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Molecular basis for antibody cross-reactivity between the hepatitis C virus core protein and the host-derived GOR protein

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SUMMARY

The presence of antibodies reactive to a recently cloned host-derived antigen GOR is highly correlated with the presence of antibodies to the hepatitis C virus (HCV). We explored the molecular basis for this observation, and address the following question: are antibodies reactive with GOR_{19-27} (QKAKSNPNR) a result of a cross-reactivity triggered by the antigenic region at residues 9–17 of HCV core (RKTKRNTNR)? We compared the relative antibody avidity between antibodies reactive to both regions, and determined the residues essential for antibody binding using substitution peptide analogues. Of 96 sera assayed, 60 were found positive for anti-HCV, and of these 55 were found to react with HCV core. Twenty-nine sera were found reactive to the GOR peptide, and these were all reactive to HCV core. In most cases the relative antibody avidity of antibodies reactive to GOR was higher for the HCV core peptide. In 21 of the GOR-reactive sera we were able to determine the essential residues for antibody binding. The essential residues in >50% of all tested sera coincided with the well conserved residues Lys^{10}, Lys^{12}, Asn^{14}, and Asn^{16}. Also, reactivity to GOR was not related to any certain serotype of antibodies to HCV. Taken together, these findings explain at the molecular level the observed cross-reactivity between these two proteins, since sequence homology *per se* is not evidence for cross-reactivity.

Keywords autoimmunity synthetic peptides antigenic regions cross-reactivity serotypes

INTRODUCTION

Recently, antibodies have been described which are thought to be cross-reactive between the hepatitis C virus (HCV) core protein and the probable host-derived gene product GOR [1–3]. The clinical significance of these antibodies lies in their possible association with autoimmune hepatitis type I [4,5].

The recently cloned structural gene of HCV [6,7] contains an antigenic region with the sequence RKTKRNTNR at residues 9–17 of the core protein [8], which is recognized by most HCV-infected humans [9–13]. This sequence holds a 63% homology with a part of the GOR sequence, QKAKSNPNR at residues 19–27 [1].

We investigated whether the origin of the GOR-reactive antibodies is indicated by an estimation of antibody avidity to these two sequences, and a determination of the recognition pattern on the level of a single amino acid.

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MATERIALS AND METHODS

Ninety-six sera were obtained from the Department of Virology at the National Bacteriological Laboratory during the period February to April 1992. All sera were tested for anti-HCV by a standard assay (Abbott Laboratories, Chicago, IL) and the results were confirmed using the Abbott Supplemental Assay (Abbott), in which each test includes one bead coated with a recombinant construct containing parts of the HCV core protein (c22) and one bead with constructs containing parts of non-structural (NS) regions 3 and 4 (c33 and C100-3).

Synthetic peptides

Three synthetic peptide analogues covering residues 1–28 (mol. wt 3195·6) and 1–18 (mol. wt 2124) of the HCV core protein [14] and residues 17–34 of the GOR clone (mol. wt 2028) were produced using a Milligen 9050 peptide synthesizer. The peptides were analysed and purified to a purity of >99% by reverse phase high pressure liquid chromatography (RP-HPLC), and the molecular weights were analysed by plasma desorption mass spectrometry. These peptides were used for

determination of the relative antibody avidities to HCV core and GOR. Ten peptide analogues covering residues 9–17 of HCV core, where each residue was sequentially substituted by alanine, were produced using simultaneous multiple peptide synthesis as previously described [15]. The sequential substitution by alanine in each case maintains the configuration of the peptide backbone but introduces the smallest carbon sidechain (CH_3). Thus, this set of substitution peptide analogues gives information about the importance of each of the existing sidechains in formation of antigen–antibody complexes containing residues 9–17 of HCV core. Using the same method, an additional set of four peptide analogues was synthesized where the Lys^{10} and Lys^{12} were substituted by amino acids with sidechains of similar size and the same charge (Arg), or similar size but lower charge (Met). All substitution peptide analogues of residues 9–17 of HCV core were analysed by RP-HPLC using a Pep-S5μ column (Pharmacia, Uppsala, Sweden), and because of their similar overall charge were found to elute within the same retention time range (mean 14·15 ± 0·25 min). All were >67% pure, except for the Met containing substitution analogues which were adjusted to that purity when tested in enzyme immunoassay (EIA). In conclusion, the coating and inhibition performances of the substitution peptide analogues are assumed to be similar, and were therefore tested using the same conditions. This assumption was recently verified using a newly developed procedure for estimating the amount peptide adsorbed on the microplate. That procedure found that the substitution analogues adsorbed within a range of 120–290 pmol/well, when the concentration for coating was 10 μg peptide per ml (Zhang *et al.*, manuscript in preparation).

