Journal of Hepatology 1996; **25**: 1–9
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Munksgaard · Copenhagen

Journal of Hepatology
ISSN 0168-8278

Production of interleukin-2 in response to synthetic peptides from hepatitis C virus E1 protein in patients with chronic hepatitis C: relationship with the response to interferon treatment

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Background/Aims: **The role of cellular immunity in the clearance of hepatitis C virus after interferon therapy has not yet been elucidated. Here, we analyzed the T cell response to peptides from hepatitis C virus E1 protein in untreated and interferon-treated patients with chronic hepatitis C virus infection.**

Methods: **We used thirty-six 15-mer synthetic peptides from hepatitis C virus E1 protein (genotype 1a) in a sensitive interleukin-2 production assay in two groups of controls (healthy seronegative individuals and patients with liver diseases unrelated to hepatitis C virus), and three groups of patients with chronic hepatitis C: nine patients who cleared the virus after interferon treatment (group 1), nine patients who failed to respond to the therapy (group 2) and nine previously untreated patients (group 3).**

Results: **None of the controls responded to any of the peptides tested, whereas 8/9 (88%) of patients from group 1 responded positively. In contrast, only 2/9 (22%) of patients from group 2 showed peptide recognition. In group 3, 5/9 patients (55%) displayed positive response against E1 peptides. When E1 peptides from the sequence corresponding to genotype 1b (the commonest in patients who were non-responders to interferon) were tested in nine additional interferon-resistant patients (group 2*) a positive response was detected in only three of them (33%).**

Conclusions: **T cell recognition of hepatitis C virus E1 peptides in patients with chronic hepatitis C who exhibit sustained response to interferon therapy is increased as compared with interferon-resistant cases, suggesting that T cell immunity to hepatitis C virus structural proteins may play a role in the clearance of this viral infection.**

Key words: **Alpha-interferon; Cellular immune response; Hepatitis C virus; Interleukin-2; Synthetic peptides.**

Hepatitis C virus (HCV), the main causative agent of non-A, non-B hepatitis (1), has a strong tendency to develop chronic infection. Chronic hepatitis C (CHC) slowly evolves to cirrhosis and there is a risk of progression to hepatocellular carcinoma (2). Although interferon (IFN) is the only accepted treatment for CHC, only around 20% of treated cases exhibit a sustained response to this therapy (3). The mechanisms underlying the tendency to chronicity of HCV infection and its resistance to IFN therapy are poorly understood.

HCV infection induces the production of antibodies against structural and non-structural proteins of the virus, but it seems that humoral immunity occurring during the natural course of the disease provides little protection (4). Moreover, it has been shown that reinfection and new episodes of acute hepatitis occur in the presence of antibodies (5,6). Although cellular immunity is an essential mechanism in the defense against viral infections, the role of the T cell response in the control of HCV infection remains obscure (7–9). In order to investigate the role of cellular immunity in the clearance of HCV induced by IFN, we have selected three groups of patients: i) untreated

Received 28 March; revised and accepted 21 November 1995

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patients with chronic hepatitis C, ii) patients who cleared the virus after IFN therapy and iii) patients who did not respond to the treatment. In all these cases we have used a sensitive assay to determine the production of interleukin-2 (IL-2) by peripheral blood mononuclear cells in response to a panel of 36 overlapping synthetic peptides from HCV E1 envelope glycoprotein. This envelope protein was chosen because it has been recently reported that immunization of chimpanzees with this protein (together with E2, the other envelope protein from HCV) has proved successful in protecting the animals against the challenge with HCV (10).

Our results show that while healthy individuals do not exhibit T cell responses against E1 peptides, positive responses are found among patients with CHC. Of interest, most of the patients who clear HCV after IFN therapy manifest T cell reactivity against peptides from E1, while the majority of IFN-resistant patients failed to respond to these antigens. Our data suggest that HCV clearance after IFN treatment is associated with the development of T cell immunity against viral structural proteins.

