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AUTOANTIBODIES BEFORE, DURING AND AFTER ADMINISTRATION OF RECOMBINANT INTERFERON-α FOR CHRONIC VIRAL HEPATITIS

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SUMMARY

This study was undertaken to investigate the presence of autoantibodies in patients with chronic viral hepatitis B and C, before, during and after interferon-α (IFN-α) therapy and to study their relation to dose and type of IFN-α and response to treatment. Fifty patients with chronic hepatitis were divided in two groups, a control-group of 21 patients (10 type B and 11 type C) who were followed for 6 months without treatment and an IFN-group consisting of 29 patients (8 type B and 21 type C) who received IFN therapy for 6 months. Serum samples were tested for a range of antibodies at the start of the study, during therapy and at the end of the 6 month period. Antibodies tested for included: antinuclear, smooth muscle, antimitochondrial, parietal cell and thyroid microsomal. Four (8%) of the total patient group had autoantibodies at the beginning of the study (two in each group). During the follow-up period no patient in the control group developed antibodies compared with 3 (11%) patients in the treatment group. Autoantibodies developed in patients treated with higher doses of IFN and were found in those patients who tended to show a poor response to IFN-therapy. Further studies are needed to establish the relationship between poor response to IFN-α and development of autoantibodies.

KEYWORDS: Viral hepatitis; Autoantibodies; Interferon-α

INTRODUCTION

Chronic viral hepatitis B and C are serious health problems worldwide. About 5-10% of adults infected with hepatitis B virus (HBV) will develop chronic infection[20], with hepatitis C (HCV) this figure is between 50 and 70%[1]. Cirrhosis, with or without hepatocellular carcinoma, occurs in approximately 20% of patients with either type of chronic hepatitis[3,6].

Improvement in serum aminotransferase levels and partial resolution of the inflammatory process are seen in most patients with chronic viral hepatitis who respond to IFN-α therapy[11,19]. However, IFN-α therapy is associated with some adverse effects, including a flu-like syndrome and other systemic disorders[17]. The induction of autoimmune disease may rarely occur, development of autoantibodies without disease is more frequent[2,5,7,12,14,15,18]. Discrepancies in reported incidence rate of autoantibodies may be due to patient's ethnic origin, type and dose of IFN used and the individual autoantibodies induced[2,5,7,14,15,18]. There are further controversies surrounding the relationship between development of autoantibodies and response to IFN therapy[2,5,7,14,15,16,18].

The aims of this study were to investigate the presence and development of antibodies in patients with

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chronic hepatitis B and C undergoing therapy with IFN-α therapy and to determine whether the appearance of autoantibodies was related to the dose and type of IFN used and to response to treatment.

PATIENTS

Fifty consecutive patients with histopathologically proven chronic viral hepatitis were divided into two groups and prospectively studied. The control group consisted of 21 patients with chronic hepatitis (10 type B and 11 type C) who were followed for 6 months without treatment. The IFN-group consisted of 29 patients with chronic hepatitis (8 type B and 21 type C) who were treated for 6 months, as described below.

Inclusion criteria for chronic hepatitis B were HBsAg, HBeAg and HBV DNA positivity, ALT elevated to at least 2.5 times the upper limit of normal and histological features of chronic hepatitis. Criteria for chronic hepatitis C were anti-HCV positivity, ALT elevated to at least twice the upper limit of normal and histological evidence of chronic active (CAH) or chronic persistent hepatitis (CPH).

Exclusion criteria were: severe hepatic, cardiac or renal disease, neuropsychiatric disorder or pregnancy. Eight patients with hepatitis B received IFN-α 2a (Roferon-A®), 4.5 million units (MU) sub-cutaneously (SC), thrice weekly for 6 months and 8 patients with hepatitis C received IFN-α 2a (3 MU) SC thrice weekly for 3 months followed by 1.5 MU for another 3 months. Thirteen patients with hepatitis C received IFN-α 2b (Intron A®); 7 received 3 MU and 6 received 1 MU SC, thrice weekly for 6 months.

For patients with hepatitis B response to IFN-α therapy was defined as disappearance of HBV-DNA, loss of HBeAg and normalization of serum aminotransferase levels and for patients with hepatitis C normalization of serum aminotransferase levels by the end of therapy.

