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Virus Isolate-specific Antibodies against Hypervariable Region 1 of the Hepatitis C Virus Second Envelope Protein, gp70

Nobuyuki Kato,[1] Takahide Nakazawa,[1,2] Yuko Ootsuyama,[1] Kazuo Sugiyama,[1] Showgo Ohkoshi[3] and Kunitada Shimotohno[1,4]

[1]*Virology Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104 and* [3]*Third Department of Internal Medicine, School of Medicine, Niigata University, 1-757 Asahimachi, Niigata 951*

Hypervariable region 1 (HVR1), located in the N-terminal region of a putative second envelope glycoprotein (gp70) of hepatitis C virus (HCV), contains immunological B-cell epitopes which might be neutralizing epitopes. To clarify whether B-cell epitopes within HVR1 are common among virus isolates or specific for the homologous virus isolate, we examined the reactivities of sera from 53 patients with chronic hepatitis or hepatocellular carcinoma/liver cirrhosis against two different HVR1 peptides (HVR1 I-1 and HVR1 Y-1) derived from patient I with sporadic acute hepatitis and an asymptomatic carrier Y, respectively, using our original assay system for the detection of anti-HVR1 antibody. All patients examined had a history of blood transfusion. Most sera showed no reactivity with either HVR1 I-1 or HVR1 Y-1 peptide. Only seven and fourteen serum samples reacted significantly, although weakly, with HVR1 I-1 and HVR1 Y-1 peptides, respectively, compared with the serum from patient I or asymptomatic carrier Y. The blood transfusions of most reactive cases had occurred more than thirty years earlier. Six cases reacted with both HVR1 I-1 and HVR1 Y-1 peptides, but further analysis revealed that only three cases reacted weakly with the peptide for either epitope I or II, identified within HVR1 I-1. These results indicate that the B-cell epitopes within HVR1 are fairly specific for the homologous virus isolate, and this may represent a serious difficulty in the development of a vaccine against HCV.

Key words: Hepatitis C virus — Humoral immune response — Hypervariable region 1 — Anti-HVR1 antibody

Hepatitis C virus (HCV) is the major causative agent of non-A, non-B hepatitis.[1,2] Most HCV infection causes chronic hepatitis (CH) and this persistent viral infection frequently develops into liver cirrhosis (LC) and hepatocellular carcinoma (HCC).[3–5] Despite comprehensive genetic analyses of HCV[6–12] and the establishment of a diagnostic system for HCV infection,[2,3] neither the mechanisms by which HCV causes these hepatic diseases nor even the mechanisms of viral persistence are yet clear.

The HCV genome contains two hypervariable regions (HVR1 and HVR2) which encode the N-terminal region of a putative second envelope glycoprotein (gp70).[13–15] HVR1, in particular, shows marked sequence variability and a quasispecies nature,[16–22] and induces anti-HVR1 antibody.[23–25] On the basis of these reports, it has been suggested that genetic alterations of HVR1 are caused by immunoselection.

Recently, we reported the strong association of genetic drift of HVR1 with viral persistence.[24,26,27] The results showed that HVR1 is the major site for genetic alterations in HCV after the onset of hepatitis,[26,27] and that HCV with amino acid(s) substitutions in HVR1 can escape recognition by pre-existing anti-HVR1 antibodies.[24,26] Furthermore, we identified two overlapping B-cell epitopes, which shifted during the clinical course of hepatitis, within the HVR1 I-1 obtained from a patient I with sporadic acute hepatitis,[26] and clearly demonstrated that HVR1 variants having amino acid substitutions within the epitopes were not absolutely recognized by pre-existing anti-HVR1 antibodies in patient I.[26] These results suggest that HVR1 contains neutralizing epitope(s) for HCV infection, although there is no direct experimental evidence to prove this. If our assumption is correct, it is very important to clarify whether HVR1s from different virus isolates are recognized by common anti-HVR1 antibodies or virus isolate-specific anti-HVR1 antibodies, in order to develop an effective vaccine against HCV.