Materials and Methods

Patients and controls

We studied 36 patients with chronic hepatitis C and two control groups consisting of six HCV seronegative healthy donors (three males and three females; age 26 to 33 years, mean age 28) and eight patients with liver diseases unrelated to HCV (three chronic hepatitis B, two primary biliary cirrhosis, two patients with alcohol-related liver disease and one non-A, non-B, non-C hepatitis; five males and three females; age 37 to 65, mean age 49). All chronic hepatitis C patients were anti-HCV positive and showed liver biopsy changes indicative of chronic hepatitis (cirrhosis was present in four cases). Patients with chronic hepatitis C were subdivided into the following groups: *Group 1*. Nine patients (eight males and one female, age 23 to 50 years, mean age 31) who exhibited a sustained response to the treatment with alpha-IFN (3 MU daily for 2 months and 3 MU 3 times a week for 8 to 10 months); all of them had normal transaminase (ALT) levels and were HCV-RNA negative in serum by polymerase chain reaction (PCR) at least 1 year after treatment. *Group 2*. Nine IFN-resistant patients (six males and three females, age 34 to 66 years, mean age 54); these patients remained HCV-RNA positive in serum and showed high ALT values at the end of IFN treatment. *Group 3*. Nine patients (five males and four females, age 26 to 59 years, mean age 46) with CHC who did not receive previous antiviral treatment. All patients from these 3 groups were tested using peptides derived from the HCV sequence corresponding to genotype 1a. Since the most common genotype encountered in patients manifesting IFN resistance is genotype 1b, an additional group of nine patients, *Group 2** (7 males and 2 females, age 27 to 65 years, mean age 42) who did not respond to IFN therapy were tested using peptides derived from the 1b sequence. All the patients who received treatment were studied at the end of the therapy (4 to 24 months after stopping IFN).

Peptide synthesis

Peptides were synthesized by the solid-phase method of Merrifield (11) using the Fmoc alternative (12). Synthesis was carried out using a manual multiple solid-phase peptide synthesizer (13) and the ninhydrin test of Kaiser was used to monitor every step (14). Couplings were repeated if necessary until a negative ninhydrin test was attained. At the end of the synthesis, peptides were cleaved, deprotected and washed six times with diethyl ether. Peptide purity was above 70% as assessed by HPLC, and peptides were used without further purification.

IL-2 production assay

Peripheral blood mononuclear cells (PBMC) were isolated from fresh heparinized blood by Ficoll-Hypaque centrifugation. Cells were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated human pooled AB serum, 100 U/ml penicillin and 100 µg/ml streptomycin, plated at 2×10^5 cells/well and cultured in triplicate in 96-well flat-bottom plates with 10 µg/ml of BT563 anti-IL-2 receptor mAb (Biotest Pharma, Germany) in the presence or absence of synthetic peptides (25 µg/ml). Mitogen stimulation of cells was carried out by incubation with phytohaemagglutinin (PHA), 1/200 final dilution (Gibco). After 7 days of culture, supernatants were harvested and stored at –20°C IL-2 content was assessed by examining the ability of the supernatant to support the growth of an IL-2-dependent CTLL mouse cell line.

CTLL cells were resuspended in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 2-mercaptoethanol (5×10^{-5} M), and were plated (8×10^3 cells/well) in a 96-well flat-bottom plate with the supernatant to be assayed (25% v/v). Following 24 h of culture, cells

