.3)	3(50.0)	1(16.7)	1(16.7)	1(16.7)	6(100)	5(83.3)	4(66.7)	1(16.7)	6(100)

Numbers in parentheses are percentages.

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TABLE 4 Comparison of ELISA results based on peptide mixture antigens with commercially available ELISA kits using mixed titer performance panel (PHV 202).

Panel ID No.	Anti-HCV ELISA[a] Abbott 2nd	Ortho 2nd	Peptide mixture assay	Confirm tests: Abbott Matrix HC-23 (NS4)	C100-3 (NS4)	HC-29 (NS3)	HC-34 (CORE)	RESULTS[b]	Ortho RIBA 2.0 5-1-1 (NS4)	C100-3 (NS4)	C33C (NS3)	C22-3 (CORE)	SOD (FUSION)	RESULTS[b]
PHV202-01	4.7	4.4	8.2	1.5	0.8	1.6	234.9	P	1+	-	3+	4+	-	P
PHV202-02	4.7	4.4	8.4	5.4	5.9	0.9	326.0	P	-	-	2+	4+	-	P
PHV202-03	4.7	4.4	8.4	83.1	72.5	3.3	326.1	P	3+	3+	3+	4+	-	P
PHV202-04	3.9	4.4	6.8	19.0	18.5	28.2	0.0	P	2+	2+	3+	-	-	P
PHV202-05	4.7	4.4	7.0	4.2	2.9	340.2	16.4	P	1+	-	4+	2+	-	P
PHV202-06	4.7	4.4	6.5	35.1	34.4	174.6	325.5	P	2+	1+	3+	4+	-	P
PHV202-07	4.7	4.4	5.3	293.5	195.3	14.6	0.0	P	3+	4+	4+	-	-	P
PHV202-08	4.7	4.4	6.5	0.0	0.1	0.6	245.9	I	-	-	-	4+	-	I
PHV202-09	4.7	4.4	4.4	71.4	62.4	217.8	0.0	P	3+	1+	4+	-	-	P
PHV202-10	2.7	2.8	0.2	1.6	1.0	103.9	0.0	P	-	-	3+	-	-	I
PHV202-11	0.3	0.3	0.1	0.0	0.1	0.0	0.0	N	-	-	-	-	-	N
PHV202-12	4.7	4.4	7.0	0.5	0.1	272.6	272.6	P	-	-	4+	4+	-	P
PHV202-13	4.7	4.4	7.5	0.4	0.2	43.8	226.0	P	-	+/-	3+	4+	-	P
PHV202-14	4.7	4.4	8.2	0.6	0.5	6.2	282.2	P	-	-	3+	4+	-	P
PHV202-15	1.9	1.3	0.1	0.3	3.3	0.0	0.0	I	-	+/-	-	+/-	2+	I
PHV202-16	4.7	4.4	3.8	0.1	0.1	104.9	233.6	P	-	-	4+	4+	-	P
PHV202-17	2.6	3.2	3.8	0.1	0.0	0.1	0.3	N	-	-	-	3+	-	I
PHV202-18	2.3	1.4	2.0	48.6	40.8	0.4	0.0	P	2+	-	1+	-	-	P
PHV202-19	0.3	0.1	0.1					NT	-	-	-	-	-	N
PHV202-20	2.8	4.2	1.6	4.8	5.1	38.4	42.8	P	-	-	2+	2+	-	P
PHV202-21	4.7	4.4	7.7	0.3	0.1	107.5	179.8	P	-	3+	4+	4+	-	P
PHV202-22	4.7	4.4	0.7	10.0	12.4	220.1	4.0	P	-	-	4+	-	-	I
PHV202-23	3.4	4.4	3.8	1.3	0.7	1.3	60.7	P	-	-	-	3+	-	I
PHV202-24	4.7	4.4	8.3	154.3	138.1	188.9	165.4	P	4+	4+	4+	4+	-	P
PHV202-25	4.7	4.4	5.4	21.5	25.6	1.2	269.2	P	2+	-	-	4+	-	P

[a] Numeric results of ELISA tsets are expressed as specimen absorbance to cutoff ratio (s/co). Specimens with s/co of >1.0 are considered positive.

[b] Interpretations are based on the criteria of the test manufacturer. P = Positive, I = Indeterminate, N = Negative, NT= Not tested.

epitopes detected were seven peptides (C2, C22, C75, NS4-1694, NS4-1719, NS4-1924, and NS5-2282), which covers 100 % of the HCV antibody positive sera. In addition, our data suggest that there are substantial variations in the profile of the antigens recognized by the immune sera in chronic liver disease samples (Table 3). Taken together, these results suggest that several epitopes of core and nonstructural protein should be used to diagnose the HCV infection because the profile of antibody production during HCV infection is diverse as observed in this study.

Comparison of assay sensitivity

Based on above observations, indirect ELISA employing the combination of seven peptides (C2, C22, C75, NS4-1694, NS4-1719, NS4-1924, and NS5-2282) was compared with commercially available ELISA kits by testing mixed titer performance panel. Our indirect ELISA gave consistent results with Abbott and Ortho second generation ELISA kits in 22 of 25 sera tested (Table 4). Two samples (PHV202-10 and 22) were positive only for NS3 protein which is not contained in the peptide mixture antigen employed in our ELISA system. These results indicates that NS3 antigen should be supplemented to the peptide mixture antigen for complete detection of HCV antibodies. In contrast, one sample (PHV202-15) was false positive which was caused by superoxide dismutase (SOD) fusion protein. Three samples (PHV202-8, 19 and 23) containing only core antibodies were detected by our indirect ELISA. It was also shown that six cases of positive samples to have core and NS3 or NS3 and NS4 region (PHV202-2, 12, 14, 16, 18 and 20) could be detected by seven peptide mixture antigens. The results reported here suggest that seven epitopes covering core, NS4 and NS5 protein are sufficient to detect HCV antibodies to these region.

In summary, we tested thirty-two linear peptides in the core, NS3, NS4, and NS5 protein. Of those peptides, three in the core protein, three in the NS4 protein, and one in the NS5 protein were verified to be the major immunodominant epitopes. Studies on antibody spectra and HCV antibody prevalence revealed that at least those seven peptides were required to identify the HCV infection. Using seven peptide mixture antigens, most HCV seropositive samples could be detected except for the sera containing NS3 antibodies only. This indicated the possibility that immunodominant epitopes in the core, NS4, and NS5 protein could suffice to detect HCV antibodies to these region. However, NS3 polypeptides in addition to the peptide mixture antigen would be required to establish assays capable of diagnosing HCV-infection conclusively.

ACKNOWLEDGEMENTS

We are grateful to Hyun Joo Doh and Seung Chan Lee for synthesis of all the peptides used in this study.

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