Clin Exp Immunol 1993; 91:489-494

Antigenic regions within the hepatitis C virus envelope 1 and non-structural proteins: identification of an IgG3-restricted recognition site within the envelope 1 protein

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(Accepted for publication 10 November 1992)

SUMMARY

Antibody binding to antigenic regions of hepatitis C virus (HCV) envelope 1 (E1; residues 183–380), E2/non-structural (NS) 1 (residues 380–437), NS1 (residues 643–690), and NS4 (1684–1751) proteins were assayed for 50 sera with antibodies to HCV (anti-HCV) and for 46 sera without anti-HCV. Thirty-four peptides, 18 residues long with an eight-amino acid overlap within each HCV region, were synthesized and tested with all 96 sera. Within the E region 183–380, the major binding site was located to residues 203–220, and was recognized by eight sera. Within the E2/NS1 region 380–437, the peptide covering residues 410–427 was recognized by two sera, and within the NS1 region 643–690, peptides covering residues 663–690 were recognized by four sera. Within the NS4 region 1684–1751, 27 sera were reactive to one or more of the NS4 peptides, and 21 out of these were reactive with peptide 1694–1711. One part of the major binding site could be located to residues 1701–1704, with the sequence Leu-Tyr-Arg-Glu. The IgG1, IgG3 and IgG4 subclasses were reactive with the five antigenic regions of HCV core, residues 1–18, 11–28, 21–38, 51–68 and 101–118. Reactivity to the major envelope site consisted almost exclusively of IgG3, and reactivity to the major site of NS4 consisted only of IgG1. Thus, a non-restricted IgG response to linear HCV-encoded binding sites was found to the core protein, whereas IgG subclass-restricted linear binding sites were found within the E1 protein, and within the NS4 protein.

Keywords HCV antigenic regions epitopes synthetic peptides

INTRODUCTION

The immune response to the hepatitis C virus (HCV) has been characterized using recombinant constructs and synthetic peptides mainly covering the core, non-structural region 3 (NS3), and NS4 [1,2]. Data so far have indicated that HCV-infected individuals mainly develop antibodies to these regions, especially to the core protein [3–5]. It has been shown that around 90% of HCV-infected individuals develop antibodies to one or more of the identified antigenic regions at residues 1–38, 51–68 and 101–118, of HCV core (amino acid numbering according to [6]; [7,8]), where the consensus site Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg at residues 9–17 elicits antibodies of several isotypes [8]. One antigenic region within NS4, at positions 120–137 of the Chiron C-100-3 protein, has also been identified [9].

We were interested to map antibody binding sites within the complete sequence of the envelope 1 (E1), and parts of non-structural proteins of HCV which previously have been shown to bind human antibodies [10]. Our aim was also to study the IgG subclasses to these, and the five antigenic regions of the core protein [7].

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MATERIALS AND METHODS

Patient sera

A total of 96 consecutive sera which were sent to the National Bacteriological Laboratory during the period March to May 1991, were selected on the basis of having been assayed for anti-HCV by the commercial assays Abbott first generation (Abbott, Chicago, IL), Abbott second generation, and/or Hepanostica C (Organon Teknika, Boxtel,The Netherlands), and/or Ortho second generation (Ortho Diagnostic Systems, Raritan, NJ). Fifty of these sera were found reactive and 46 were found negative by these commercial assays. Most (40/50) of the anti-HCV$^+$ sera were obtained from intravenous drug users (IVDUs). This material has been characterized in previous studies [7,8], where reactivity to HCV core peptides was present in 46 of the 50 anti-HCV$^+$ sera. All 96 sera were used for identification of antigenic regions within HCV E1 and NS4, and all of those found reactive to the major antigenic regions of these

proteins were subjected to IgG subclass analysis. In 14 of the anti-HCV$^+$ sera, all from IVDUs and with a varying recognition of HCV core peptides [7], the IgG subclass distribution to HCV core was studied.