TABLE 1
Synthetic peptides from HCV E1

Peptide number	Sequence 1a	Sequence 1b
1	YQVRNSTGLYHVTND	YEVRNVSGLIHVTND
6	STGLYHVTNDCPNSS	VSGLIHVTNDCSNSS
11	HVTNDCPNSSIVYEA	HVTNDCSNSSIVYEA
16	CPNSSIVYEAHDAIL	CSNSSIVYETADMIM
21	IVYEAHDAILHTPGC	IVYETADMIMHTPGC
26	HDAILHTPGCVPCVR	ADMIMHTPGCVPCVR
31	HTPGCVPCVREGNVS	HTPGCVPCVREGNSS
36	VPCVREGNVSRCWVA	VPCVREGNSSRCWVA
41	EGNVSRCWVAMTPTV	EGNSSRCWVALTPTL
46	RCWVAMTPTVATRDG	RCWVALTPTLAAKDA
51	MTPTVATRDGKLPAT	LTPTLAAKDASIPTA
56	ATRDGKLPATQLRRH	AAKDASIPTATIRRH
61	KLPATQLRRHIDLLV	SIPTATIRRHVDLLV
66	QLRRHIDLLVGSATL	TIRRHVDLLVGAAAF
71	IDLLVGSATLCSALY	VDLLVGAAAFCSAMY
76	GSATLCSALYVGDLC	GAAAFCSAMYVGDLC
81	CSALYVGDLCGSVFL	CSAMYVGDLCGSVFL
86	VGDLCGSVFLIGQLF	VGDLCGSVFLVSQLF
91	GSVFLIGQLFTFSPR	GSVFLVSQLFTFSPR
96	IGQLFTFSPRRHWTT	VSQLFTFSPRRHQTV
101	TFSPRRHWTTQGCNC	TFSPRRHQTVQDCNC
106	RHWTTQGCNCSIYPG	RHQTVQDCNCSIYPG
111	QGCNCSIYPGHITGH	QDCNCSIYPGHVSGH
116	SIYPGHITGHRMAWD	SIYPGHVSGHRMAWD
121	HITGHRMAWDMMMNW	HVSGHRMAWDMMMNW
126	RMAWDMMMNWSPTAA	RMAWDMMMNWSPTAA
131	MMMNWSPTAALVMAQ	MMMNWSPTAALVVSQ
136	SPTAALVMAQLLRIP	SPTAALVVSQLLRIP
141	LVMAQLLRIPQAILD	LVVSQLLRIPQAVVD
146	LLRIPQAILDMIAGA	LLRIPQAVVDMVAGA
151	QAILDMIAGAHWGVL	QAVVDMVAGAHWGVL
156	MIAGAHWGVLAGIAY	MVAGAHWGVLAGLAY
161	HWGVLAGIAYFSMVG	HWGVLAGLAYYSMVG
166	AGIAYFSMVGNWAKV	AGLAYYSMVGNWAKV
171	FSMVGNWAKVLVVLL	YSMVGNWAKVLVVML
176	NWAKVLVVLLLFAGV	NWAKVLVVMLLFAGV

Peptide sequences correspond to HC-J1, described by Okamoto et al. (18), classified as genotype 1a and to a viral isolate classified as 1b (David Parker, Wellcome Laboratories, personal communication). Peptides contain an extra Ala at the C-terminus (not shown) that was added for synthesis convenience.

were pulsed with 1 μCi/well of [^{3}H]thymidine for 18 h and thymidine incorporation was determined. The results are expressed as stimulation index (SI), cpm incorporated in response to a peptide divided by the cpm incorporated in the absence of peptide. The response against a peptide was considered positive when SI >3.

Determination of HCV-RNA

This was performed using the polymerase chain reaction (PCR) as previously described (15).

Identification of HCV genotypes

Genotyping was carried out using a hybridization technique with specific probes for HCV genotypes 1a, 1b, 2a, 2b and 3a, according to Simmonds et al. (16), and the amplified HCV core region by nested PCR. The same probes and primers described by Viazov et al. (17) were used, with the following technical modifications: the primers for the second PCR were 5′ labelled with digoxigenine, and hybridization was detected using an anti-digoxigenine peroxidase-labelled antibody (Boehringer Mannhein).

HLA-DR typing

HLA-DR determination was done by using the Standard INNO-LIPA HLA DR kit (Innogenetics, Belgium). Assays were performed according to the manufacturer's instructions.

Statistical analysis

Differences between groups were analyzed by Fisher's exact test. Relative risk associated to some HLA-DR was estimated using the odds-ratio (obtained with SPSS for Windows).

Results

To analyze the reactivity of T cells against antigenic determinants from the HCV envelope protein E1, we used 36 15-mer overlapping peptides (10 amino acids overlap) encompassing the entire sequence of this glycoprotein to stimulate PBMC from controls (healthy individuals and patients with non-HCV-related liver disease), from patients with chronic HCV infection and from patients who cleared the virus after IFN treatment. The peptide sequence, shown in Table 1, corresponds to HC-J1 (18), genotype 1a.