Peptides to be used for serotyping of antibodies to HCV core and NS4 were produced according to HCV core types 1, 2, and 3, residues 68–81 [16,17] and NS4 types 1a, 1b, 2a, 2b, and 3, residues 1692–1705 [18].

Enzyme immunoassays

The EIAs for detection of anti-HCV core and GOR, and the testing of the substitution peptide analogues, were performed according to a previously described protocol [8]. In brief, peptides were passively adsorbed to microtitre plates at a concentration of 1 μg/ml in 0·05 M sodium carbonate buffer pH 9·6. Sera were added at a dilution of 1:100 in PBS with 0·05% Tween 20, 1% bovine serum albumin (BSA) and 2% goat serum. Bound IgG was indicated by incubation with goat anti-human IgG conjugated to alkaline phosphatase (Sigma Chemical Co., St Louis, MO) diluted 1:1500. The plate was then developed by addition of the substrate dinitro-phenylene-diamine (Sigma).

Only reactions exceeding the mean OD at 405 nm of the anti-HCV negative sera ($n = 36$) by more than 7 s.d. were regarded as reactive. The 7 s.d. assure a high specificity in the assays.

When using the substitution peptide analogues coated to solid phase, a residue was considered as essential if the substitution by alanine decreased the OD at 405 nm by more than 50% than that of the peptide having the original sequence. Accordingly, when using the substitution peptide analogues to inhibit the binding to the original peptide, a residue was considered as essential if the substitution peptide did not decrease the OD at 405 nm by more than 50% of the non-inhibited peptide.

Avidity EIA

Plates were coated as described above. Before addition of 50 μl of human sera diluted 1:50, a 50-μl portion of serial four-fold dilutions of peptides, range 9·9–0·002 nmol, were added to the wells. The mixture was incubated for 60 min at 37°C and developed as described above using the Sigma conjugate and substrate.

All sera were tested for inhibition at a final dilution of 1:100 in all avidity EIAs. Relative avidity is defined as the inhibiting peptide concentration giving 50% inhibition (IC_{50}) compared with the non-inhibited control. As long as the antibody concentrations are constant, the amount of inhibiting peptide giving >50% inhibition is reversely correlated to the antibody avidity. This definition follows the Langmuir equation, simplified by Steward *et al.* [19], where the avidity, or affinity constant (Ka) is estimated as follows: Ka = 1/(mole of antigen giving >50% inhibition). We only compared the IC_{50} for each of the two peptides obtained with one serum tested at a single dilution. This approach allows only an intraserum comparison of the obtained avidity values, since the antibody concentrations are held at constant levels, and since the peptides used were of maximum purity. This method is, however, not appropriate for comparing the avidities obtained between different sera, since the exact concentration of specific antibody is unknown. Also, we do not claim that these obtained avidity values correspond to an actual avidity. Values were used only for intraserum comparisons.

Serotyping of antibodies to HCV core and NS4

Antibodies to HCV core and NS4 were typed according to the methods described by Machida *et al.* [16] and Simmonds *et al.* [18]. In brief, type-specific peptides were adsorbed on microplates as described. Before addition of sera a 25-μg mixture of peptides, different from the types adsorbed to the plate, was added. Thus, the reactions that were not inhibited were

Table 1. Relation between outcome from testing by anti-GOR_{17-34} and (a) anti-hepatitis C virus (HCV) $core_{1-28}$, and (b) Abbott Supplemental Assay, in 96 human sera

	Anti-GOR_{17-34}		
HCV status	+	−	
a.			
Anti-$Core_{1-28}^{+}$	29	23	52
Anti-$Core_{1-28}^{-}$	0	44	44
Total	29	67	96
b. Abbott Supplemental*			
$S+/NS^{+}$	28	14	42
$S+/NS^{-}$	1	12	13
$S-/NS^{+}$	0	5	5
$S-/NS^{-}$	0	36	36
Total	29	67	96

*S, Bead coated with HCV core (c22); NS, bead coated with HCV NS3 (c33) and NS4 (C100-3).