METHODS

Sera were drawn before treatment started, during IFN therapy and at the end of the study period; all samples were stored at -20°C. Samples were initially tested in a dilution of 1:10 for the following autoantibodies : antinuclear (ANA), smooth muscle (SMA), antimitochondrial (AMA), parietal cell (PCA) using indirect immunofluorescence microscopy. ANA was assayed on mice liver imprint and on *Crithidia luciliae*. SMA, PCA and AMA were detected utilizing rat liver and kidney tissue. Thyroid microsomal antibodies (TMA) were detected by radioimmuno-assay-RIA (DYNOtest® - Henning, Berlin).

HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe were assayed before and after IFN-α therapy by ELISA

TABLE 1

Demographic and histopathologic data of all patients with chronic hepatitis B and C.

FEATURES	CONTROL-GROUP	IFN-GROUP	*p*VALUE
n	21	29	
Demographic			
male sex	15 (71%)	20 (69%)	NS
age (median)	37 (21-66)*	40 (25-70)*	NS
white race	17 (81%)	24 (83%)	NS
Histopathologic			
CLH	1 (5%)	0	NS
CPH	2 (10%)	3 (10%)	NS
CAH	18 (86%)	26 (90%)	NS

* years NS-not significant
CLH - chronic lobular hepatitis
CPH - chronic persistent hepatitis
CAH - chronic active hepatitis

(Abbott Laboratories, North Chicago, IL). HBV-DNA was detected by PCR using a technique previously described[9]. Anti-HCV was measured by ELISA -2nd generation (Abbott Laboratories, North Chicago, IL). Serum IgM anti-HAV, anti-HDV and anti-HIV were tested for at the beginning of the study by ELISA (Abbott Laboratories, North Chicago, IL).

This study was approved by the local Ethical Committee. Results were compared using the Chi-square test, T-test and Fisher's exact test. A two-tailed p value of less than 0.05 was considered statistically significant.

RESULTS

Demographic and histopathologic data are shown in Table 1. There were no statistical differences in relation to sex, race or histologic data between the two groups.

All patients were anti-HDV, IgM anti-HAV and HIV negative, but 1 patient with chronic hepatitis B in the IFN-group was anti-HIV positive. Among the 29 treated patients 2 (25%) with chronic hepatitis B and 9 (43%) with chronic hepatitis C were considered to be responders to IFN-therapy.

Four (8%) of the 50 patients in the total group (1/18 type B and 3/32 type C) presented with autoantibodies at the start of the study, 2 in each group. None of the control group developed autoantibodies during follow-up, compared with 3 (11%) in the treatment group (all of whom were previously negative). The profile of autoantibodies titers of the 3 (1/8 type B and 2/21 type C) patients in the IFN-group are shown in Figure 1. The point prevalence of antibodies before therapy and their incidence during the 6 month follow-up are summarized in Table 2.

No correlation was found between the presence of autoantibodies and the type of hepatitis, nor with the type of IFN-α (2a or 2b) used. Autoantibodies developed only in cases treated with higher doses (3 and 4.5 MU). Presence of autoantibodies was not significantly correlated with IFN response, however, a trend in that direction was observed. Five of the 18 non-responders had autoantibodies compared with none of the 11 responders (p=0.07).

During treatment we did not observe any clinical manifestation suggestive of an autoimmune disease, except in one patient who had chronic hepatitis B and a family history of type 2 diabetes mellitus. She received IFN-α 2a (4.5 MU) three times per week for 6 months. Before therapy her borderline glucose tolerance was controlled by diet and her fasting blood glucose was 5 mmol/L. After 1 month of IFN polydipsia and polyuria developed and her fasting blood glucose rose to 12.9 mmol/L. The introduction of glibenclamide (5 mg per day) led to an improvement of her symptoms and a decreased in her blood glucose, without any changes having been made to the IFN regimen. In addition to the autoantibodies evaluated in this study, which were negative, we also tested her for islet-cell and glutamic acid decarboxylase antibodies by protein A immunoperoxidase and immunoprecipitation respectively - both were negative. On the basis of the clinical features and serological results an IFN-in-

TABLE 2

Point prevalence and incidence of autoantibodies in patients with chronic hepatitis B and C - Prevalence data in the whole group and incidence rate in the IFN-group.

