

Sequence uniqueness and sequence variability as modulating factors of human anti-HCV humoral immune response

Darja Kanduc · Luciana Tessitore ·
Guglielmo Lucchese · Anthony Kusalik ·
Emanuel Farber · Francesco M. Marincola

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Abstract We recently compared the HCV polyprotein to the human proteome in order to test whether amino acid sequences unique to the virus could represent immunodominant epitopic determinants of the human humoral immune response against HCV. We identified a relatively limited number of HCV fragments with no/low similarity to the human host that represented exclusive HCV motifs. In this study, the peptides corresponding to low/zero similarity sequences were synthesized and assayed with HCV-infected sera. With different patterns, the synthetic HCV peptides corresponding to low/zero similarity sequences were found to be immunoreactive. In particular, the HCV E1 (315–323) HRMAWDMMM, HCV E2/NS1 (547–555) NWFSGTWMN, and HCV NS5 (2638–2646) YDTRC FDST sequences were immunodominant in the HCV-infected cohort under study. These three peptides correspond to sequences that are endowed with low-similarity to

the human proteome, are highly conserved among various HCV strains, and have, potentially, a scarce susceptibility to proteolytic attacks. These data may be of help in defining the multiple factors which concur in the modulation of the human immune response against HCV, eventually providing information for the design of effective anti-HCV vaccines.

Keywords Unique peptide sequences · Conserved peptide sequences · Sequence-to-sequence peptide matching · Proteasomal cleavages · HCV-related immunity

Introduction

Recently we undertook a comparative study of HCV and *Homo sapiens* proteomes to obtain data on peptide sequence (dis)similarity between the HCV polyprotein and human proteins. We found a high degree of amino acid motif sharing between HCV and human proteomes, with only a limited number of viral peptide motifs restricted to HCV with no counterpart in the human proteome [22]. More specifically, 57.6% of the approximately 34,000 human proteins included at the time of the download in the human proteome database at EBI's Integr8 site contain viral HCV pentamers. High level of similarity persists even when the stringency of the search parameters is increased by extending the HCV peptide length to 6- or 7-mers.

We interpret such ample similarity as a possible factor limiting the immune reactions of the host against HCV, partly explaining the host's tolerance of the virus responsible for the chronic infection. Indeed, viral peptide sequences identical to those found in self-proteins should be immunotolerated, since autoantigen-specific lymphocytes are deleted during the establishment of self-identity

D. Kanduc (✉) · F. M. Marincola
Infectious Diseases and Immunogenetics Section,
Department of Transfusion Medicine, Clinical Center,
National Institutes of Health, Bethesda, MD, USA
e-mail: kanducd@cc.nih.gov; d.kanduc@biologia.uniba.it

D. Kanduc · G. Lucchese
Department of Biochemistry and Molecular Biology
"Ernesto Quagliariello", University of Bari, Bari, Italy

L. Tessitore
DISCAFF, University A. Avogadro, Novara, Italy

A. Kusalik
Department of Computer Science,
University of Saskatchewan, Saskatoon, Canada

E. Farber
Department of Pathology,
University of South Carolina, Columbia, SC, USA

[4, 9, 17]. Consequentially, sharing of common motifs might be one cause of limited immunogenicity of HCV. As a corollary, the data also question a possible direct relationship between HCV molecular mimicry to human proteins and human HCV-associated autoimmune diseases. Molecular mimicry predicts that HCV similarity to human proteins should lead to autoimmune phenomena since immune response against the infectious agent may result in formation of cross-reacting antibodies that bind the shared epitopes on the normal cell and result in the auto-destruction of the cell [30]. The molecular mimicry hypothesis implies that HCV infection should be a practically infinite source of autoimmune diseases since HCV 5-, 6-, and 7-mer matches are widely disseminated throughout more than half of the human proteome while actually HCV-associated autoimmune diseases have a much lower incidence than expected [27].

