Interferon Plus Ribavirin in Patients with Hepatitis C Virus Positive Mixed Cryoglobulinemia Resistant to Interferon

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ABSTRACT. ***Objective.*** Only a small fraction of patients with hepatitis C virus (HCV) positive mixed cryoglobulinemia achieve longterm recovery after interferon (IFN) therapy; we evaluated the effectiveness and safety of combination therapy (interferon plus ribavirin) in nonresponders or those who relapsed after one or more courses of IFN.

Methods. Twenty-seven patients with HCV positive mixed cryoglobulinemia were studied. All were treated with IFN-α2b (3 MU 3 times weekly) for one year, plus daily oral ribavirin 1000 or 1200 mg.

Results. Five patients (18.5%) obtained complete recovery from viral infection and from all signs and symptoms of disease. During treatment, most patients (85%) improved clinically. All 5 responders were "relapsers" to the first treatment(s).

Conclusion. Combination therapy of IFN plus ribavirin could be useful for patients with mixed cryoglobulinemia resistant to IFN as monotherapy. (J Rheumatol 2003;30:1775–81)

Key Indexing Terms:
MIXED CRYOGLOBULINEMIA HEPATITIS C VIRUS RIBAVIRIN
α-INTERFERON NON-HODGKIN'S LYMPHOMA

Mixed cryoglobulinemia is a rare lymphoproliferative disease characterized by a well defined clinical syndrome of purpura, arthralgias, and weakness, and by the presence of serum cold-precipitable immune complexes (IgG and/or IgM)[1]. More than 90% of patients are found to have hepatitis C virus (HCV) infection (with or without liver involvement), suggesting that this virus could be the etiologic agent of mixed cryoglobulinemia[2-5]. Although usually benign, the disease is sometimes associated with multiorgan involvement, and a fraction of patients can undergo severe or even life-threatening complications, such as membranoproliferative glomerulonephritis[6,7] or non-Hodgkin's lymphomas[8,9].

Treatment of mixed cryoglobulinemia includes diet[10] and/or drug therapy, e.g., steroids[11], cyclosporine[12], colchicine[13], plasmapheresis[14], and others[15]. In the last 10 years, many investigators[16-23] reported good efficacy of α-interferon (IFN), a compound endowed with antiviral and antiproliferative activity, able to inhibit HCV replication and to reduce cryoglobulin production as well. Despite different IFN schedules, only a minority of patients (10–20%) obtain a complete response, while most do not respond or experience relapse after treatment, even if most patients experience good relief from clinical symptoms under therapy. Before the introduction of ribavirin, the most common approach to retreatment of HCV infected patients was to increase the dose or change the type of IFN[24-29]. In accord with this approach, we had treated a group of mixed cryoglobulinemia patients who were relapsers and nonresponders with leukocyte IFN with unsatisfactory results[30]. However, the most recent clinical trials in chronic HCV infection suggest that the best approach for refractory HCV infection is combination therapy of IFN plus ribavirin[31,32]. The studies of combination therapy in HCV-chronic hepatitis resistant to IFN indicated that a response rate of 22 to 40% could be expected[33-36]. Since the data on the safety and efficacy of the combination therapy in mixed cryoglobulinemia are limited (24 patients over 3 studies with different treatment schedules)[37-39], we investigated the effect of IFN plus ribavirin in patients with HCV positive mixed cryoglobulinemia who were nonresponders or relapsers after one or more courses of IFN as monotherapy.

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MATERIALS AND METHODS

Patients. Twenty-seven patients (15 women, 12 men, mean age 48 ± 12 yrs) with mixed cryoglobulinemia were studied. The diagnosis was based on standard criteria[1]. The median duration of disease before the first course of IFN therapy was 2 years (range 1–6). All patients had been treated previ-

ously: 10 underwent a single course of low dose recombinant IFN (3 MU 3 times/week), while 17 had been treated with 2 courses (recombinant and subsequently leukocyte IFN), but a complete response had not been obtained. Among 10 subjects treated with a single course, 4 could be considered "relapsers" and 6 "nonresponders"; among the 17 patients treated twice, 6 were nonresponders to both treatments, 2 were relapsers to the first course and nonresponders to the second, while the remainder (9 cases) relapsed after each therapy. All 27 patients were HCV-RNA positive and showed active disease at enrolment to this trial. In addition to usual symptoms (weakness, arthralgias, variable degree of purpura), 6 patients showed Raynaud's phenomenon, 2 had "sicca syndrome," one peripheral neuropathy, and one membrano-proliferative glomerulonephritis. All patients were Caucasian heterosexuals and had no history of intravenous drug use or ethanol abuse. All patients gave informed consent before entry into the study; the study protocol was approved by the ethical committee of Friuli Venezia Giulia.

