

Rheumatoid factor after antiviral therapy in patients with HCV chronic hepatitis

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ABSTRACT

Positivity of rheumatoid factor (RF) in the course of Hepatitis C virus HCV infection has been described in many papers, with percentages between 30% and 80%, but no data are reported on the behaviour of this parameter during the treatment. In the present retrospective study, 66 patients with HCV infection and positivity for RF were observed between March 2001 and January 2004; they had received combined therapy with Peg-IFN alpha-2b 1.5 mcg/kg/weekly and ribavirin 800-1200 mg/daily (on the basis of body weight). Before treatment, all of them had presented hypertransaminasemia for at least 6 months and high viral load. No patient suffered from other hypersensitivity disorders. The follow-up period lasted for a mean period of 26±7 months, after which only 34 (51.5%) revealed normal transaminases activity with negativity of HCV-RNA (long-term responders, LTR), while the remaining 32 (48.5%) were classified as non responders (NR). In both groups significant variations of RF values were observed. Moreover, RF remained positive in 6 (17.6%) of the LTR group and in 17 (53.1%) of the NR group patients. These data suggest a possible inhibiting action of the combined therapy on the exaggerated immune response. This effect appears partially unrelated to the antiviral action of the therapy.

Introduction

The role played by HCV in inducing autoimmunity has been suggested by several studies (1-3). The frequent extrahepatic involvement in the course of HCV infection, such as cryoglobulinemia (4), arthritis (5, 6) and Sjögren's syndrome (7) have been related to autoimmune virus-induced processes and confirmed by the positivity of various indexes of autoimmunity found in the course of HCV infection (1-3, 8). Various mechanisms have been hypothesized as responsible for the onset of autoimmunity during HCV infection, but the most likely causes seem to be the strong lymphotropism of HCV (9) and the enhanced T-cell apoptosis, which contribute to viral persistency (10); moreover, autoimmunity appears

related to the resistance of CD5+ B cell population to apoptosis (11).

Among the autoimmunity indexes, the presence of RF has been reported as a very frequent finding in the serum of HCV positive patients, with percentages ranging from 30% to 80% (2, 3), usually associated to the presence of cryoglobulins. The pathogenic role of such factor is still unclear, since its presence is often unrelated to the severity of hepatic damage, but the most recent opinions on this aspect indicate the host immune response to be an important factor in the pathogenesis of hepatocellular injury, rather than the direct viropathic effects (12).

However, certain data regarding the behaviour of RF in the course of antiviral treatment have not yet been reported in the literature. Therefore, in this retrospective study, the Authors present the variations of RF titers in the serum of a group of HCV infected patients, with similar degree of hepatic involvement, treated with α -interferon and ribavirin. The possible meaning of these variations is discussed in relationship to the effects of the treatment on the virological outcome.

Methods

Patients

Sixty-six patients (34 females), mean (\pm SD) age 46.9 (6.6) years, affected by HCV-related chronic hepatitis, genotype 1, with high viral load (>6 log-copies/ml), all characterized by positivity for RF, were treated at the Hepatology Unit of our department, in the period March 2001 – January 2004. Although all of them presented hypertransaminasemia for at least six months, the time of HCV infection remained unknown in all the cases. None of them presented arthritis or other autoimmune diseases. The patients were submitted to liver biopsy, which showed a mean (\pm SD) grading of 5.4 (2.6) and a staging of 2.4 (0.8), sec. Ishak. Cryoglobulins were present in 44 patients (66%) in two of whom antinuclear antibodies (ANA) resulted positive at low titers ($<1:160$). A mild hypocomplementemia (C4 reduction) was evidenced in four patients (6%), while 26 patients (39%) showed a moderate hypergammaglobulinemia.

Competing interests: none declared.

Treatment

All patients received Peg-IFN alpha-2b, 1.5 mcg/kg/weekly, combined with ribavirin 800-1200 mg daily, on the basis of body weight. The duration of therapy was determined by the virological response, lasting up to 48 weeks in the case of undetectable viral load after six months.

Subjects who failed to achieve virological response at the sixth month of full treatment were labelled as NR patients. LTRs belonged to the group of patients who, for at least an eighteen-month period, remained HCV-RNA negative, after therapy discontinuation.

Laboratory methods

Diagnosis of HCV infection was made by positivity for antibodies against HCV, either an enzyme immunoassay (EIA; Ortho HCV 3.0) or a recombinant immunoblot (RIBA 2; Ortho HCV RIBA II Orthoclinical Diagnostic), and then confirmed in all the patients by positivity of polymerase chain reaction (PCR; Amplicor, Roche Diagnostic System Inc.) (over 600 copies/ml). HCV genotype was studied by amplification of the core region of HCV with PCR using genotype specific primer. RF was determined by ELISA (reference value less than 15 U/ml).

Statistics

The considered variables were uniformly distributed and were expressed as mean \pm SD.

To study frequencies, the chi-square test was used. The paired *t*-test was used to compare the RF levels before and after antiviral therapy. A level of

probability <0.05 was considered as significant.