Based on these observations, we reasoned that a host anti-HCV immune response should be predominantly directed toward amino acid motifs unique to the virus, so collimating with the general acceptance of the preferential immunorecognition of non-self sequences. Therefore, we started this study by testing the potential of HCV peptide sequences not shared with the human proteome to raise the humoral immune response in HCV-infected patients. Synthetic peptides corresponding to HCV polyprotein displaying low sequence similarity to the human proteome were immunoassayed against HCV-infected sera. Synthetic peptides with medium/high similarity to the human proteins were used as controls. We report that, among the HCV peptides we tested, those hosting sequences unique to the HCV proteome were predominant targets of immune recognition by HCV-infected sera. Additionally, we found that the immunoreactivity was further restricted to HCV peptide sequences highly conserved among the different HCV strains and hosting a low number of potential proteolytic sites, so indicating a multiple control pathway in the human anti-HCV response.

Materials and methods

Computational analyses

The HCV polyprotein sequence selected for this study corresponds to the hepatitis C virus, genotype 1a, as reported at <http://www.expasy.org>, accession P26664. The polyprotein includes the following proteins: capsid C; envelope glycoprotein E1; envelope glycoprotein E2; p7; protease NS2-3; serine protease/NTPase/helicase NS3; non-structural (NS) 4A; NS4B; NS5A; and RNA-directed RNA polymerase, for a total length of 3,010 amino acids. The human proteome was obtained from EBI's Integr8 site

(<http://www.ebi.ac.uk/integr8>) and consisted of 34,044 nonredundant proteins at the time of download. Hypothetical and/or unidentified protein sequences were included.

Sequence similarity analysis comparing the viral polyprotein sequence to the human proteome was conducted using HCV 5-mers as probes to scan the *Homo sapiens* proteome looking for exact matches. Overlaps between the human proteome and each viral protein were determined as already described [22, 34].

The comparative peptide similarity analysis was conducted by multiple sequence alignment of the following different HCV isolate genotypes. The HCV types are listed here with the Swiss-Prot accession number in parentheses: 1a, isolate 1 (P26664); 1a, isolate H (P27958); 2a, isolate JFH-1 (Q99IB8); 2a, isolate HC-J6 (P26660); 1b, isolate Taiwan (P29846); 1b, isolate BK (P26663); 1b, isolate Con1 (Q9WMX2); 1b, isolate Japanese (P26662); 1b, isolate HC-JT (Q00269); 2b, isolate HC-J8 (P26661); 2b, isolate JPUT971017 (Q9DHD6). Sequence alignment was conducted using the T-Coffee program (see <http://www.expasy.org/tools>) [29]. Proteasomal cleavages (made by human proteasome type III) in HCV-1A were predicted using the PAMProC program [23].

Synthetic peptides and sera

The HCV synthetic peptides used for immunoassays were: aa315–323 HRMAWMMMM; aa440–448 LFYHHKFNS; aa482–491 RPYCWHYPP; aa547–555 NWFQCTWMN; aa741–750 SQAEAALENL; aa786–794 VYTFYGMWP; aa1603–1611 WQMWKCLI; aa1628–1637 GAVQNEI TLT; aa1760–1768 WAKHMWNFI; aa 2050–2058 TCRNMWSGT; aa 2182–2191 AEAAGRRLAR; aa2638–2646 YDTRCFDST. Peptides were synthesized using standard Fmoc (N-(9-fluorenyl) methoxycarbonyl) solid phase peptide synthesis and were obtained from Primm srl, Milan, Italy. Peptide purity (>90%) was controlled by analytical HPLC, and the molecular mass of purified peptides confirmed by fast atomic bombardment mass spectrometry. Peptides were dissolved in 0.9% NaCl, aliquoted and stored at -20°C .