Methods. Liver function and hematological variables were determined by usual laboratory methods. Cryoglobulin determination was by standard methods, as reported[1,8]. Rheumatoid factor (RF) and C3 and C4 fractions of complement were measured by rate nephelometry. Mixed cryoglobulins were classified as type II on the basis of the presence of monoclonal IgM immunoglobulins with RF activity complexed with polyclonal IgG, and as type III in the presence of polyclonal immunoglobulins.

Purpura scoring system. A simple clinical scoring system was used to assess the severity of vasculitis. A score of zero indicated the absence of skin lesions, score of 1 the presence of < 10 purpuric spots on the lower limbs, score of 2 the presence of > 10 spots on the lower limbs, score of 3 indicated extension of the spots to the trunk and/or upper limbs, and a score of 4 the presence of skin ulcers and/or gangrene.

Histology. A bone marrow biopsy was performed in all patients with a Jamshidi-like needle (Trapsystem, Treviso, Italy). One patient refused bone marrow biopsy. The sample was placed in B5 solution and 2 h later in 70% ethanol. After decalcification, samples were stained following standard methods. The bone marrow biopsy was performed in all cases before the beginning of treatment and at the end of the followup, 12 months after the end of therapy. On the basis of histological and immunological findings, bone marrow was classified as follows: as normal in the absence of lymphocyte infiltration; as "reactive lymphocyte infiltration" in the presence of paratrabecular foci of small lymphocytes (with or without lymphoplasmacytic features) observed to be polyclonal on FACS scan (see below); as "monoclonal lymphocyte infiltration" in the presence of lymphocyte monoclonal on FACS; and as non-Hodgkin's lymphoma in the presence of massive (> 50%) infiltration by plasmacytic lymphocytes[40].

A liver biopsy was obtained only from patients with biochemical and/or clinical signs of chronic liver disease. Samples were placed in buffered formalin, stained with hematoxylin and eosin and, for reticulum, with Gomori stain. In each biopsy the disease activity and fibrosis were assessed according to Metavir[41]. The liver biopsy was performed once, before the first IFN therapy.

Phenotyping. Mononuclear cells from marrow aspirate were separated on a Ficoll density gradient. As reported, cells were stained with specific monoclonal antibodies, and after incubation and washing, immunofluorescence was measured by FACScan flow cytometry (Becton Dickinson, Mountain View, CA, USA). The monoclonality of marrow lymphocytes was evaluated by FACS determination of the surface light-chain distribution.

Virological studies. Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) markers were detected by ELISA using commercial kits. The presence of anti-HCV antibodies was assayed by the 2nd generation (4 antigen) immunoenzymatic screening test Ortho-HCV (Ortho Diagnostic Systems, Raritan, NJ, USA). The presence of HCV-RNA in serum was assessed by polymerase chain reaction (PCR) amplification of the conserved 5' untranslated region (5' UTR) of HCV. Amplification was performed using 2 steps, the so-called nested PCR[42].

The HCV genotype was determined by PCR amplification of the core region as described by Okamoto, *et al*[43].