To clarify this point, we examined the reactivities of sera from 53 patients with CH or HCC/LC (HCC accompanied by LC) against two HVR1 sequences, HVR1 I-1[24] and HVR1 Y-1,[21] derived from patient I with acute hepatitis and an asymptomatic carrier Y, respectively.

[2] Present address: Gastroenterology Division of Internal Medicine, School of Medicine, Kitasato University, 1-15-1 Kitasato, Sagamihara-shi, Kanagawa 228.

[4] To whom correspondence should be addressed.

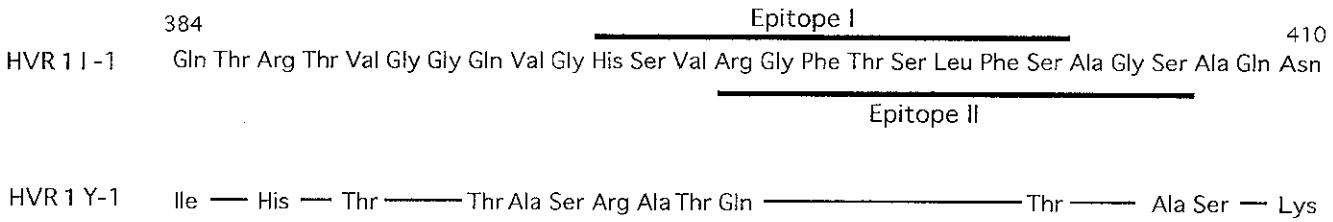

Fig. 1. Amino acid sequences of HVR1 I-1 and HVR1 Y-1. Amino acid sequences are in the three-letter code. The lines in the sequence of HVR1 Y-1 indicate amino acid identity with the same positions in HVR1 I-1. The positions of B-cell epitopes I and II within HVR1 I-1, identified previously,[26] are shown by lines above and below the sequence of HVR1 I-1, respectively.

Table I. Clinical Data on Patients and the Results of the Anti-HVR1 Antibody Assays

No.	Sex	Diagnosis	BT[a]	Anti-HVR1 antibody[b]		No.	Sex	Diagnosis	BT[a]	Anti-HVR1 antibody[b]	
				HVR1 I-1	HVR1 Y-1					HVR1 I-1	HVR1 Y-1
I	F	AH[c]	None	100	ND	27	F	HCC/LC	24	ND	ND
Y	M	ASC[d]	None	ND[h]	100	28	M	CH	25	<1	ND
1	M	AH	<1	ND	ND	29	M	HCC/LC	27	ND	ND
2	M	AH	<1	ND	ND	30	M	CH	28	<1	ND
3	M	AH	<1	ND	ND	31	M	CH	29	<1	ND
4	M	AH	<1	<1	ND	32	M	CH	29	ND	ND
5	F	CH[e]	1	ND	ND	33	M	HCC/LC	29	ND	ND
6	M	CH	1	ND	ND	34	F	CH	30	ND	ND
7	M	CH	1	ND	ND	35	M	HCC/CH[g]	30	ND	18
8	F	CH	1	ND	ND	36	M	HCC/LC	30	ND	ND
9	M	CH	2	ND	ND	37	M	HCC/LC	31	4	ND
10	M	CH	2	ND	2	38	F	HCC/LC	31	<1	ND
11	F	CH	2	ND	ND	39	F	HCC/LC	31	ND	ND
12	F	CH	3	ND	24	40	M	CH	33	<1	ND
13	M	CH	4	ND	ND	41	M	CH	33	1	31
14	M	CH	6	ND	ND	42	F	CH	34	<1	9
15	M	CH	9	<1	ND	43	M	HCC/LC	34	7	6
16	F	CH	9	ND	ND	44	F	CH	36	2	4
17	M	CH	10	ND	ND	45	M	HCC/LC	36	ND	ND
18	M	CH	10	ND	ND	46	M	CH	37	<1	4
19	M	CH	10	ND	ND	47	M	CH	37	18	8
20	M	CH	13	ND	ND	48	M	HCC/LC	38	ND	7
21	F	CH	16	ND	ND	49	M	CH	39	ND	2
22	M	CH	17	ND	ND	50	F	HCC/LC	40	ND	ND
23	F	CH	18	ND	43	51	F	HCC/LC	41	ND	ND
24	M	CH	22	ND	ND	52	F	CH	41	1	6
25	M	HCC/LC[f]	23	ND	ND	53	M	HCC/LC	42	5	3
26	M	CH	24	ND	ND						