Serum samples from patients with HCV infection were kindly made available by Dr. Luisa Gennero and Prof. Antonio Ponzetto, Dept. of Internal Medicine, University of Turin, Division of Gastroenterology, San Giovanni Battista Hospital, Turin, Italy. HCV studies had been approved by the Hospital Institutional Review Board. We analyzed only sera from patients who had not yet received therapeutical treatments. Control sera from healthy subjects were available in the lab. Blood donors' sera were obtained from the Blood Bank, University Hospital of Bari, following donors' informed and signed consent. Sera were treated with RNase/DNase for 2 h at 37°C , and then partially purified by

precipitation with 40% saturated $(\text{NH}_4)_2\text{SO}_4$ (x2). The precipitate was dissolved in phosphate-buffered saline (PBS), dialyzed against PBS with several changes for 24 h at 4°C, aliquoted and stored at -20°C until assay. For pre-absorption experiments, sera from blood donors were pooled, partially purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation and then incubated with peptide 1 (or 4 or 5 or 9) at a threefold excess (w/w) in PBS -1% BSA for 1 h at room temperature.

ELISA immunoassays

Serum reactivity against synthetic HCV peptides was tested by ELISA assays. Multiwell plates were coated with synthetic HCV peptides by adding to each well 100 μl 0.5% glutaraldehyde cross-linked synthetic peptide solution at a concentration of 25 μM . Following incubation for 2 h at RT, wells were blocked with 5% BSA in PBS, and incubated with human partially purified serum. HPR-conjugated goat anti-human antibodies were used as secondary antibody. Peroxidase activity was detected by adding 3,3',5,5'-tetramethylbenzidine dihydrochloride as substrate. Results are expressed as effective absorbance. The absorbance was measured at wavelengths of 450 and 620 nm. The effective absorbance was calculated subtracting A_{620} from A_{450} .

Results

Identification of HCV-restricted protein sequences and selection of putative epitopes

Similarity analysis identifies amino acid sequences that could represent putative low-similarity epitopes, quantifying the number of perfect peptide matches between the

proteomes of the test organisms, in this case, HCV and human. Basic to this analysis is the definition of the length of the minimal immunogenic peptide determinant, i.e., the length of the shortest sequence that can constitute a linear determinant. According to the literature, 5 to 6 amino acids peptides represent sufficient minimal antigenic determinants [13, 14, 25, 28, 37] and, therefore, a putative epitopic peptide is reasonably defined to have a minimal length of five amino acids. Thus, the similarity level of a peptide sequence to a set of proteins can be calculated as the number of times each peptide pentamer occurs in the analysed set of proteins. The similarity level of a peptide is zero when the 5-mers forming the peptide are absent in the proteins under analysis. The similarity level of a peptide is incrementally higher as the 5-mer sequence is repeatedly represented in the proteome investigated.

In this study, the HCV genotype 1a proteome from a previous report [22] was further analysed for similarity to the human proteome searching for low similarity peptide fragments to be evaluated as antigens in immunoassays with sera from HCV-infected patients. A set of nine 9-mer HCV fragments with no more than 2 matches at the 5-mer level were synthesised and used as antigens. The low-similarity peptide sequences thus identified are described in Table 1. The Table also shows three HCV peptides used as controls because of their medium/high similarity to the human proteome.

Analysis of peptide sequence variability among distinct HCV Types

This study was focused on the HCV polyprotein sequence corresponding to genotype 1a, since (1) genotype 1a is distributed with a relatively high frequency among the HCV