Each peptide from Table 1 was tested in PBMC from patients with HCV infection and controls by measuring IL-2 production as described in Methods. None of the peptides gave a positive response in any of the individuals belonging to the control groups (HCV seronegative healthy donors and patients with liver disease unrelated to HCV) (data not shown). The recognition of peptides by the different groups of patients with chronic hepatitis C was as follows:

As shown in Fig. 1, 8/9 (88%) of patients from group 1 (those having cleared HCV following treatment with IFN) responded to peptides from E1. The number of peptides recognized by each of the react-

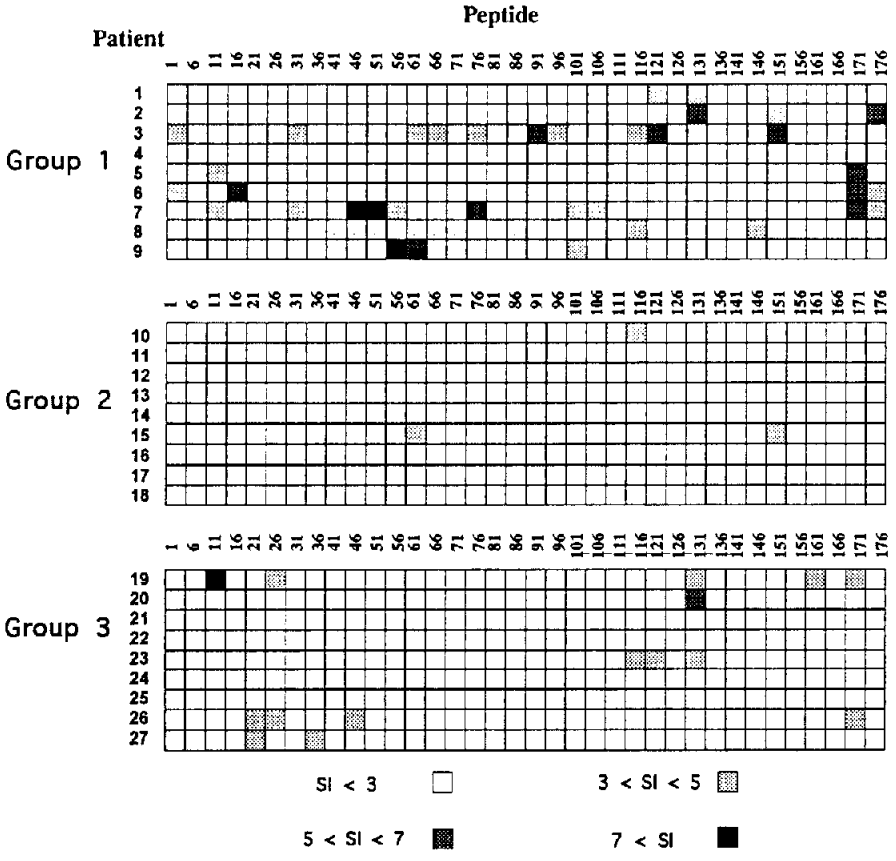

Fig. 1. IL-2 production in different groups of HCV patients against HCV E1 synthetic peptides (sequence 1a). Peptide sequences correspond to those shown in Table 1. Group 1 to 3 are as follows: (1) Patients having cleared HCV following IFN treatment. (2) Patients who at the end of IFN treatment still had high ALT levels. (3) Patients not having been subjected to IFN treatment. Data are represented as stimulation index, SI (cpm with peptide/cpm without peptide) as indicated.

ing patients varied between 2 and 10 (mean 4.5). There was a great variability between the different individuals of this group with respect to the identity of the specific peptides which were recognized as well as to the values of the stimulation index (SI). Peptides inducing positive responses were distributed all along the E1 sequence. Of the 36 peptides tested 21 (58%) elicited a positive response in at least one of the patients from this group. The most commonly recognized peptides were 171 and 176 which were both detected in three out of nine of the patients studied. The mean SI for positively recognized peptides was 4.9, ranging from 3 to 8.

Only 2/9 (22%) of the patients from group 2 (IFN resistant cases) exhibited a positive response to some of the peptides, a proportion which is significantly lower than in group 1 ($p<0.01$). The number of peptides recognized by patients from group 2 was also lower than in group 1 ($p<0.01$). Only 3 peptides elicited T cell response in patients from group 2: one of the subjects recognized 1 peptide and the other patient 2 peptides. The SI for these peptides was very low, just above the cut-off value of 3.