Table 2. Relative antibody avidity between antibody binding to GOR_{17-34} and hepatitis C virus (HCV) $core_{1-18}$, and the recognition pattern of antibody binding to residues 9–17 of HCV core, in 29 sera reactive to GOR

Sera ID	OD at 405 nm in EIA coated with indicated peptide		Minimal amount of peptide (nmol) required for > 50% inhibition of binding to				Serotype of HCV antibodies	Essential residues for antibody binding to HCV $core_{9-17}$								
			GOR_{17-34}		$Core_{1-18}$											
			Inhibiting peptide		Inhibiting peptide											
	GOR_{17-34}	$Core_{1-18}$	GOR_{17-34}	$Core_{1-18}$	GOR_{17-34}	$Core_{1-18}$		R	K	T	K	R	N	T	N	R
1139	2·348	> 2·5	0·04	< 0·002	> 9·9	0·15	1	R	K	—	—	—	—	—	N	—
1179	2·151	> 2·5	0·010	< 0·002	> 9·9	0·62	2	—	K	—	—	—	—	—	N	—
1183	0·501	0·288	> 9·9	> 9·9	> 9·9	2·47	ND					ND				
1322	0·470	1·305	> 9·9	> 9·9	> 9·9	0·62	1	R	—	—	—	—	N	—	—	—
1331	0·530	1·897	9·9	> 9·9	> 9·9	0·04	1	—	K	—	K	R	N	T	N	—
1336	1·534	2·382	0·04	0·010	> 9·9	0·62	ND	—	K	—	K	—	—	T	N	—
1341	0·663	2·143	0·62	9·9	> 9·9	2·47	1/3	—	—	—	—	—	N	—	—	—
1344	1·581	2·178	0·62	< 0·002	> 9·9	0·01	1/3					ND				
1377	0·295	1·194	> 9·9	> 9·9	> 9·9	0·04	1					ND				
1379	0·377	> 2·5	> 9·9	> 9·9	> 9·9	0·62	1/3					ND				
1382	2·223	2·347	0·04	< 0·002	> 9·9	0·62	ND	—	K	—	K	R	—	T	N	—
1394	1·293	> 2·5	0·010	< 0·002	> 9·9	0·62	1/3	—	K	—	—	—	N	T	N	—
1396	0·429	1·942	0·62	> 9·9	> 9·9	0·15	ND	—	—	—	—	—	—	T	N	—
1406	0·848	2·112	0·04	< 0·002	> 9·9	2·47	2	—	K	T	K	R	N	—	N	—
1407	0·623	1·717	> 9·9	> 9·9	> 9·9	9·9	ND	—	K	—	K	R	N	T	N	—
1436	1·982	> 2·5	0·04	0·04	> 9·9	0·15	1	—	K	—	K	R	N	—	N	—
1439	2·455	2·316	0·04	< 0·002	> 9·9	0·15	1					ND				
1440	2·167	2·314	0·04	< 0·002	> 9·9	0·04	1 or 3	R	—	T	—	—	—	—	—	—
1441	2·476	2·301	0·04	< 0·002	> 9·9	0·62	1					ND				
1443	1·260	2·193	0·15	> 9·9	> 9·9	2·47	ND				Not tested					
1518	0·648	2·205	0·010	0·62	> 9·9	0·62	ND	—	K	—	K	R	—	T	N	—
1521	0·433	1·889	0·010	9·9	> 9·9	2·47	1	—	K	—	K	—	N	T	N	—
1522	0·500	1·737	2·47	> 9·9	> 9·9	2·47	3	—	—	—	—	R	—	—	—	—
1523	2·145	2·000	0·04	0·010	> 9·9	0·62	ND	—	K	—	K	—	—	—	N	—
1527	0·459	> 2·5	0·04	> 9·9	> 9·9	0·62	1 or 3	R	—	—	—	—	N	—	—	—
1529	0·743	1·379	0·62	2·47	> 9·9	0·15	ND	R	—	—	K	—	N	—	—	—
1532	0·239	1·733	0·010	0·04	> 9·9	0·62	ND					ND				
1552	0·638	1·708	0·04	0·62	9·9	0·15	ND	—	K	—	K	—	—	—	N	—
1713	1·232	2·029	0·010	0·010	9·9	0·62	ND	—	K	—	K	—	—	—	N	