Table 1 Description of the HCV peptides used in this study

Peptide	Aa position	Viral protein	Sequence	Similarity to human proteome (number of 5-mer matches)
Controls				
C1	741–750	E2/p7	SQAEAALENL	289
C2	1628–1637	NS3	GAVQNEITLT	66
C3	2182–2191	NS5A	AEAAGRRLAR	263
Low-similarity				
1	315–323	E1	HRMAWDMMM	1
2	440–448	E2/NS1	LFYHHKFNS	2
3	482–491	E2/NS1	RPYCWHYPP	0
4	547–555	E2/NS1	NWFGCTWMN	1
5	786–794	NS2	VYTFYGMWP	1
6	1603–1611	NS3	WDQMWKCLI	0
7	1760–1768	NS4A	WAKHMWNFI	0
8	2050–2058	NS5	TCRNMWSGT	0
9	2638–2646	NS5	YDTRCFDST	2

infected Italian population [3, 7, 11], and (2) it has been reported that individuals infected with this virus evoke a stronger anti-HCV immune response to several immunodominant epitopes of HCV compared to individuals infected with HCV 1b [5]. On the other hand, it has been well demonstrated that HCV exhibits complex genetic variability that can be classified into four hierarchical strata: genotypes, sub-genotypes, isolates, and quasi-species [39, 40]. Moreover, infected patients may present multiple HCV genotype infections or experience changing patterns of HCV sequence variability over time [33].

Thus, amino acid sequence variability of various HCV types might alter peptide immunoreactivity. To comprehensively define the relationship between HCV sequence variability, its similarity to the human proteome and peptide immunoreactivity, the peptide sequences selected for this study were compared by sequence alignment to those of various HCV types and evaluated for homology level. Table 2 shows that peptides 2, 5 and 8 are highly variable sequences across the HCV type sequences studied while the other peptide sequences tend to be conserved.

Table 2 Peptide sequence and similarity level changes among different HCV isolate genotypes

HCV type	Peptide 1		Peptide 2		Peptide 3	
1a	HRMAWDMMMN	(1)	LFYHHKFNS	(2)	RPYCWHYPP	(0)
1a (H)	HRMAWNMMMN	(1)	LFY Q HKFNS	(16)	RPYCWHYPP	(0)
2a (JFH-1)	HRMAWDMMMN	(1)	LFY TNR FNS	(16)	RPYCWHYPP	(0)
2a (HC-J6)	HRMAWDMMMN	(1)	LFY T HSFNS	(14)	RPYCWHYPP	(0)
1b (Taiwan)	HRMAWDMMMN	(1)	LFYA HR FNA	(6)	RPYCWHY A P	(2)
1b (BK)	HRMAWDMMMN	(1)	LFY T HSFNS	(14)	RPYCWHYPP	(0)
1b (Con1)	HRMAWDMMMN	(1)	LFY V HKFNS	(10)	RPYCWHY A P	(2)
1b (Japanese)	HRMAWDMMMN	(1)	LFYA HR FNA	(6)	RPYCWHY A P	(2)
1b (HC-JT)	HRMAWDMMMN	(1)	LFYA H KFNS	(12)	RPYCWHY A P	(2)
2b (HC-J8)	HRMAWDMMLS	(1)	LFY T HKFNS	(14)	RPYCWHYPP	(0)
2b (JPUT971017)	Q RMWDMML N	(2)	LFYA N KFNS	(10)	RPYCWHYPP	(0)
HCV type	Peptide 4		Peptide 5		Peptide 6	
1a	NWFGCTWMN	(1)	VYTFYGMWP	(1)	WDQMWKCLI	(0)
1a (H)	NWFGCTWMN	(1)	VY A LYGMWP	(18)	WDQ M RKCLI	(15)
2a (JFH-1)	SWFGCTWMN	(2)	TY CL T GLWP	(25)	WD A MWKCLA	(6)
2a (HC-J6)	SWFGCTWMN	(2)	TYS L T GL W S	(79)	WD V MWKCL T	(0)
1b (Taiwan)	NWFGCTWMN	(1)	A Y A LYG V WP	(19)	WDQMWKCL T	(0)
1b (BK)	NWFGCTWMN	(1)	T Y A LYG V WP	(20)	WDQMWKCLI	(0)
1b (Con1)	NWFGCTWMN	(1)	A Y A LYG V WP	(19)	WDQMWKCLI	(0)
1b (Japanese)	NWFGCTWMN	(1)	A Y A LYG V WP	(19)	WDQMWKCLI	(0)
1b (HC-JT)	NWFGCTWMN	(1)	A Y A LYG V WP	(19)	WDQMWKCLI	(0)
2b (HC-J8)	A WFGCTWMN	(1)	TYS V L GL W S	(83)	WD V MWKCL T	(0)
2b (JPUT971017)	A WFGCTWMN	(1)	TYS V L GL W S	(83)	WD V MWKCL T	(0)
HCV type	Peptide 7		Peptide 8		Peptide 9	
1a	WAKHMWNFI	(0)	TCRNMWSGT	(0)	YDTRCFDST	(2)
1a (H)	WAKHMWNFI	(0)	TC K NMWSGT	(0)	YDTRCFDST	(2)
2a (JFH-1)	WA R HMWNFI	(0)	TC M N T W Q GT	(6)	YDTRCFDST	(2)
2a (HC-J6)	WAKHMWNFI	(0)	TC M N I W Q GT	(1)	YDTRCFDST	(2)
1b (Taiwan)	WA N DMWNFI	(0)	TC S N T W H GT	(6)	YDTRCFDST	(2)
1b (BK)	WAKHMWNFI	(0)	TC S N T W H GT	(6)	YDTRCFDST	(2)
1b (Con1)	WAKHMWNFI	(0)	TC S N T W H GT	(6)	YDTRCFDST	(2)
1b (Japanese)	WAKHMWNFI	(0)	TC S N T W H GT	(6)	YDTRCFDST	(2)
1b (HC-JT)	WAKHMWNFI	(0)	TC S N T W H GT	(6)	YDTRCFDST	(2)
2b (HC-J8)	WAKHMWNFI	(0)	TC L N L W Q GT	(12)	YDTRCFDST	(2)
2b (JPUT971017)	WA R HMWNFI	(0)	TC L N M W Q GT	(1)	YDTRCFDST	(2)