Results

The follow-up period lasted for a mean period of 26 ± 7 months, during which no other autoimmune disorders, possible causes of RF positivity, occurred. There were 34 out of 66 (51.5%) LTR and 32 out of 66 (48.5%) NR patients. RF was determined at the end of follow-up period and was present in 17 out of 32 (53.1%) NR individuals, whereas 6 out of 34 (17.6%) LTR patients showed a positivity for RF presence, (chi-square 7.6, $p=0.006$).

The mean pre-antiviral therapy RF value, evaluated in the whole population, was statistically different from the one after therapy (184.7 ± 162.8 vs. 45.8 ± 50.8 , $p=0.0001$). Similar behaviour was discovered when analyzing the mean value of the pre- and post-antiviral therapy RF in the NR and LTR subgroups, respectively, *i.e.*, 214.7 ± 161.8 vs. 56.6 ± 53.7 , $p=0.0001$, and 145.9 ± 159.1 vs. 31.9 ± 44.3 , $p=0.002$. The RF data are evidenced in Table I.

Discussion

As referred to in the introduction, serological markers of exaggerated immune response are often positive in course of HCV infection, but no close correlation between them and hepatic cytolysis has been found, even if both hepatic and extrahepatic damage appear to be linked to immune-mediated mechanisms (10). This apparent discrepancy could probably be explained on the basis of the fact that the hepatic injury is due to both mechanisms (direct viropathic effect,

but to a lesser extent, and immune-mediated action) which do not appear to be strictly linked to each other.

Our results show an overall significant reduction of serum RF levels after antiviral therapy. Interestingly, in the follow-up period, RF negative values were observed with different frequency in the LTR and NR patients; in fact, only a minority of LTR cases remained positive for the RF and at a significantly lower level, while, in the NR group, more than 50% maintained similar levels. This suggests that the IFN plus ribavirin counteracts the exaggerated immune response sensibly, independently of viral outcome. Alternatively, it could be suggested that a reduction of RF levels in LTR is due to the effect of antiviral therapy on viral replication, since the RF production is an effect of HCV.

Possible hypotheses regarding this aspect appear difficult, since the absence in our patients of extrahepatic manifestations caused by HCV, in which a pathogenic function of RF could be advanced, and the recent literature data do not help to explain the real mechanisms played by RF in this condition. We think that further studies on the typification of RF and its links to the behaviour of the pattern of interleukins (IL) could give substantial elements on the means of this factor in HCV infection. In this regard, recent studies demonstrate that IFN-ribavirin associated treatment develops both direct antiviral and indirect immune-mediated effects, through different mechanisms: IFN therapy can affect the intrahepatic T-cell response and eventually alter the cytokine profile, inhibiting the production of IL 10 but maintaining that of IL 12 and tumour necrosis factor (TNF)-alpha (13), meanwhile ribavirin suppresses IL 10, IL 12 and TNF-alpha production (14). Since the synthesis of RF is the expression of a polyclonal activation of B cells, due to stimulation by T cells (15, 16), it could be hypothesized that the changes induced by the combined treatment in the cytokine pattern determine a reduction of RF synthesis through the regulation of the mechanism of stimulation T cells-B cells. Probably, in some cases polyclonal B-cell hyperactivity

Table I. Rheumatoid factor values in patients with HCV-related chronic hepatitis.

Patients n=66	RF Before Therapy	RF After Therapy	Statistical significance
NR n=32	M (\pm SD) 214.7 (161.8) Positive n=32 (100%)	M (\pm SD) 56.6 (53.7) Positive n=17 (53.1%)	* $p=0.0001$ ** $p=0.006$
LTR n=34	M (\pm SD) 145.9 (159.1) Positive n=34 (100%)	M (\pm SD) 31.9 (44.3) Positive n=6 (17.6%)	* $p=0.002$ ** $p=0.006$

RF: Rheumatoid factor; NR: Non responder patients; LTR: Long-term responder patients.

*chi-square test; **paired *t*-test.

escapes partially, with still unknown mechanisms, the immune modulation effects of the therapy, so that RF synthesis persists after clinical improvement and HCV viral clearance. Thus, in these cases the immune-mediated effect appears to be independent of the direct antiviral action of the therapy.

On the other hand, it is noteworthy to stress that the serological negativity of HCV-RNA does not mean complete viral clearance, since genomic material in peripheral blood mononuclear cells of patients with HCV chronic hepatitis has been found after successful antiviral treatment (17) and therefore, in such circumstances, the ongoing immune-stimulation could not be excluded, also in presence of serum HCV negativity. To the best of our knowledge, this report (over a long period of observation) firstly shows a different behaviour of the antiviral therapy in modulating the hepatic manifestation and the exaggerated immune response of HCV infection. Accordingly, the immune-modulation role of IFN plus ribavirin is hypothesized.

Unfortunately, ANA and other immunological parameters of autoimmunity were not followed up, therefore it has been impossible to know the behaviour

of such important features of immune response because only the basal values were known. Further studies focusing on other immunological aspects, as well as IL pattern and its eventual correlations with the main autoimmunity indexes, are being carried out by our group.

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