Table 1. Amino acid sequences of the envelope 1 (E1), E2/non-structural region 1 (NS1), NS1 and NS4 regions of hepatitis C virus (HCV) according to Choo *et al.* [6], which were used for peptide synthesis

Amino acid sequence of HCV region [6]	Residues of region
Envelope 1	
SCLTIPASAYEVRNVSGIYHVTNDCSNSSI	183–212
VYEAADVIMHAPGCVPCVRENNSSRCWVAL	213–242
TPTLAARNASVPTTTLRRHVDLLVGTAAFC	243–272
SAMYVGDLCGSVFLISQLFTFSPRRHETVQ	273–302
DCNCSIYPGHVSGHRMAWDMMMNWSPTAAL	303–332
VVSQLLRIPQAVMDMVAGAHWGVLAGLAYY	333–362
SMVGNWAKVILVMLLFAG	363–380
E2/NS1	
GVDAETHVTGGSAGHTVSGFVSLLAPGAKQ	380–409
NVQLINTNGSWHLNSTALNCNDSLNTGW	410–437
NS1	
ACNWTRGERCDLEDRDRSELSPLLLTTTQW	643–672
QVLPCSFTTLPALSTGLI	673–690
NS4	
GRVVLSGKPAIIPDREVLYREFDEMEECSQ	1684–1713
HLPYIEQGMMLAEQFKQKALGLLQTASRQA	1714–1743
EVIAPAVQ	1744–1751

To cover each region, 18-amino acids long peptides with an eight-residue overlap were produced.

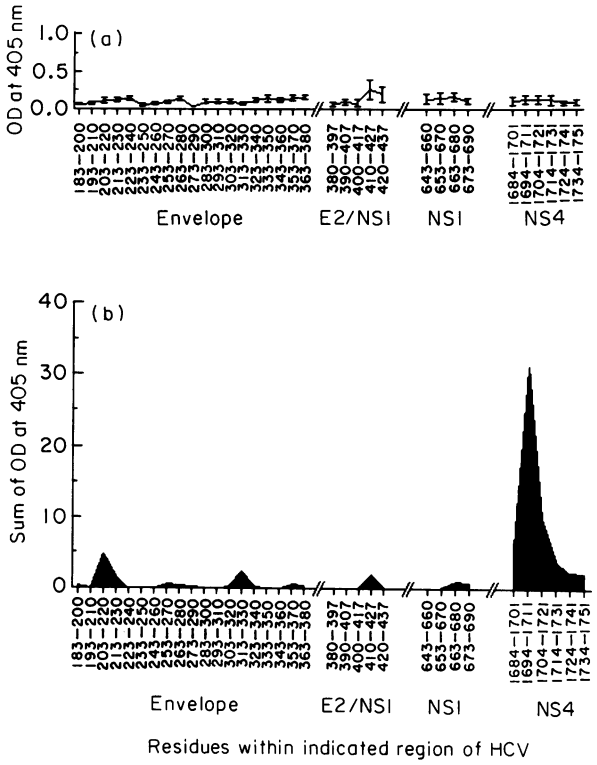

Fig. 1. Mapping of antibody binding sites within the HCV envelope 1 (E1) amino acids 183–300, E2/non-structural region 1 (NS1), amino acids 380–437, the NS1 region, amino acids 643–690, and NS4 amino acids 1684–1751 with 46 anti-HCV-negative (a) and 50 anti-HCV-positive sera (b). In (a), mean optical densities (OD) at 405 nm, and s.d. of the 46 anti-HCV-negative sera when tested with each peptide are given. In (b), values are given as the sum of the OD at 405 nm of reactive sera.