Accession numbers of HCV genotypes are given under “Materials and methods”. The peptide sequences numbered from 1 to 9 are aligned with the corresponding peptide portions present in the different HCV genotypes. Sequences are reported with changed amino acid bold underlined. Peptide similarity level (as total number of pentamer matches between the peptide and human proteome) in parentheses

Table 3 Predicted proteolytic pattern of low-similarity HCV peptides

Peptide	Aa Position	Viral protein	Sequence ^a	No. of Proteolytic sites
1	315–323	E1	↓HR↓MAWDMMM	2
2	440–448	E2/NS1	↓LF↓Y↓HHK↓F↓N↓S	6
3	482–491	E2/NS1	↓RKYCWHYPP	1
4	547–555	E2/NS1	↓N↓WF↓GCTWMN	3
5	786–795	NS2	VYTFY↓G↓M↓W↓P	4
6	1603–1611	NS3	↓WD↓Q↓MWK↓C↓LI	5
7	1760–1768	NS4A	↓WA↓K↓H↓M↓WN↓FI	6
8	2050–2058	NS5	T↓CRN↓MWS↓GT	3
9	2638–2646	NS5	↓Y↓DTRC↓FDST	3

^a The proteolytic sites are indicated by arrows

Proteolytic pattern of low-similarity HCV peptide sequences

The selected low-similarity HCV fragments described in Table 1 were further analyzed for proteolytic sites as a possible additional factor affecting peptide epitopicity. Indeed, it has been reported that mutations within HCV epitopes also cause their destruction by changing the pattern of proteasome digestion [21]. The importance of intracellular antigen processing in the definition of antigenic determinants has been established [38], and cleavage at a single processing site can be crucial for effective antigen presentation [24]. Therefore, to reach a better comprehensive understanding of the molecular basis of HCV antigenicity, the predicted proteolytic pattern of the selected HCV motifs was analyzed. The distribution of potential proteolytic sites in the low-similarity sequences is illustrated in Table 3. It can be seen that sequences 1, 3, 4, 8 and 9 appear to host a low number of potential proteasomal cleavage sites (≤ 3), suggesting a potential higher stability of the corresponding peptides.