Reverse transcription/PCR analysis of µCDR3/FW4 region. The presence of an expanded monoclonal B cell population was investigated in peripheral blood mononuclear cells (PBMC) or bone marrow aspirates using the "immunoglobulin gene fingerprinting" technique with primers from variable (V_H) and constant (C_H) heavy chain regions. Total cellular RNA was isolated by the procedure of Chomczynski and Sacchi[44] from PBMC obtained by fractionation of whole blood (or bone marrow) on Ficoll/Hypaque gradient. One microgram of mRNA was reverse transcribed (RT) using oligo-dT and the GeneAmp RNA/PCR kit (Perkin-Elmer Cetus, Norwalk, CT, USA), following the procedure recommended by the manufacturer. The entire RT product was subjected to PCR with 50 pmol of oligonucleotide primers hV2 5'CTGAGGACACGGCCGTGATTACTG 3', from a conserved sequence in the V_H Framework 3 (codons 84 to 92) and hµ1 5'GTCCTGTGCGAGGGCAGCCAA 3', from the first exon of the heavy chain constant µgene (codons 139 to 145). The PCR was carried out with a "hot start," followed by 35 cycles: 1 min of denaturation at 95°C, 1 min of annealing at 62°C, and 1 min of extension at 72°C in a Perkin-Elmer Cetus thermal cycler. Fifteen microliters of the PCR product were next radioactively labeled by adding 5 pmol of an internal ^{32}P 5'end-labeled oligonucleotide from constant region of µgene (hµ3: 5'GGAAAAGGGTTGGGGCGGAT 3') and performing one more PCR cycle under the same conditions as above, except for longer denaturation (5 min) and extension (12 min). This reaction was done in a 25 µl total volume that contained fresh Taq polymerase and dNTP mixture. The hµ3 oligonucleotide was previously 5' end-labeled to high specific activity with ^{32}PγATP (Amersham, Buckinghamshire, UK) and T4 Polynucleotide Kinase (New England Biolabs, Beverly, MA, USA). Two microliters of each reaction were analyzed on a denaturing 6 M urea-6% polyacrylamide sequencing gel, as described[45,46]. Analysis of B cell monoclonality was performed in PBMC and bone marrow from all patients before the treatment and at the end of the followup. Determinations in PBMC only were performed during the therapy (at 3rd and 6th month).

Therapy. Since the disease is rare and the number of patients low, we did not consider a randomization between 2 arms, one with and one without ribavirin. Further, our broad experience in the treatment of patients with noncryoglobulinemic HCV positive chronic hepatitis indicated that a second (or third) IFN course would not be effective without ribavirin[47,48], thus we considered a control arm with IFN as monotherapy would be unethical. On the basis of these considerations, all the patients followed the same treatment schedule: 3 million IU of recombinant IFN (Intron-A, Schering-Plough, city) 3 times per week for 6 months combined with daily oral ribavirin (Rebetol, Schering-Plough, Kenilworth, NJ, USA) 1000 mg for patients with body weight < 70 kg or 1200 mg for those weighing > 70 kg for 6 months.

Evaluation criteria. Responses to treatment were classified at the end of therapy and the followup period (one year after the end of therapy) by clinical and laboratory criteria. Considering the difficulties of finding homogeneous criteria to evaluate the different aspects of the disease, evaluation was split into 4 separate categories: (1) viral response; (2) biochemical response; (3) immune response; and (4) clinical response, as follows.

Viral response: the effect of the therapy on HCV-RNA. Complete response was defined as undetectable of HCV-RNA at the end of treatment and the end of followup. Relapse was defined as undetectable HCV-RNA at the end of treatment, but HCV-RNA still positive at the end of the followup. No response was defined as persistent positivity during therapy and at the end of followup.

Biochemical response: the effect of therapy on liver function tests. Complete response: normalization of the serum ALT concentration during the treatment, followed by normal ALT values lasting for 12 months after discontinuation of therapy. Partial response: reduction (but not normalization) of ALT. No response: ALT stable or reduction of less than 50% during treatment. Relapse: normalization of serum ALT level during treatment,

followed by return to pretreatment values at the end of followup. In some patients this response was not valuable since ALT level was normal at the beginning of treatment.

Immune response: the effect of therapy on serum RF concentration and cryocrit concentration. Complete response: normalization of serum RF concentration and elimination of circulating cryoglobulins. Partial response: reduction (but not normalization) of RF and cryoglobulins by more than 50%. No response: Reduction of RF and cryocrit by less than 50% or achievement of stable levels. Relapse: normalization of serum RF and cryoglobulins during therapy followed by return to pretreatment values after the end of treatment.