a) Years after blood transfusion.
b) Relative titers of anti-HVR1 antibodies against HVR1 I-1 or HVR1 Y-1 peptides. The maximum antibody titers against HVR1 I-1 and HVR1 Y-1 were 8,800 PSL and 1,500 PSL, respectively, with exposure for 2 days. PSL is the unit of radiation dose used in the BAS2000 (Fuji Photo Film Co.). Percentage values versus the maximum antibody titers obtained in patient I or Y are indicated.
c) Acute hepatitis.
d) Asymptomatic carrier.
e) Chronic hepatitis.
f) Hepatocellular carcinoma accompanied with liver cirrhosis.
g) Hepatocellular carcinoma accompanied with chronic hepatitis.
h) Not detected.

All 53 patients had histories of blood transfusion a few months to 42 years previously, and had high titers of antibody against HCV (second-generation, Abbott, North Chicago, IL). Patient I is a 22-year-old female diagnosed with acute non-A, non-B hepatitis without a history of blood transfusion. Asymptomatic carrier Y is a 41-year-old male with normal serum alanine aminotransferase values (< 30 IU/liter for over 16 months) and no histological findings of hepatitis. Detailed clinical data on patient I and carrier Y were given previously.[17,21] The amino acid sequences of HVR1 I-1 and HVR1 Y-1 are shown in Fig. 1. Anti-HVR1 antibodies against the HVR1 I-1 or HVR1 Y-1 peptides were detected by our original assay system as described previously.[24] Briefly, an expression plasmid, pTZ19RSVdhfrl, was used to express a fusion protein of HVR1 (27 amino acids) and dihydrofolate reductase (DHFR) derived from *Escherichia coli* by *in vitro* transcription and translation using [^{35}S]methionine for radiolabeling. Immunoprecipitates of the fusion protein with sera from patients with CH or HCC/LC were analyzed by SDS-PAGE. Since we have demonstrated that the electrophoretic band intensities of samples obtained by immunoprecipitation depend on the titers of antibodies in patients' sera,[24] this assay system is suitable for the comparative analysis of antibody titers from different serum sources.

Using this system, we detected antibodies that reacted significantly with HVR1 I-1 or HVR1 Y-1 peptides in sera from 53 patients with CH or HCC/LC. The results of the anti-HVR1 antibody assay are summarized in Table I. The titer of anti-HVR1 antibody against HVR1 I-1 in the serum from patient I was about six times higher than that of HVR1 Y-1 in the serum from asymptomatic carrier Y (see legend to Table I).

Sera from most patients showed no significant reactivity with HVR1 I-1. This result supports our previous finding[24] of sequence-specific anti-HVR1 antibodies in sera from three patients with hepatitis. However, seven cases (#37, 41, 43, 44, 47, 52 and 53) showed distinct electrophoretic signals in their immunoprecipitates, although the intensities of the bands obtained were much lower (1 to 18%) than that from patient I (Table I). It is noteworthy that in all seven cases more than thirty years had passed since blood transfusion, suggesting that HCV species with HVR1 amino acid sequences similar to that of HVR1 I-1 were produced during the course of persistent HCV infection.