HCV peptide immunoreactivity assay

Peptides corresponding to the HCV sequences motifs described above were synthesized, and tested as potential antigenic epitopes in ELISA immunoassays using sera from HCV-infected and healthy subjects. The immunoreactivity pattern of the HCV peptides monitored by using sera from five healthy controls, 10 blood donors, and 29 HCV patients, is detailed in Table 4. It can be seen that the majority of the HCV low-similarity peptides showed a clear response, although to different extents. For example, it is evident from Table 4 that peptide 1 (i.e., peptide E1_{315–323} HRMAWDMMM) is immunodominant. This epitope is a conserved sequence among the HCV strains (see Table 2) and hosts a low number of potential proteolytic sites (see Table 3). Analogously, amino acid sequence conservation as well as scarce susceptibility to proteolytic fragmentation,

i.e., a low number of proteolytic sites, are shown by the peptides 4 and 9 which are frequently recognized by the HCV-infected sera (Table 4). One exception is peptide no. 3, corresponding to the low-similarity HCV_{482–491} RPYCWHYPP sequence, which was unreactive with almost all of the sera tested, even if endowed with a high level of sequence conservativeness and low potential to proteolytic degradation. In this context it may be relevant that HCV_{482–491}RPYCWHYPP is located in the viral CD81-binding domain spanning from aa 481 to aa 493 [10], suggesting that the low similarity HCV_{482–491}RPYCWHYPP peptide might potentially represent a cryptic epitope.

Moreover, positive reactions are observed using sera from healthy subjects and blood donors too. This immunoreactivity has repeatedly been reported in the medical literature and remains unexplained. Among the many possible causes, cross-reactivity between HCV and Influenza A virus [44] as well as the quality of life in volunteer blood donors [42] have been suggested. In this study, the signals produced by peptides 1, 4, 5 and 9 (see Table 4) could be specifically blocked by pAb preabsorption with the relevant peptide (Fig. 1), so indicating specific detection. ELISA data from Table 4 and Fig. 1 are confirmed by immuno-dot-blot assays (not shown).

Discussion

The Public Health implications of HCV infection are severe. Chronic hepatitis C, hepatic fibrosis, cirrhosis and hepatocellular carcinoma may sequentially characterize HCV progression [1, 12, 16]. Antiviral treatment of chronic hepatitis C has evolved from interferon monotherapy to a combined regimen (i.e., interferon plus ribavirin) with a concomitant improvement in the clinical management of the viral infection [6, 46]. However, adverse effects remain severe [2, 32] and, most importantly, no therapy has been shown to determine permanent viral eradication [20, 35]. Consequently, great efforts are directed towards therapeutic