Clinical response: the effect of therapy on the clinical manifestations of the disease (including purpura, arthralgias, and weakness). Complete response: elimination of all clinical signs of the disease. Partial response: improvement of clinical symptoms, i.e., reduction of the global score > 50%. No response: reduction of the global score < 50% or stable. Relapse: normalization of clinical symptoms during therapy followed by return to pretreatment score after the end of treatment.

Controls. Biochemical and clinical measures were determined each month under therapy and every 2 months after discontinuation of treatment. Autoantibodies were determined every 3 months and thyroid function tests every 6 months. Determinations of HCV-RNA were performed before the beginning of therapy, at the end of the treatment, and at the end of followup. All patients were followed for at least 6 months after the end of therapy.

Statistical analysis. Data are expressed as mean ± standard deviation. Statistical analysis was carried out using the statistical package SPSS for Windows[49]. The analysis of variance between 2 groups was calculated (one-way) where p represents the probability of Snedecor's F that the means interval-dependent variables of the 2 groups are unequal for numerical continuous ones. For categorical variables, a cross-tabulation with Pearson chi-square was used to test that the row and the column variables were independent. Partial associations were studied by hierarchical log-linear analysis in a multiway cross-tabulation.

RESULTS

Biochemical and histological findings. Patients' main clinical, laboratory, and histological findings are indicated in Table 1. Patients' ages refer to the start of therapy (mean age 48 ± 12 yrs). All patients had low C4 concentration. The monoclonal component was IgMk in all subjects, while in 3 cases no monoclonal component was found; accordingly in the latter the mixed cryoglobulinemia was defined as type III.

Liver biopsy was performed in 24 patients (89%). In all cases a chronic liver disease of variable severity (from A1F0 to A3F4) was found. The bone marrow histology was normal in 12 patients (48%), while lymphocyte infiltration of variable degrees was present in the remaining 14 patients (52%). In these cases, the FACS determination of surface light-chain distribution confirmed the presence of a monoclonal infiltrate in 9 cases (34%), whereas in the other 5 (19%) a non-monoclonal (reactive) infiltrate was found. Among the 9 subjects showing monoclonal infiltrate, 3 (15%) had histological findings suggestive for the diagnosis of non-Hodgkin's lymphoma: this diagnosis was supported by the presence, in 2 patients, of greater than 50% CD19 and CD20 positive infiltrate and negative CD5 findings (lymphoplasmacytic immunocytoma-like)[40]. In the third patient of this group, a CD5/CD19 positive infiltrate was found (chronic lymphocytic leukemia-like)[40]. All 3 subjects with type III mixed cryoglobulinemia showed normal bone marrow.

Virological findings. HCV-RNA was detected in all cases (100%) before therapy. HCV genotyping (performed in 25 cases) showed the presence of type 1a in one case (4%), 1b in 14 cases (52%), type 2a in 4 cases (16%), 2b in one case (4%), 2c in 5 cases (20%), and type 3a in one case (4%). Coinfections were not found.

Effects of the therapy. During therapy HCV-RNA became undetectable in 5 patients, and at the end of the followup these patients remained negative and were considered complete responders (18%). No case of virological relapse was found (Table 2).

At the end of therapy, a reduction of cryocrit was observed in 21 (78%) patients. The mean cryocrit concentration was significantly reduced at the end of treatment (from 5.3 ± 6.5% to 2.2 ± 3.4%; $p < 0.01$), and even at the end of the followup the mean cryocrit level remained significantly lower than the basal value (1.5 ± 2.2%; $p < 0.005$). Rheumatoid factor showed similar results, from 197 ± 186 IU/ml to 129 ± 148 IU/ml, but to a lesser extent ($p < 0.05$) than serum cryoglobulins. Based on the evaluation criteria given above, during the treatment 9 patients (33%) achieved a complete immunological response and 12 (44%) a partial response, while the others could be considered nonresponders. At the end of followup 5 patients (18%) showed complete response and 6 (22%) obtained partial responses.