ND, Not determined.

considered to be caused by type-specific antibodies, since only cross-reactive antibodies should be inhibited. Both the HCV core and NS4 systems were used for serotyping. If none of the systems gave clear results the sample was considered to be not typable by serotyping EIA.

RESULTS

Reactivities to HCV proteins/peptides and the GOR peptide

Results from testing the 96 human sera for anti-HCV using the Abbott supplemental assay and the HCV $core_{1-28}$ EIA are given in relation to outcome in the GOR EIA in Table 1. As shown, the 29 sera initially reactive in the GOR EIA were all reactive for anti-HCV core in the Abbott Supplemental Assay, and the peptide-based HCV $core_{1-28}$ assay. This shows a significant relationship between anti-GOR and anti-HCV core ($P < 0{\cdot}001$, Fisher's exact test). Of the initial 29 GOR-reactive sera, 24 could be verified by inhibition with the GOR peptide, whereas in all of the 29 sera, the HCV $core_{1-18}$ reactions could be verified by inhibition using the corresponding HCV core peptide.

The relative avidities of each of the initial 29 GOR-reactive sera are given in Table 2 and Fig. 1. As shown in Fig. 1, the GOR reactivity of six of these sera could be inhibited by lower amounts of HCV $core_{1-18}$ peptide than of GOR peptide. This illustrates the higher avidity for the $core_{1-18}$ peptide.

As shown in Table 2, 11 of the 24 GOR reactivities were more efficiently inhibited by the HCV $core_{1-18}$ peptide than the GOR peptide. In two sera the GOR reactions were equally inhibited by the HCV_{1-18} peptide and the GOR peptide, and in 11 sera the GOR peptide was the most efficient inhibitor.

The 29 tested HCV $core_{1-18}$ reactivities could all be inhibited by addition of the corresponding core peptide, whereas only two sera showed inhibition > 50% by addition of the GOR peptide at maximum concentration (Table 2).

Sera which bound to the GOR-coated plates, and with a higher relative avidity for the inhibiting GOR peptide than for the HCV_{1-18} peptide, had significantly lower OD at 405 nm when tested at a 1:100 dilution with the GOR peptide ($P < 0{\cdot}001$; Mann–Whitney rank sum test).

Anti-GOR reactivity in relation to HCV serotype

Using published serotyping systems for antibodies to HCV core and NS4 we were able to serotype the reactivities of most of the sera. It has been suggested that genotypes I and II (or 1a and 1b) correspond to serotype 1, genotypes III and IV (or 2a and 2b), to serotype 2, and genotype V (or 3) to serotype 3 [16–18].

In 33 out of the 54 anti-HCV-positive sera type-specific antibodies could be detected. All three serotypes were represented among our samples, 12 were of type 1, four of type 2, and one of type 3. Antibodies of more than one serotype were detected in 16 sera. No serotype of antibodies to HCV core was found to be significantly correlated to reactivity with the GOR peptide (Fisher's exact test).

Identification of essential residues for antibody binding

Results from testing all GOR-positive sera with the substitution peptide analogues covering residues 9–17 of HCV core are given in Table 2. If a substitution by alanine at a certain position caused the peptide reactivity to decrease by more