Table 4 HCV peptide antigenic pattern in HCV infected patients

Serum	HCV peptide			1	2	3	4	5	6	7	8	9
	C1	C2	C3									
None	-	-	-	0.27	-	-	0.35	0.39	-	-	-	0.21
Healthy												
1	-	0.23	-	0.52	-	-	0.33	0.29	-	-	-	0.49
2	-	-	-	0.39	-	-	0.24	0.30	-	0.27	0.33	0.28
3	-	0.23	-	0.48	-	-	0.73	0.45	0.22	0.21	0.24	0.66
4	0.22	-	-	0.25	-	-	0.44	0.28	-	-	-	0.94
5	-	-	-	0.30	-	-	0.53	0.30	-	-	-	0.62
Blood donor												
1	-	0.22	-	0.36	-	-	0.34	-	-	-	-	0.33
2	-	0.27	0.24	0.38	-	-	0.42	0.23	-	-	-	-
3	-	-	-	0.37	-	-	0.69	0.50	0.27	-	-	0.48
4	-	-	-	0.35	-	-	0.42	-	-	-	-	0.64
5	-	-	-	0.21	-	-	0.49	0.47	-	-	-	0.71
6	-	-	-	0.48	-	-	0.22	0.39	-	-	-	0.48
7	-	-	-	-	-	-	0.53	0.27	0.22	0.22	0.31	0.33
8	-	-	-	-	-	-	0.88	0.38	0.44	-	-	0.73
9	0.21	-	-	-	-	-	0.39	0.50	-	-	0.27	0.59
10	0.24	-	0.33	0.23	-	-	0.41	0.36	-	-	-	0.65
HCV infected												
1	-	0.29	0.28	0.91	-	-	0.40	0.38	0.23	0.28	-	0.44
2	0.31	-	-	0.59	0.23	-	-	-	0.40	0.49	0.58	0.21
3	-	-	-	1.06	-	-	0.33	0.27	-	-	-	0.60
4	-	-	-	0.93	0.30	-	0.58	0.50	-	-	-	0.66
5	-	-	-	0.88	0.21	-	0.31	0.61	-	-	-	0.70
6	-	-	-	0.54	0.30	-	0.24	0.29	0.30	0.65	0.71	0.55
7	-	0.21	-	1.17	0.33	-	0.27	0.45	-	-	-	0.82
8	-	-	0.33	1.31	-	-	0.60	0.23	-	-	0.67	0.36
9	-	-	-	-	-	-	-	0.21	0.22	-	0.33	0.46
10	-	-	-	0.38	-	-	0.33	0.48	0.39	-	0.39	0.41
11	-	-	-	0.34	0.29	-	0.41	-	-	-	0.44	0.42
12	-	-	-	1.16	-	-	0.45	0.46	-	0.89	0.82	0.48
13	-	-	-	0.47	-	-	-	0.29	-	-	-	-
14	-	0.33	-	-	-	-	0.27	0.46	-	-	0.28	0.35
15	-	-	-	0.28	-	-	0.33	0.51	0.27	-	-	0.30
16	0.22	-	-	1.28	0.40	-	-	0.90	0.46	0.60	0.83	0.34
17	0.26	-	-	0.78	-	-	0.30	0.48	0.47	-	-	0.79
18	-	-	-	1.19	0.48	-	0.48	-	-	0.58	0.23	0.34
19	-	0.27	-	0.83	0.27	-	0.38	-	0.33	0.73	0.85	0.53
20	-	-	0.23	0.42	0.50	-	0.77	0.43	-	-	-	0.48
21	-	-	-	0.46	-	-	0.38	0.33	0.21	-	0.49	0.58
22	-	-	-	0.31	-	-	0.44	-	-	-	0.58	0.77
23	0.23	-	-	1.22	-	-	0.45	0.60	-	0.96	0.71	0.80
24	-	-	-	0.38	-	-	0.24	0.23	-	-	-	0.31
25	0.22	-	-	0.43	-	-	-	0.39	-	-	0.26	-
26	-	-	-	0.26	-	-	0.50	0.44	0.31	-	-	0.36
27	-	-	-	1.38	0.38	0.23	-	0.58	0.50	0.55	0.83	0.71
28	-	-	-	0.91	-	-	0.48	0.56	0.46	-	-	0.88
29	-	-	0.28	1.25	0.39	-	0.52	-	-	0.55	-	-

Peptide sequence and similarity level to human proteome are detailed under Table 1. Results are expressed as effective absorbance. The absorbance was measured at wavelengths of 450 and 620 nm. The effective absorbance was calculated subtracting A_{620} from A_{450} . Samples were considered to be negative (-) when effective absorbance was ≤ 0.20 . Data are average values of two independent assays