Three patients (Patients 1, 22, and 26) had normal ALT serum concentration at the beginning of the study, thus these cases were not valuable for the biochemical response. During treatment, 12 of 24 (50%) patients responded to IFN treatment with normalization of ALT, but 5 of them (42%) relapsed immediately after suspension of IFN. At the end of the followup period, 8 patients (33%) had a sustained normalization of ALT and were considered biochemical complete responders; one of them (Patient 16) was a nonresponder at end of treatment, but the ALT level normalized spontaneously during followup, and therefore he was considered a "late responder." The anti-HCV antibody concentrations in responders and nonresponders/relapsers did not change after therapy.

In most patients (23 cases, 85%), a complete remission or a marked improvement of the main clinical disease manifestations (skin manifestations, weakness, arthralgias) occurred within 2 or 3 weeks of treatment. Regretably, most patients (16 cases, 70%) relapsed within a few weeks after the end of treatment. At the end of the followup, only 5 patients (18%) had obtained a complete response, and 4 patients maintained the partial response.

Fever, fatigue, and flu-like syndrome were observed in most patients during the first 2–3 weeks of treatment. These symptoms usually improved with acetaminophen pretreatment. In 3 patients we suspended the treatment because of side effects: one for severe depression (at 5th month), one

Table 1. Clinical, virological, and histological features of patients.

Patient	Age, yrs, sex	Monoclonal Component	Liver Histology	Bone Marrow Histology	HCV Genotype	Outcome of Previous Therapies
1	48 M	IgMK	NP	NHL	NP	Rel/Rel
2	47 F	IgMK	A1F1	ML	1b	NR/NR
3	67 F	IgMK	A2F1	N	1b	Rel/NR
4	36 M	IgMK	A1F1	N	1b	NR
5	64 M	IgMK	A1F0	N	2c	NR
6	25 M	IgMK	A2F1	N	1b	Rel
7	52 M	IgMK	A2F2	RL	1b	NR
8	44 F	Absent	A1F1	N	2b	Rel
9	38 F	IgMK	A2F1	RL	1b	Rel
10	57 F	Absent	A1F1	N	2a	Rel/NR
11	41 F	IgMK	A3F2	ML	1b	NR/NR
12	46 F	IgMK	A1F1	ML	1b	Rel
13	65 F	IgMK	A1F1	N	1b	Rel/Rel
14	47 F	IgMK	A2F1	RL	2c	NR
15	43 F	IgMK	A1F0	ML	2c	Rel/Rel
16	62 M	Absent	A3F4	N	2c	Rel/Rel
17	48 F	IgMK	A2F2	NHL	2c	Rel/Rel
18	56 M	IgMK	A3F3	NHL	1b	Rel/Rel
19	29 M	IgMK	A1F0	ML	2a	Rel/Rel
20	47 F	IgMK	A1F1	N	1b	NR/NR
21	33 F	IgMK	A1F0	N	3a	NR/NR
22	65 M	IgMK	NP	RL	2a	NR/NR
23	37 M	IgMK	A2F1	N	1a	Rel/Rel
24	34 M	IgMK	A2F1	N	1b	NR
25	60 M	IgMK	A3F4	RL	1b	Rel/Rel
26	54 F	IgMK	NP	ML	1b	NR
27	49 F	IgMK	A2F1	ML	1b	NR
Means	48 ± 11					

NP: not performed, ML: monoclonal lymphocytosis; N: normal, NHL: non-Hodgkin's lymphoma, RL: reactive lymphocytosis; NR: nonresponder; Rel: relapser. Liver histology was evaluated according to the Metavir scoring system: activity (A) was graded according to intensity of necro-inflammatory lesions; A0: no activity, A1: mild activity, A2: moderate activity, A3: severe activity. Stage of fibrosis (F) was graded as follows; F0: no fibrosis, F1: portal fibrosis without septa, F2: portal fibrosis with some septa, F3: portal fibrosis with numerous septa, F4: cirrhosis.

for diffuse erythema (6th month), and one for severe anemia (2nd month). Thrombocytopenia (platelets < 100×10^6/l) occurred in 5 cases, but therapy was not discontinued. Five patients developed normocytic anemia, and ribavirin was consequently reduced to 1000 and 800 mg/day in 2 and 3 patients, respectively. No patient developed thyroid dysfunction requiring therapy or discontinuation of antiviral treatment.