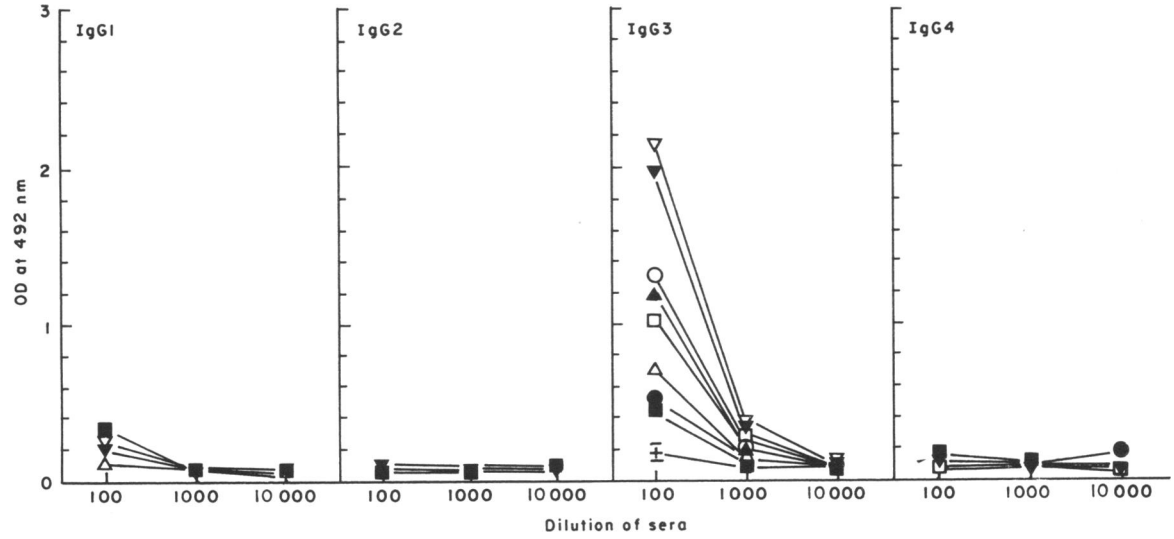

Fig. 2. IgG subclass reactivity of eight sera reactive to residues 203–220 of HCV envelope 1 protein. Values are given as the optical density (OD) at 492 nm. +, Mean OD at 492 nm and s.d. of six sera negative for anti-HCV. ○, 2835; ●, 2840; □, 3554; ■, 3555; △, 3596; ▲, 3855; ▽, 3865; ▼, 3897; +, mean and s.d. of NS.

Synthetic peptides

A total of 34 peptides were synthesized, all 18 amino acids long with an eight-amino acid overlap within each region (Table 1), covering the complete sequence of E1 residues 183–380, parts of E2/NS1 residues 380–437, NS1 residues 643–690, and NS4 residues 1684–1751 based on the sequence of the US HCV clone [6]. The latter regions were selected according to previous findings [10]. Synthesis of the five peptides containing the antigenic regions within residues 1–18, 11–28, 21–38, 51–68, and 101–118 of HCV core, have previously been described [7]. Peptides were simultaneously synthesized using a recently described method [11]. In brief, synthesis was carried out on 30 mg of Polyhipe resin (NovaSyn PR 500, NovaBiochem, Läufl-ingen, Switzerland) in bags of polypropylene screening. Amino acids were prepared in stock solutions and were dispensed at each coupling step. Peptides were cleaved and deprotected using

Table 2. Mapping of antigenic regions within the hepatitis C virus (HCV) envelope (E) residues 183–380, E2/non-structural region (NS) 1 residues 380–437, NS1 residues 643–690, and NS4 residues 1684–1751 in 50 sera with anti-HCV