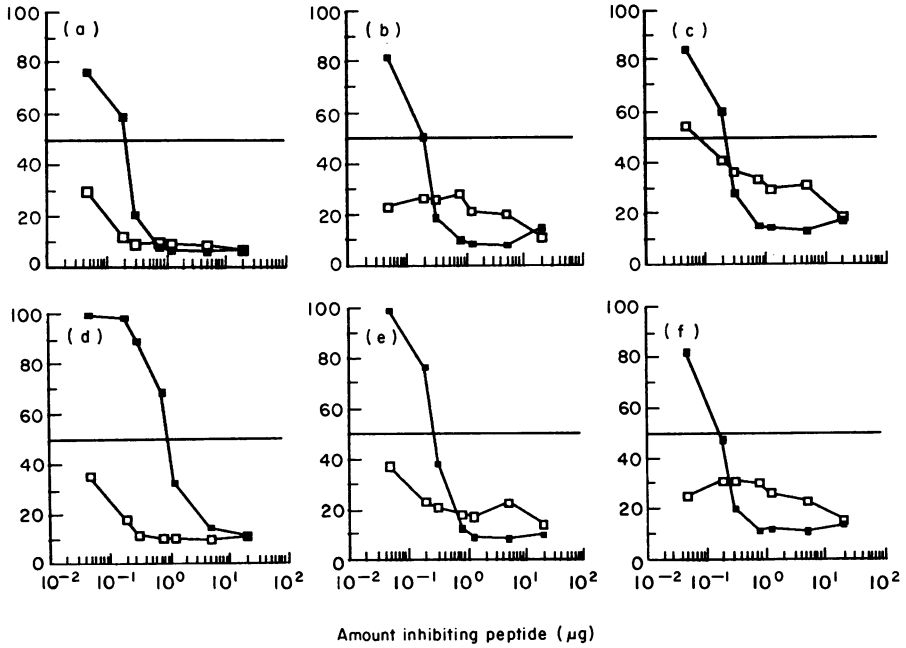

Fig. 1. Results from inhibition of anti-GOR reactivity in six sera (a, 1139; b, 1179; c, 1336; d, 1344; e, 1382; f, 1394) using increasing amounts of either GOR_{19-36} peptide (■) or HCV $core_{1-18}$ peptide (□). Results are given as per cent residual binding, that is the OD at 405 nm of each inhibited reaction divided by the non-inhibited reaction.

Table 3. Analysis by substitution peptide analogues of the importance of the sidechains at residues 10 and 12 of hepatitis C virus (HCV) core protein in the binding of human antibodies

Serum	Amount (nmol) of peptide with indicated substitution giving 50% inhibition							Sidechain criteria at indicated residue	
	K^{10}, K^{12} (original)	A^{10}	R^{10}	M^{10}	A^{12}	R^{12}	M^{12}	10	12
1713	0·20	>6·68	>6·32	>6·42	>6.68	1·39	5·41	K	s,c
1523	0·54	>6·68	4·35	>6·42	>6·68	1·16	4·32	s,c	s,c
1394	0·41	>6·68	>6·32	>6·42	2·81	0·50	0·26	K	s
1320	0·27	>6·68	>6·32	>6·42	4·10	0·93	0·57	K	s
1436	0·65	>6·68	5·27	>6·42	5·19	0·46	1·58	K	s
1336	0·17	>6·68	5·04	>6·42	3·18	0·33	0·71	K	s
1382	0·78	>6·68	4·24	>6·42	5·21	0·33	0·38	K	s
1439	0·06	6·03	1·33	>6·42	1·58	0·05	0·26	K	s
1440	0·20	0·57	0·34	1·69	0·27	0·04	0·05	None	s
1391	0·30	1·41	0·42	0·25	0·57	0·23	0·23	s	None

K, Only Lys allowed; s, size of sidechain important; c, charge of sidechain important.

than 50%, compared with the peptide with the original amino acid sequence, that residue was considered to be essential for antibody binding. The essential residues were most often the Lys^{10} (15/25), the Lys^{12} (14/25), the Asn^{14} (14/25), and the Asn^{16} (17/25; Table 4). All these four residues were almost completely conserved between the different strains of HCV core and the GOR proteins (Table 4).