Analysis of μCDR3/FW4 region. Analysis of B cell monoclonality was positive in either PBMC or bone marrow in all cases of type II mixed cryoglobulinemia, while the 3 patients with type III were negatives. Interestingly, even the patients showing mild or absent bone marrow lymphocyte infiltration were positive. During therapy, the monoclonal bands obtained from PBMC showed different patterns: in some cases (8 patients) monoclonality disappeared, in some cases (10 patients) the intensity of the bands was reduced but they were persistently present, while in the others (9 cases) no change was observed. Elimination of monoclonality was obtained in all HCV-RNA negative patients (5 cases) and in a small fraction of nonresponders (3 cases, 14%). After the conclusion of treatment, monoclonality remained undetectable in either PBMC or bone marrow in 4 of the 5 responder patients. One responder developed a faint band that remained unchanged over time (at least in PBMC). In the 3 nonresponders a strong band indicating B cell monoclonality was detectable again in either PBMC or bone marrow at the end of followup.

DISCUSSION

This study shows that combination therapy is effective in the treatment of patients with mixed cryoglobulinemia who were nonresponders or relapsers in response to a previous interferon treatment. These results show the possibility of a complete recovery from HCV infection in previously treated patients. Interestingly, no patient who had been considered a nonresponder to one or 2 IFN treatments showed complete response to combination therapy. By contrast, the cases that obtained complete virological response (5 patients) were relapsers during the first IFN course. These results are quite different from those obtained with combination therapy in patients with HCV positive chronic hepatitis without mixed cryoglobulinemia — indeed, in cryoglobulinemic patients the efficacy of the treatment was less impressive[50-52].

In this report, the response to treatment was split into 4

Table 2. Effects of combination therapy at the end of followup.

Patient	Response			
	Viral	Biochemical	Immune	Clinical
1	NR	NV	PR	PR
2	NR	NR	Rel	Rel
3	NR	Rel	PR	PR
4	NR	NR	Rel	Rel
5	NR	CR	Rel	Rel
6	CR	CR	CR	CR
7*	NR	NR	NR	NR
8	CR	CR	CR	CR
9	CR	CR	CR	CR
10	NR	CR	PR	Rel
11	NR	Rel	Rel	Rel
12	NR	NR	Rel	Rel
13	NR	Rel	Rel	Rel
14	NR	NR	NR	NR
15	CR	CR	CR	CR
16	NR	LR	PR	Rel
17	NR	NR	NR	Rel
18	NR	Rel	PR	Rel
19	NR	NR	NR	NR
20	NR	Rel	PR	PR
21*	NR	NR	NR	Rel
22	NR	NV	Rel	Rel
23	CR	CR	CR	CR
24	NR	NR	Rel	Rel
25	NR	NR	Rel	PR
26	NR	NV	Rel	Rel
27*	NR	NR	NR	NR

NR: nonresponder, PR: partial response, CR: complete response, Rel: relapse, NV: not valuable. * Patient with early treatment suspension.

categories to separately evaluate the effects of IFN on the different aspects of the disease. The virological response is somehow unsatisfactory: only 5 patients cleared the virus definitively, while the majority (82%) could be considered nonresponders. Interestingly, no patient could be considered a relapser — this might indicate that the first course(s) of IFN selected highly resistant HCV strains. Indeed, the fraction of patients who obtained a virological response under therapy was larger (46%) during the first IFN treatment than during the combination therapy (18%).

Concerning liver function tests, a small number of patients (3 cases) could not be evaluated since they showed normal concentrations of serum AST/ALT/GGT/ALP before treatment. But normalization was obtained in 12 patients during therapy. The normalization of liver function tests did not occur only in the cases who eliminated the virus, but even in a fraction of patients (2 cases) with persistent HCV-RNA positivity (Patients 5 and 10). These 2 cases showed variable concentrations of cryoglobulins and RF during therapy or relapsed at the end of treatment, thus in terms of immune response they were considered a relapser and a partial responder, respectively. These findings indicate the possibility of different effects of IFN on the liver and on the immune system. While elimination of viral replication is almost always associated with normalization of liver function tests, these biochemical measures could normalize under therapy even in the presence of viral replication and persistent cryoglobulin production. In accord with these observations, in one case (Patient 16) liver function tests normalized spontaneously several months after the end of treatment. Similar results have been reported in patients under IFN therapy for chronic hepatitis C without cryoglobulins, i.e., patients with normal liver function tests despite circulating levels of HCV-RNA[53].