Five out of nine (55%) of the patients from group 3 (untreated patients with CHC) recognized peptides from E1. The number of peptides eliciting a positive T cell response varied between 1 and 5 per patient (mean 3). Ten of the 36 peptides tested (27%) were recognized by at least one of the patients from this group. The mean SI for recognized peptides was 4.4. Fig. 1 shows the specific peptides eliciting positive responses in group 3: it can be seen that peptide 131 was the most commonly recognized peptide (3 out of 9 patients) in this group. Six of the patients from group 3 were then subjected to IFN therapy (patients #19, 21, 22, 23, 26, 27). Two of them (#21 and 22) tested negatively for all the peptides, while four (#19, 23, 26, 27) showed a positive response to some E1 peptides. Interestingly, the former two failed to respond to IFN, while patients #19, 26 and 27 exhibited a positive response to the therapy.

We determined the genotype of HCV in untreated patients from the sera collected during the year before the assay and in those patients who completed IFN therapy from the sera collected during the year before treatment. As shown in Table 2, most patients who did not respond to IFN were infected with HCV genotype 1b. In contrast, in the group of patients who cleared the virus following IFN treatment, the genotypes were more evenly distributed. Thus in group 1, from a total of nine patients, two, three, three and one were found to be infected with genotypes 1a, 1b, 3

TABLE 2

Genotyping of HCV in patients with CHC

Group 1		Group 2		Group 3		Group 2*	
Patient[a]	Genotype	Patient[a]	Genotype	Patient[a]	Genotype	Patient[a]	Genotype
1	1a	10	1a	19	3	28	1b
2	1b	11	1a	20	1b	29	1b
3	3	12	1b	21	1b	30	1b
4	1b	13	1b	22	1a	31	1b
5	3	14	1b	23	1b	32	1b
6	1b/3	15	1b	24	ND	33	3
7	1a	16	1b	25	1b	34	3
8	1b	17	1b	26	1a	35	1b
9	3	18	3	27	1b	36	1b

Genotype of HCV in untreated patients was done from the sera collected during the last year before the assay and in those patients who completed IFN therapy from the sera collected during the last year before treatment. [a] Patient numbers correspond to those from Fig. 1 and Fig. 2. * This group of patients is equivalent to group 2, but it was tested against peptides from genotype 1b. ND: Not determined.

TABLE 3

Production of IL-2 after mitogen stimulation (PHA)

Group 1		Group 2		Group 3		Group 2*	
Patient	IL-2 (SI)	Patient	IL-2 (SI)	Patient	IL-2 (SI)	Patient	IL-2 (SI)
1	56	10	160	19	90	28	172
2	70	11	57	20	167	29	116
3	170	12	35	21	270	30	77
4	162	13	132	22	164	31	91
5	192	14	240	23	417	32	70
6	129	15	83	24	103	33	131
7	103	16	139	25	141	34	77
8	220	17	225	26	48	35	171
9	121	18	81	27	52	36	123
Mean (SD)	135 (51)		128 (67)		161 (111)		114 (36)

Results are expressed as stimulation index, SI (cpm with PHA stimulation/cpm without PHA stimulation).

and 1b+3, respectively. Untreated patients (group 3) were infected with genotype 1b (five patients), 1a (two patients) and 3 (one patient).

Since non-responders to IFN treatment were mainly infected with genotype 1b (group 2; Fig. 1 and Table 2), we decided to test a new group of IFN-resistant patients (group 2*, n=9), predominantly infected with genotype 1b (Table 2) against a set of 36 peptides corresponding to the sequence of genotype 1b (Table 1). As shown in Fig. 2, a positive