Further analysis of the importance of residues Lys^{10} and Lys^{12} was performed using peptide analogues where the Lys residues were substituted by amino acids with similar sizes of sidechains and with a similar positive charge (Arg), or with a non-polar side chain (Met). The peptides were assayed both coated to microplates and as inhibitors in solution. The results of both assays were similar. If the amount needed for obtaining 50% inhibition using a substitution peptide analogue was at least five times higher than the concentration needed of the original peptide, the substitution was considered to affect the recognition significantly. As shown in Table 3, the Lys^{10} seems to be more essential than the Lys^{12} in antibody binding since substitutions are rarely allowed. With regard to the Lys^{12}, it was often found that the Arg and Met substitutions were well accepted with retained recognition, suggesting the size of the sidechain as a major factor for antibody binding. For two sera the Lys^{12} could be substituted by Arg, but less well by Met, which would suggest that in these two cases the positive charge of the sidechain is an important factor for antibody binding (Fig. 2).

In Table 4 the sequences of HCV $core_{9-17}$ corresponding to

Table 4. Alignment of the amino acid sequences of hepatitis C virus (HCV) $core_{9-17}$ corresponding to published genotypes and GOR_{19-27}, in relation to positions essential for antibody binding

Region	HCV strain*	HCV type	Sequence								
HCV $core_{9-17}$	HC-J1	I	R	**K**	T	**K**	R	N	T	N	**R**
	HCV-1	I	K	—	N	—	—	—	—	—	—
	HCV-H	I	—	—	—	—	—	—	—	—	—
	HC-J4	II	—	—	—	—	—	—	—	—	—
	HC-J6	III	—	—	—	—	—	—	—	—	—
	HC-J8	IV	—	—	—	—	—	—	—	—	—
	E-b1	V	—	—	—	—	—	—	—	I	—
GOR_{19-27}			Q	**K**	A	**K**	S	N	P	N	**R**
Most essential†			—	K	—	K	—	N	—	N	—
No. of occasions as essential† ($n = 25$)			5	15	2	14	9	14	10	17	0
(%)			(20)	**(60)**	(8)	**(56)**	(36)	**(56)**	(40)	**(68)**	—

* Sequence alignment according to Machida *et al.* [16] and Chan *et al.* [17].

† Residues most often occurring as a residue that cannot be substituted with retained antibody binding to HCV $core_{9-17}$.

Letters written in bold indicate residues conserved between the HCV core and GOR.

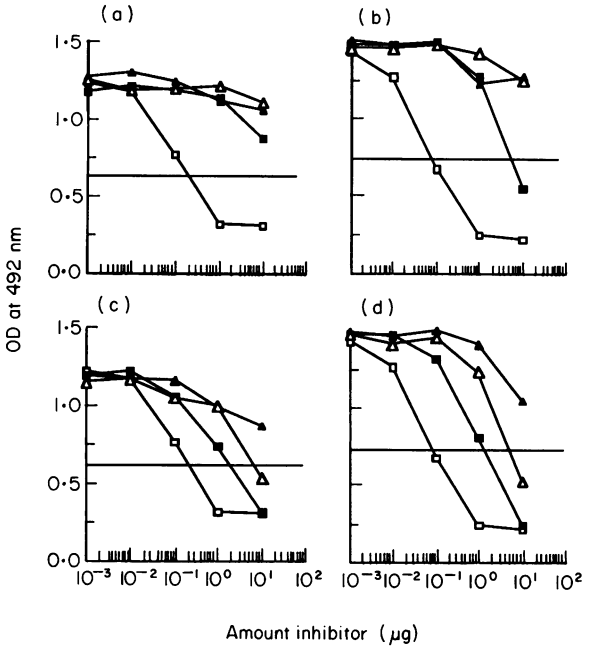

Fig. 2. Results from inhibiting the antibody binding in sera 1713 (a and c) and 1523 (b and d) to peptide HCV core$_{7-19}$ by increasing amounts of the substitution peptide analogues where the Lys^{10} (a and b) and Lys^{12} (c and d) were sequentially substituted by Ala, Arg, and Met. Results are given as the OD at 405 nm. The Lys^{10} and Lys^{12} line indicates the original peptide. The vertical lines indicate IC_{50} cut off. (a, b) □, Lys^{10} and Lys^{12}; ▲, Lys^{10} →Ala; ■, Lys^{10} →Arg; △, Lys^{10} →Met. (c, d) □, Lys^{10} and Lys^{12}; ▲, Lys^{12} →Ala; ■, Lys^{12} →Arg; △, Lys^{12} →Met.

different HCV strains have been given in relation to residues 19–27 of GOR, and in relation to the most essential residues for antibody binding. As shown, four out of the five conserved residues are essential.