Similar results were obtained with regard to HVR1 Y-1. In this case, sera from fourteen patients showed significant bands in their immunoprecipitates, but none showed higher antibody titers than serum from asymptomatic carrier Y (2 to 43% of the level in serum from carrier Y). In eleven of the fourteen patients, over thirty years had also passed since blood transfusion. Sera from six patients (#41, 43, 44, 47, 52 and 53) reacted significantly with both HVR1 I-1 and HVR1 Y-1. The anti-HVR1 antibodies in the sera from these six patients may recognize a single common epitope within HVR1, since positions 398 to 406 of both HVR1s show similar amino acid sequences, as shown in Fig. 1.

Table II. Reactivities of Sera from Patients with Hepatitis against Epitopes I and II within HVR1 from Patient I

No.	Relative antibody titer[a]		
	HVR1 I-1 (27 aa)	HVR1 I-1-bc25 (12 aa) Epitope I	HVR1 I-1-bc51 (13 aa) Epitope II
I	100[b]	100[b]	100[c]
37	4	< 1	< 1
41	1	< 1	< 1
43	7	< 1	19
44	2	< 1	ND[d]
47	18	< 1	< 1
52	1	< 1	1
53	5	4	6

a) Relative titers of antibodies against HVR1 I-1, HVR1 I-1-bc25, or HVR1 I-1-bc51 peptide. Percentage values versus the maximum antibody titer obtained from patient I are indicated. The amino acid sequences of HVR1 I-1-bc25 (positions 394 to 405) and HVR1 I-1-bc51 (positions 397 to 409) contain epitopes I (11 amino acids, positions 394 to 404) and II (11 amino acids, positions 397 to 407) identified by Kato *et al.*,[26] respectively.
b) Serum from patient I at 6 months postdiagnosis showed maximum antibody titer. PSL values against HVR1 I-1 and HVR1 I-1-bc25 were 8,800 and 15,700, respectively, with exposure for 2 days.
c) Serum from patient I at 8 months postdiagnosis showed maximum antibody titer. PSL value against HVR1 I-1-bc51 was 6,100 with exposure for 2 days.
d) Not detected.

In the case of the seven patients whose sera reacted significantly with HVR1 I-1, we further examined the reactivities against peptides containing epitope I or II, both previously identified within HVR1 I-1.[26] Epitopes I and II are each composed of 11 amino acids located at amino acid positions 394 to 404 and 397 to 407, respectively, as shown in Fig. 1. Sera from two patients (#43 and #52) reacted with the peptide (HVR1 I-1-bc51) containing epitope II and serum from patient #53 reacted with both peptides (HVR1 I-1-bc25 containing epitope I and HVR1 I-1-bc51). This suggests that these three patients (#43, 52 and 53) were infected with HCV having an HVR1 sequence similar to that of epitope I or II. Alternatively, it remains possible that quasispecies of HVR1 spread and diversified for a few decades after blood transfusion, and that the HCV variant that induced the production of anti-HVR1 antibodies showing reactivities against both HVR1 I-1 and HVR1 Y-1 arose from this diversified population of HCV. However, the remaining four cases (#37, 41, 44 and 47) showed no significant reactivities against either epitope I or II (Table II); nevertheless, the immunoprecipitates from these four cases showed distinct signals against the whole HVR1 I-1 peptide. This suggests that sera from these four patients contain antibodies that recognize positions within HVR1 I-1 different from those of epitopes I or II. In addition, we did not find any patients whose sera reacted more strongly than that of patient I or carrier Y. This result indicates that the B-cell epitope within HVR 1 is fairly specific for the homologous virus isolate. So far, there are several reports[23–26] suggesting that HVR1 contains a neutralizing epitope. Therefore, if we assume that the anti-HVR1 antibody acts as a neutralizing antibody, the results obtained in this study reveal a serious problem for the development of an effective vaccine against HCV. An assay system, including cell culture, needs to be developed to determine the neutralizing epitope against HCV.

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