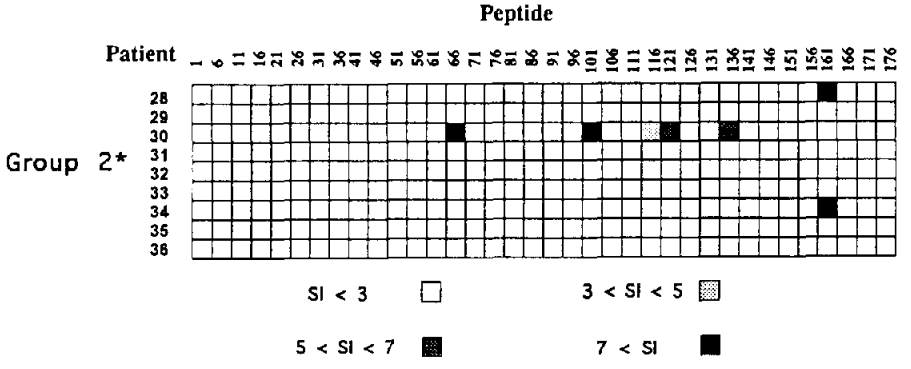

Fig. 2. IL-2 production against HCV E1 synthetic peptides in a group of patients who still had high ALT levels at the end of IFN treatment. The sequence of peptides corresponds to an isolate of genotype 1b. As in Fig. 1, data are expressed as SI.

TABLE 4
HLA-DR typing of HCV patients

Group 1		Group 2		Group 3		Group 2*	
Patient	HLA- DR	Patient	HLA-DR	Patient	HLA-DR	Patient	HLA-DR
1	DRB1*0403/DRB4*01011 DRB1*0701/DRB4*01011	10	DRB1*0701/DRB4*01011 DRB1*0401/DRB4*01011	19	DRB1*03011/DRB3*0202 DRB1*0101	28	ND
2	DRB1*0103 DRB1*1102/DRB3*0202	11	DRB1*03011/DRB3*0201 DRB1*16/DRB5*0101	20	DRB1*0406/DRB4*01011	29	DRB1*03011/DRB3*0202 DRB1*1303/DRB3*03033
3	DRB1*0403/DRB4*01011	12	DRB1*0701/DRB4*01011 DRB1*16/DRB5*0101	21	DRB1*0406/DRB4*01011	30	ND
4	DRB1*0403/DRB4*01011	13	DRB1*1301/DRB3*0101 DRB1*1302/DRB3*0101	22	DRB1*1501/DRB5*0101 DRB1*1104/DRB3*0202	31	ND
5	DRB1*1113/DRB3*0202 DRB1*16/DRB5*0102	14	DRB1*0701/DRB4*01011 DRB1*0403/DRB4*01011	23	DRB1*0103 DRB1*1302/DRB3*0301	32	ND
6	DRB1*0701/DRB4*01011 DRB1*16/DRB5*0101	15	DRB1*0701/DRB4*01011 DRB1*0406/DRB4*01011	24	ND	33	ND
7	DRB1*0103	16	DRB1*0103 DRB1*0701/DRB4*01011	25	ND	34	DRB1*0102 DRB1*0701/DRB4*01011
8	DRB1*0403/DRB4*01011 DRB1*0407/DRB4*01011	17	DRB1*0701/DRB4*01011 DRB1*1301/DRB3*0101	26	DRB1*0304/DRB3*0202 DRB1*0406/DRB4*01011	35	DRB1*0403/DRB4*01011 DRB1*0701/DRB4*01011
9	DRB1*0402/DRB4*01011 DRB1*0701/DRB4*01011	18	DRB1*0407/DRB4*01011 DRB1*1308/DRB3*0301	27	ND	36	DRB1*0407/DRB4*01011

ND: Not determined.

response was found in only three of them (33%) with a mean of 2 peptides per patient being recognized. Moreover, only 6 of the 36 (16%) peptides tested were recognized in this group of patients. These results are comparable to those found in the other group of IFN-resistant patients (group 2) tested with peptides from the genotype 1a sequence.

To exclude general reduction of T lymphocyte reactivity in the group of non-responders to IFN, we also stimulated PBMC from these patients with a mitogen (PHA) and we compared the degree of stimulation with that obtained in the other groups. As is shown in Table 3, the amount of IL-2 produced after PHA stimulation was similar in the different groups of patients, giving a mean SI of around 130 in all the groups.