DISCUSSION

Yoshizawa *et al.* [20] reported in 1991 that the GOR EIA might be used to screen for anti-HCV among blood donors. Although the inclusion of a GOR peptide might increase the sensitivity of the first C100-3 assay, the sensitivity of the GOR peptide is not satisfactory compared with assays containing the HCV core represented by recombinant proteins or peptides. Here we used the estimation of antibody avidity and a set of substitution peptide analogues to determine the possible origin of antibody reactivity to GOR. In most cases the GOR reactivity could be inhibited by addition of HCV core$_{1-18}$ peptide. In contrast, only two out of 24 anti-HCV core$_{1-18}$ reactions could be inhibited by addition of GOR peptide. This indicates a primarily anti-HCV core$_{1-18}$ response, where most antibodies cross-react with GOR. Further, of the 24 anti-GOR peptide reactions, 11 were more efficiently inhibited by the HCV core$_{1-18}$ peptide than the GOR peptide. Conversely, 13 anti-GOR peptide reactions were equally or more efficiently inhibited by the GOR peptide. This raises the following question: are the 11 sera showing a slightly higher relative avidity for the GOR peptide held on solid-phase specific for GOR? This finding can be attributed to the variation in the human immune recognition of the HCV core site at residues 9–17 [8].

We also analysed the molecular basis for the observed cross-reactivity. In 21 of the initially 29 anti-GOR-reactive sera we were able to determine the essential residues for antibody binding. The essential residues in >50% of all tested sera coincided with the observed residues Lys^{10}, Lys^{12}, Asn^{14}, and Asn^{16}. We found the Lys^{10} to be the most essential residue, since the substitution by Arg holding a sidechain of similar size and charge was not allowed. However, for the Lys^{12} in most cases only the size of the sidechain was crucial, and in two cases both the size and the charge of the sidechain were crucial. Taken together, these findings explain on the single amino acid level the observed cross-reactivity between these two proteins. Even if two sequences hold a high degree of homology, one prerequisite for cross-reactivity is that the residues essential for antibody binding are the ones conserved within the two sequences. Thus, as we show here, the sequence homologies between HCV core and GOR meet this criterion. Also, these four residues are almost completely conserved among known sequences of the HCV core protein, except for the E-b1 type V/3 strain [17]. However, since we found anti-GOR reactivities in all three different serotypes of HCV, this would suggest that anti-GOR reactivity is not related to any serotype of antibodies to HCV, despite the fact that the type 3 E-b1 strain has an Ile instead of an Asn at position 16.

Also, our finding may shed light on the factors affecting the formation of this antigen–antibody complex. In many cases the Lys^{10} could not be substituted by a similar amino acid, indicating that even minor changes in the structure destroy the recognition of the antigenic region. Both lysine and arginine, owing to their amino and guanidino groups, can form salt bridges with COOH groups (aspartic or glutamic acids) if such are present at a correct proximity in the complementarity determining regions of the antibody. In this particular antigenic region all of the residues essential for antibody binding contain polar sidechains, indicating that except for hydrogen bonds, van der Waals forces and hydrophobic interactions, the charge could be one of the important forces in forming and maintaining this antigen–antibody complex.

Taken together, our findings indicate that the observed GOR reactivities ought to be of HCV core$_{9-17}$ origin. This is also supported by the observation that we found only GOR reactivities in anti-HCV-positive samples. With two exceptions [4,21] other studies have only detected GOR reactivities in anti-HCV-positive samples [22,23]. Using inhibition experiments, Hosein *et al.* [23] also reached the conclusion that anti-GOR reactivities are of anti-HCV core origin. The GOR translation product has only been identified in transformed cells [3]. If identification is possible only in transformed cells, then the antibodies to HCV core$_{9-17}$ which cross-react with the GOR protein would have no biological significance. Studies that present data on the role of these antibodies in autoimmune hepatitis are certainly warranted, since this would have important implications for the preferred treatment.

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