	IgG reactivity to indicated region of HCV											
	Core Envelope			E2/NS1	NS1		NS4					
Sera	1–190*	203–220	313–330	410–427	663–680	673–690	1684–1701	1694–1711	1704–1721	1714–1731	1724–1741	1734–1751
2089	+	−	−	−	−	−	++	++	+	−	−	−
2090	+	−	+	−	−	−	−	++	−	+	+	+++
2264	+	−	−	−	−	−	++	+	−	−	−	−
2269	+	−	−	−	−	−	−	+++	−	+	−	−
2271	+	−	−	−	+	−	−	−	−	−	−	−
2272	+	−	−	−	+	−	+	−	−	−	−	−
2276	+	−	−	−	−	−	−	−	+	−	+	−
2311	+	−	−	−	−	−	−	+	−	−	−	−
2378	+	−	−	−	−	−	−	−	+	++	−	−
2382	+	−	−	−	−	−	−	++	−	−	−	−
2426	+	−	−	−	−	−	−	+++	+	−	−	−
2435	+	−	−	−	−	−	−	++	−	−	−	−
2457	+	−	−	−	−	−	−	++	−	−	−	−
2823	+	−	−	−	−	−	−	−	++	−	−	−
2835	+	+	−	−	−	−	−	−	−	−	−	−
2840	+	+	−	+	−	−	+	++	−	−	+	−
3554	+	+	−	−	−	−	−	−	−	−	−	−
3555	+	+	−	−	−	−	−	−	−	−	−	−
3596	+	+	−	−	−	−	−	−	−	−	−	−
3601	+	−	−	−	−	−	−	+	−	−	−	−
3733	+	−	−	−	−	−	−	+	−	−	−	−
3754	+	−	−	−	−	−	−	+++	+	−	−	−
3764	+	−	+	−	−	−	−	+	−	−	−	−
3830	+	−	−	+	−	−	−	+++	+	−	−	−
3842	+	−	−	−	−	−	−	+++	++	−	−	−
3850	+	−	−	−	−	−	−	++	−	−	−	−
3855	+	+	−	−	−	+	−	++	++	−	−	−
3865	+	+	−	−	−	−	−	−	−	−	−	−
3891	+	−	−	−	−	−	−	++	+	−	−	−
3897	+	++	−	−	−	−	−	++	−	−	−	−
4952	+	−	−	−	−	−	−	−	−	+	−	−
5068	+	−	++	−	−	−	++	++	−	−	−	−
5074	+	−	−	−	−	−	−	−	+	+	−	−
5096	+	−	−	−	−	+	−	−	−	−	−	−
No. of sera reactive		8	3	2	2	2	5	21	11	5	3	1

* Reactivity to HCV core peptides according to Sällberg *et al.* [7].

+, OD_{405} value over cut-off but < 1·0; ++, OD_{405} value of > 1·0; +++, OD_{405} value of > 2·0, as determined by peptide enzyme immunoassay (EIA).

Only sera reactive with one or more peptides are listed.

trifluoracetic acid containing the appropriate scavengers [11], lyophilized and dissolved in distilled water at a concentration of 1 mg/ml, and subsequently analysed by high pressure liquid chromatography.

Peptide enzyme immunoassays

Peptide enzyme immunoassays (EIA) were performed as previously described [7]. In brief, peptides were coated overnight at 4°C to microtitre plates (Nunc Maxisorp 96F Certificate, Nunc, Denmark) at 1 μg/well in carbonate buffer, pH 9·6. Before testing, the additional binding sites were blocked by addition of 2% bovine serum albumin (BSA) for 2 h at room temperature. Sera were added at a 1:100 dilution and incubated for 45 min at +37°C. Bound serum IgG was indicated by addition of alkaline phosphatase-labelled goat anti-human IgG (A-3150, Sigma Chemicals, St Louis, MO) diluted 1:1500. All dilutions were prepared in PBS containing 2% goat serum, 2% BSA, and 0·05% Tween 20. The IgG subclass levels to the antigenic regions were determined according to a previously reported protocol [8], where the peptide-reactive immunoglobulin was recognized by MoAbs specific for the four human IgG subclasses. The MoAbs used were clone NL16 for IgG1, HP6014 for IgG2, ZG4 for IgG3, and RJ4 for IgG4. All MoAbs were obtained from Unipath Ltd, UK, except for the HP6014 from CDC (Atlanta, GA). Sera were tested at dilutions 1:100, 1:1000, and 1:10000 in the IgG subclass assays. Only sera giving absorbances exceeding the mean of the anti-HCV$^-$ sera by >7 s.d. were regarded as reactive [7].