To study a possible relationship between the peptides recognized by the different groups of patients and the HLA-DR molecules, determination of HLA-DR alleles was performed in most patients with CHC (Table 4). We found that the frequency of patients with CHC presenting DRB1*04 molecules (50%) was significantly higher ($p<0.001$) than the frequency in the normal population (16%, using 219 randomly selected Spanish Caucasian individuals, ref. 19). Since the design of our experiment is a case-control study and not a cohort study, the approximated relative risk was estimated using the odds ratio. This analysis showed that relative risk of developing CHC is approximately 5 times higher in individuals bearing HLA DRB1*04 (95% confidence limits: 2.2–12.7). Peptides 116 and 121 (10 aminoacids overlapped) were recognized by three individuals in group 1 (patients #1, 3 and 8). Interestingly, all of them have the allele DRB1*0403/DRB4*01011, suggesting that in some cases the recognition of this linear epitope might be associated with this allele. On the other hand, DRB1*0701/DRB4*01011 was found in eight out of 13 IFN non-responder patients, but only in two patients from group 1 (patients #1 and 9). Despite the apparent predominance of DRB1*0701/DRB4*01011 in the group of IFN-resistant patients, the difference from the prevalence of this allele in the general Spanish population (18%, ref. 19) did not reach statistical significance, probably because of the small number of cases studied.

Discussion

Interferon has, in addition to its direct antiviral properties, immunopotentiating activities which are thought to contribute to the clearance of viral infections (20). In chronic hepatitis C only about 20–30% of the patients respond to this treatment (3). In this disease the mechanisms responsible for the resistance to IFN and the role played by T cell immunity in the resolution of HCV infection remain poorly under-

stood. In the present work we show that most of the patients responding to IFN with sustained negativization of viremia demonstrate positive T cell reactivity against synthetic peptides from the envelope protein E1 while only a minority of IFN resistant patients recognized these peptides ($p<0.01$). In addition, the number of peptides inducing IL-2 production in the cases exhibiting positive test results was higher ($p<0.01$) among IFN-responders that among IFN-resistant patients. Therefore, successful IFN therapy appears to be associated with the development of T cell response to this viral structural protein.

These results, concerning T cell response and the outcome of IFN treatment, agree with those reported by Hoffmann et al. (21) for core protein. These authors show that a strong T cell response against core protein before and during IFN therapy may be a predictor of sustained response. Other authors have also reported data about T cell response against HCV antigens, but they have not analyzed this response in IFN-responder and IFN-resistant patients. Botarelli et al. (7) studied the proliferative T-lymphocyte response to different HCV recombinant proteins in three groups of subjects: patients with chronic hepatitis C, patients who eliminated the virus after IFN treatment and healthy HCV-seropositive individuals. These authors found that the percentage of IFN-responders who recognized the different structural proteins tested (core, E1 and E2) was very low (18%) and was similar to that observed in the group of patients with chronic hepatitis C. The discrepancy between these results and ours (88% of IFN-responsive patients recognizing peptides from E1) might be explained by differences in methodology. While the authors mentioned used a proliferative assay to test the T cell response to viral antigens, we employed a system based on the production of IL-2 in the presence of a monoclonal antibody against the IL-2 receptor to avoid the consumption of this cytokine. This system has been shown to be able to detect IL-2 production even in cases where the proliferative response is absent (22). It is worth noting that despite the sensitivity of our assay none of the controls recognized any of the peptides tested. The negativity of the test for all peptides in these individuals supports the specificity of our findings. Saracco et al. (23) have reported that anti-E1 antibody levels increased in 81.8% of long-term responders to IFN therapy. This, in conjunction with our data on the increased T cell response to E1, suggests that the immune response against E1 protein might be associated with viral clearance following IFN treatment.

An intermediate situation between IFN-resistant and IFN-responder patients concerning the reactivity to E1 peptides is observed in patients with untreated chronic hepatitis C with 55% of these cases recognizing the peptides from the envelope protein. Interestingly, in six of these patients who were then treated with IFN it was found that the two who failed to recognize E1 peptides manifested resistance to the therapy while three out of four who reacted positively against E1 peptides responded favourably to the treatment. These preliminary data in conjunction with unpublished work from our group (García-Granero et al., manuscript in preparation), demonstrating a high rate of T cell response to core peptides in patients who responded to IFN therapy and a reduced response rate in IFN-resistant cases, further support the idea that there is a link between the ability to mount a T cell response to viral structural proteins and the outcome of IFN treatment.