Despite the disappointing results in terms of virological and immunological responses, most patients improved clinically. Fourteen patients (52%) showed complete relief from clinical symptoms and 9 further cases (33%) had a partial response, while a minority (4 cases, 15%) did not experience any improvement. One patient (Patient 27) could not be fully evaluated due to the early suspension of treatment. It is not easy to explain these results. One possible explanation is the decrease of the viral load during therapy; indeed, the reduction of the viral particles (even in nonresponders) decreases the amount of cold-precipitable immunocomplexes constituted by HCV-RNA/anti-HCV IgG polyclonal antibodies/anti-IgG IgM monoclonal antibodies. To confirm this hypothesis, quantitation of the HCV-RNA would be useful, but the presence of a large amount of cryoglobulin able to bind the virus prevents reliable measurement of HCV-RNA[54,55]. However, other effects of IFN on the immune system could explain the favorable clinical results.

In contrast to our previous results[56,57], the HCV genotype does not seem to be the most relevant factor. Among the 5 cases with complete responses, 2 (Patients 6 and 9) were carriers of genotype 1b, which is widely considered the most aggressive HCV genotype, although the number of cases is too low for statistical analysis. In addition to the viral factors, host factors such as liver histology or biochemical variables could be important. As for liver histology, although no patient with cirrhosis (A3F3 or A3F4) showed a sustained response, the low number of such patients (3 cases) again does not allow a statistical analysis. However, the pretreatment cryocrit concentrations as well as other laboratory variables were not different between responders (4.9 ± 3.8%) and nonresponders (5.3 ± 4.2%; p: nonsignificant).

To summarize our results, the elimination of HCV-RNA was nearly always associated with disappearance of all clinical, biochemical, and immunological signs of disease. However, a small number of patients had normalized ALT and normal liver function tests despite persistent detectable levels of HCV-RNA, while others also obtained lasting relief from clinical symptoms. This should be due to the antiproliferative properties of IFN and its ability to reduce the HCV associated lymphoproliferative disorder. Indeed, B cell monoclonality measurement showed that all patients with type II mixed cryoglobulinemia bear a monoclonal B cell population not only in bone marrow, but even in PBMC.

These findings, confirmed by other authors with different methods[58,59], indicate a role of HCV in the development of some lymphoproliferative disorders. The therapy seems to result in elimination of B cell monoclonality from PBMC in most patients, although the result was transient[60-62]. This effect of therapy could explain why the incidence of lymphoproliferative disorder in patients with chronic hepatitis C is low. Most of these patients are currently treated with antiviral drugs, and until recovery occurs they undergo several courses of IFN (sometimes combined with ribavirin). These treatments, independently from the effects on HCV replication, are able to control B cell proliferation. Thus, mixed cryoglobulinemia is quite rare in patients undergoing antiviral treatment for chronic hepatitis C, while the disease is much more frequent in patients unable to undergo antiviral treatment. We have observed the appearance of only 2 cases of mixed cryoglobulinemia among more than 500 patients (0.4%) treated with IFN for chronic hepatitis C in 10 years, while the prevalence and incidence of mixed cryoglobulinemia are much higher (7–14%) in untreated patients.

Combination therapy seemed to induce a complete recovery from mixed cryoglobulinemia in patients previously treated with IFN as monotherapy. However, the results with combination therapy in mixed cryoglobulinemia are less impressive than those in HCV patients with chronic hepatitis without cryoglobulins[63]. It is likely that the less favorable results are due to patients' older age, to long-standing disease, and to other presently unknown factors. Combination therapy seems useful only in patients who relapsed after use of IFN as monotherapy, while in nonresponders it was of limited, if any, efficacy. In these patients new therapeutic approaches should be tried, such as the pegylated interferons[64,65] with ribavirin (and/or combined with amantadin); these may be the most promising combinations for the near future.

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