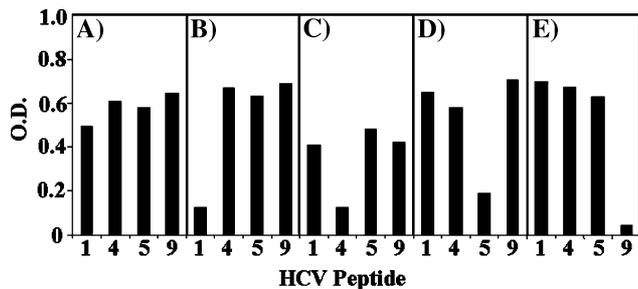


Fig. 1 Recognition of HCV peptides with low-similarity to the human proteome by pAbs from blood donors. Sera from 10 blood donors were pooled and pAbs were immunoassayed by ELISA (see under “Materials and methods”). HCV peptides were assayed with pAbs **a** untreated, **b** pre-treated with peptide n. 1, **c** pre-treated with peptide n. 4, **d** pre-treated with peptide n. 5, **e** pre-treated with peptide n. 9. Peptide numbering is described under Table 1. Results are expressed as optical density (O.D.) and correspond to effective absorbance. The absorbance was measured at wavelengths of 450 and 620 nm. The effective absorbance was calculated subtracting A_{620} from A_{450} . Data are average values of two independent assays

vaccine approaches by targeting the HCV proteins that play key roles in favoring the continuous presence of the virus in the hepatocytes. One example is the HCV core protein which is characterized by anti-apoptotic activity [19, 26, 41] that can protect the infected cell from immune-mediated destruction. However, the development of an efficient HCV vaccination strategy faces as a main obstacle the high mutational rate of HCV-RNA [39]: the rate of nucleotide variant appearance is high (approximately 10^{-3} substitutions per site per year), with a large share of HCV sequence variation concentrated within two hypervariable regions, HVR1 and HVR2, located at the N-terminus of the E2 envelope glycoprotein [15]. This explains the many different viral types and subtypes, the resulting heterogeneity of viral antigen proteins and, possibly, the lack of an effective immune response in HCV infection.

As a matter of fact, we still do not understand the molecular mechanisms of antibody-mediated neutralization and its impact for HCV pathogenesis. Indeed, the acute phase of HCV infection is often subclinical, and 70% of infected individuals develop a chronic infection. This suggests that an anti-HCV immune response is weak or absent. In particular, the role of humoral immune response is unclear. Previous experiments showed that serum from a chronically infected patient could neutralize HCV infectivity in a chimpanzee model, so suggesting the existence of specific anti-HCV antibodies [8]. However this report has remained an *isolated datum*. In general, whereas neutralizing antibodies directed against HCV are present in Igs made from anti-HCV-positive plasma, nonetheless these HCV-specific Igs are largely ineffective *in vivo*. The mechanism for the poor effectiveness is currently unknown. Of interest is the report of a close association between sequence diversity in the

hypervariable region 1 and the appearance of HCV specific antibodies in the sera of subjects with acute infection [18]. This association together with the analysis of the rate of hypervariable region evolution suggest that variation is a function of the immune pressure exerted by the Ab response [43, 45]. Finally, recently it has been proposed that enrichment of neutralization epitope-specific antibodies might permit a better definition of neutralizing antibodies in immune prophylaxis of HCV infection [47].

In this context, the present study offers support to possible anti-HCV immunotherapeutical approaches. Indeed, the similarity profile of HCV proteins to the human proteome may allow the definition of a HCV peptidome platform theoretically able to evoke humoral immune response without concomitant harmful cross-reactions. Moreover, utilizing highly conserved sequences among HCV-specific motifs would offer the potential therapeutic advantage of a potentially broader vaccine effectiveness. In particular, the highly conserved HCV E1_{315–323}HRMAWDMMM sequence interestingly displays an immunodominant role among HCV-infected sera confirming previous reports about its central role in modulating anti-HCV humoral responses [31, 34, 36]. As an additional notation, screening for proteasomal cleavages would provide peptidome sets endowed with the necessary stability so further increasing the potential vaccine efficacy.

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