Close analysis of individual responses to peptides from E1 shows that none of them seem to play the role of immunodominant T cell epitope. This might be due to the heterogeneity of the HLA-DR molecules of the patients. Moreover, the role played by HLA class II molecules other than HLA-DR cannot be ignored. As is well known, these structures are responsible for peptide presentation to T cells, thus influencing the immunogenicity of a specific peptide in each individual. As mentioned, we found that the three IFN-responder patients who reacted positively against the peptides 116–121 (10 aminoacids overlapped) possessed the allele DRB1*0403/DRB4*01011, suggesting that HLA class II molecules may determine the response against specific linear epitopes from HCV. We also observed a significant preponderance of DRB1*04 molecules in the total group of patients with CHC. Also we found a high, although non-significant proportion of patients with the allele DRB1*0701/DRB4*01011 among the patients showing IFN resistance. Clearly, the implication of HLA-DR molecules in the evolution of HCV infection deserves further study.

One of the perplexing features of this study is the absence of T cell responses to E1 antigens in many patients with chronic hepatitis C and in most IFN-resistant cases, despite continued exposure to viral antigens. This lack of response is not due to a general immunological depression since T cell function, as estimated by the proliferative response to PHA, is similar in all groups of patients with CHC and in controls. Although from our data it cannot be entirely excluded that the differences between the sequence of the infecting virus and that of the peptide used might explain some negative test results, the very low reac-

tivity to peptides from E1 in IFN-resistant patients appears not be attributable to differences between the virus sequence and the peptides tested. In fact, IFN-resistant patients (groups 2 and 2*), mainly infected with viral genotype 1b, responded almost equally to peptides from genotype 1a and 1b. Moreover, comparing the degree of homology between the E1 sequences used in this paper and other published sequences (23 from genotype 1a and 52 from genotype 1b), we found a degree of homology of 94% and 92% for genotype 1a and 1b, respectively, indicating a high degree of conservation of the peptides used in this study.

One of the possible explanations for the low response to E1 peptides in groups 2, 2* and 3 is that E1 might not be a strong immunogen. In fact, in patients with chronic hepatitis C a higher stimulatory index was obtained when using core peptides than when using E1 peptides in the assay system (García-Granero et al. unpublished observations). Similarly, Ferrari et al. (9) have shown that chronic hepatitis C patients respond more intensely to recombinant core protein than to recombinant E1. Nevertheless, with the sensitive IL-2 production assay used in the present work we were able to detect positive responses to E1 in almost all the patients who cleared the virus after IFN therapy, suggesting that E1 determinants are capable of inducing cellular immunity. Therefore, hypotheses other than the low immunogenicity of E1 should be considered to explain the lack of responses to this immunogen observed in patients with chronic HCV infection. Further studies are needed to evaluate whether HCV infection might cause deletion of specific reactive T cell clones or whether HCV can evade immunoclearance through the induction of immunosuppressive cytokines. In this respect it has been recently shown (24) that the expression of TGF-β 1, a strong immunosuppressor, is markedly increased in the liver of patients with chronic hepatitis C.

Another point deserving comment is the finding of T cell reactivity against E1 in half the cases with untreated chronic hepatitis C and in some chronic hepatitis C patients with continued disease activity after IFN treatment. These findings indicate that HCV can avoid immunoclearance despite T helper cell recognition. The mechanisms of this escape from the immune system are not well understood. As in other viral infections, cytotoxic T cells are thought to be the main effectors in the control of viral infection and in the elimination of virus-infected cells. It is conceivable that modulation of the cytokine network by HCV might result in inhibition of the cytotoxic T cell response even in the presence of appropriate T helper cell reactivity. Studies are at present underway in our laboratory to analyze the cytotoxic T cell response in similar groups of patients to those studied in the present paper.

Acknowledgements

This work was supported by Gobierno de Navarra (grant 840), by CICYT (grant BIO94-0245) and by Fundación Ramón Areces. The authors thank the Blood Bank of Navarra for providing human AB serum and Madaus, for providing anti-IL-2 receptor mAb. We also thank Dr M. Garcia-Granero for the statistical advice.

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