RESULTS

Recognition of envelope, E2/NS1, NS1, and NS4 regions by human sera

The human recognition of the regions within the E1, E2/NS1, NS1, and NS4 is depicted in Table 2 and Fig. 1. The major recognition sites within the E1 sequence were located at residues 203–220 and residues 313–330, and were recognized by eight and three sera, respectively. These sera were all reactive to HCV core peptides, and 10 were obtained from IVDUs, and one from a homosexual man.

Six sera had recognition sites within regions E2/NS1 and NS1, and of these all had antibodies to HCV core peptides (Table 2 and Fig. 1).

Most reactivities found to peptides covering the NS4 region (26/50), were located at residues 1684–1721 covered by three peptides (Table 2 and Fig. 1). Eleven sera were found to recognize the peptide 1694–1711, and not the adjacent peptides 1684–1701 and 1704–1721. This suggests that residues 1701–1704, with the sequence Leu-Tyr-Arg-Glu, is part of an antigenic site. However, nine sera recognized both peptide 1694–1711 and one or both of the adjacent overlapping peptides, indicating a varying recognition of this antigenic region. The most commonly detected peptide, residues 1694–1711, was recognized by 21 of the 50 (42%) anti-HCV$^+$ sera. In 27 (54%) of the anti-HCV$^+$ sera, reactivity to one or more of the NS4 peptides could be found. None of the 46 anti-HCV$^-$ sera was found to react with any of the 26 peptides.

Table 3. Relative IgG subclass levels to antigenic regions within hepatitis C virus (HCV) core and non-structural 4 (NS4) proteins in 14 sera from intravenous drug users with antibodies to HCV

	Immunodominant regions within HCV coded proteins covered by peptides																	
	Core*															NS4		
	1–18			11–28			21–38			51–68			101–118			1694–1711		
Serum	IgG1	IgG3	IgG4	IgG1	IgG3	IgG4	IgG1	IgG3	IgG4	IgG1	IgG3	IgG4	IgG1	IgG3	IgG4	IgG1	IgG3	IgG4
2089	+	−	−	++	−	+	+	−	+	+	−	−	+	−	−	+	−	−
2090	++	−	−	++	−	−	+	−	−	++	−	−	+	−	−	+	−	−
2264	++	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−
2269	++	++	−	++	−	−	−	−	−	+	−	−	+	−	−	+	−	−
2271	++	+	−	+	−	−	+	−	−	+	−	−	+	−	−	−	−	−
2272	+++	+	−	++	+	−	+	+	−	+	+	−	+	−	−	−	−	−
2276	+	++	−	++	+	−	+	+	+	+	+	−	+	−	−	−	−	−
2382	++	++	+	++	+	−	+	+	+	+	+	−	+	+	−	+	−	−
2435	++	−	+	++	−	−	+	−	−	++	−	−	++	−	−	++	−	−
2823	++	−	−	+++	+	+	+	−	−	++	−	−	++	−	−	−	−	−
3818	++	++	−	++	+	−	++	+	−	++	−	−	+	−	−	−	−	−
3850	++	++	−	++	−	−	++	−	−	++	−	−	+	−	−	+	−	−
4952	++	−	−	+	−	−	+	−	−	++	−	−	+	−	−	−	−	−
5086	+	−	−	++	−	−	+	−	−	+	−	−	+	+	−	−	−	−
Total	14	7	2	13	5	2	13	4	2	13	3	0	14	2	0	7	0	0

* Regions were selected according to Sällberg *et al.* [7].

+, A positive reaction at a dilution of serum 1:100; ++, positive reactions at dilutions of serum 1:100 and 1:1000; +++, positive reactions at dilutions of serum 1:100, 1:1000 and 1:10000.

IgG subclasses to antigenic regions within the core, E1, and NS4
The IgG subclasses to all antigenic regions within HCV core were determined in 14 sera from IVDUs, previously shown to react with HCV core peptides [7]. The dominant subclasses were found to be IgG1, followed by IgG3 (Table 3). IgG4 was only detected to antigenic regions within residues 1–38. By the IgG2 assays only borderline values were found in three sera (data not shown).

Of the eight sera with IgG to the envelope 1 residues 203–220, all were found to have IgG3 to this region. Three sera were reactive at a dilution of 1:1000, and five sera were reactive only at a dilution of 1:100. Borderline values were found by the IgG1 assay in four of the eight sera (Fig. 2). We tested all 96 sera for IgG3 reactivity to the E1 peptide 203–220, and no additional sera were reactive (data not shown).

All 21 sera reactive with peptide 1694–1711 of NS4, had only IgG1 to this region. In Table 3, the IgG1 levels are depicted for seven of these sera obtained from IVDUs. None of the anti-HCV$^-$ sera was reactive in any of the IgG subclass assays.

DISCUSSION

The major recognition site within the E region was found in the area of residues 203–220, and the recognition of this site seemed to be restricted to IgG3. No relation was found between the presence of these antibodies and other serological HCV tests, or available epidemiological data. The region 203–220 of E1 was not identified by any of six human and chimpanzee sera in another study [12], possibly due to the fact that pin-bound decapeptides give a high background noise when assaying polyclonal sera. Matsuura *et al.* [13] concluded that antibodies to a recombinant E1 protein may have some protective properties. However, recent data suggest that chimpanzees with previous HCV infection are not protected upon challenge [14]. We have recently also found HCV RNA in human sera at the same time as antibodies to the major antigenic region of HCV E1 (Sällberg *et al.*, manuscript in preparation).

The antigenic regions assayed here within the E2/NS1 and NS1 regions were recognized only by a minority of the sera. Weiner *et al.* [15] found individual variation of the recognition of the hypervariable antigenic region within the E2/NS1 residues 384–414 [16]. Antibodies against the E2/NS1 of the host's own HCV strain, which do not cross-react with the E2/NS1 of the US HCV strain [6], could explain the low prevalence of reactivities to this region observed in our material. This will be further investigated by using peptides corresponding to sequenced E2/NS1 genes of the infected host, according to the approach by Albert *et al.* [17].

Antibodies to the region of NS4 covered by our peptides were found in 54% of all anti-HCV$^+$ sera, in agreement with the finding of antibodies to recombinant proteins C-100–3 and 5-1-1 in 48 out of 81 (59%) sera [18].

As we have previously reported [7], antibodies to HCV core peptides were not found in four out of the presently evaluated 50 anti-HCV$^+$ sera, and none of these reactive to the E or NS peptides. Thus, the reactivities of these sera remain unclear. Cerino & Mondelli [9] have identified an antigenic region with the sequence Val-Leu-Tyr-Arg-Glu-Phe within NS4. Corroborating their observations, we found a major site within residues 1694–1711 of NS4 with the sequence Leu-Tyr-Arg-Glu.

Similar to the antibody response against the major discontinuous determinant on hepatitis B virus (HBV) core protein [19,20], the detected subclasses to HCV core were IgG1, IgG3 and IgG4. Only a minority of human sera recognize linear binding sites in the HBV core protein [11,21]. The envelope protein of HBV has been shown to elicit mainly IgG1 and IgG3 subclasses [22]. Only IgG3 was found to react with our HCV E1 peptide. To the two non-structural proteins, HBeAg of HBV [19,20] and the NS4 of HCV, IgG1 seem to be the major subclass. Thus, a slightly different IgG subclass distribution of the responses to the envelope/NS and to the core proteins is observed for these two hepatotropic viruses. The IgG responses to HBV and HCV envelope/NS proteins are generally of lower levels and of fewer subclasses than to the core proteins, possibly reflecting a lower immunogenicity of the envelope/NS proteins.

We have identified two IgG subclass-restricted antigenic regions within the HCV envelope and NS4 protein, in contrast to regions within HCV core which were recognized by all IgG subclasses. It will be of interest to investigate the kinetics and duration of these antibodies and to correlate them to the presence of HCV RNA.

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