FREQUENCY	AUTOANTIBODIES					TOTAL (%)
	ANA	SMA	AMA	PCA	TMA	
PREVALENCE (n=50)	0	2	0	2	0	4 (8.0)
INCIDENCE (n=27)	2	0	0	1	0	3 (11.1)

ANA - antinuclear
SMA - smooth muscle
AMA - antimitochondrial
PCA-parietal cell
TMA - thyroid microsomal

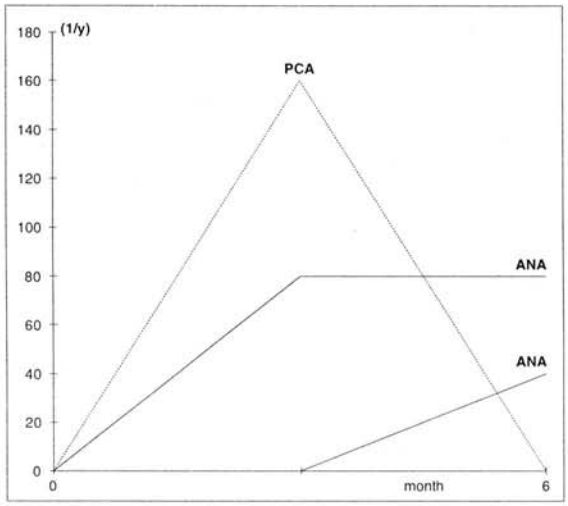

Fig. 1 - Curve of autoantibodies titers (1/y) in 3 patients with chronic viral hepatitis during IFN-therapy.

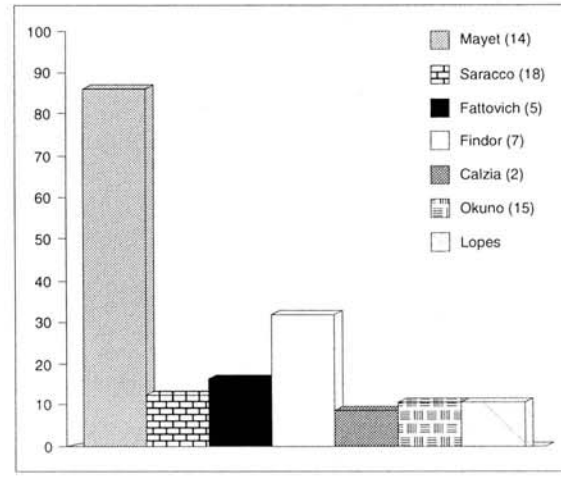

Fig. 2 - Date reported by several authors about development of autoantibody during IFN-therapy of chronic viral hepatitis [author (reference)].

duced exacerbation of type 2 diabetes mellitus was diagnosed[13].

DISCUSSION

Among the 18 cases with chronic hepatitis B, only 1 (5.5%) presented with SMA at the beginning of the study. Several publications report a high prevalence of autoantibodies in this type of viral hepatitis, ranging from 10 to 25%[5,14,18]. Among 32 cases of chronic hepatitis C we found 3 (9.4%) with autoantibodies at the start of the study (2 were PCA positive and 1 SMA positive). SARACCO et al.[18] reported an autoantibody prevalence of 14.5% among 55 cases of chronic non-A, non-B hepatitis. FRIED et al.[8] found a prevalence of 21% for ANA and 55% for SMA in 62 patients with chronic hepatitis C.

None of our patients had AMA or TMA before or during IFN-therapy, in contrast TRAN et al.[22], using RIA to detect TMA, observed a prevalence of 11% in 72 cases of chronic hepatitis C and of 1.6% in 60 cases of chronic hepatitis B.

During follow-up none of the control group developed autoantibodies. Of the 2 cases who presented with autoantibodies, one lost them and the other showed no change in PCA titers. The observed incidence of autoantibodies of 11% in the IFN-group is similar to that found by some authors[2,15], but differs from others[7,14] (see Figure 2). Some of these observed differences may be due to the higher IFN dosage regimens used in some studies[7,14]. We only observed autoantibodies developed in patients who received higher IFN doses. SARACCO et al.[18] in a reply to MAYET et al.[14] suggested that the discrepancy between results may have been due to a difference in the susceptibility of distinct populations to autoimmune disorders.

No differences in autoantibodies incidence were observed in relation to the type of IFN-α used (2a or 2b). In contrast, a recent paper from Germany showed a high incidence of autoantibodies induced by IFN-γ[23]. Development of autoimmunity is mediated by the immunomodulatory mechanisms of IFN and IFN-γ has the unusual property of exerting a relatively greater immunoregulatory effect than IFN-α or β, including induction of HLA class-II antigen expression[4]. Increased expression of HLA class-II antigens is found in several autoimmune diseases[21].

The association between presence of autoantibodies and poor response to IFN therapy has been previously reported by some authors[15,16], but is not universally accepted[5,14,18]. SARACCO et al.[18] observed that patients with chronic non-A, non-B hepatitis, who developed autoantibodies during IFN-therapy, usually showed a good response to treatment.

LISKER-MELMAN et al.[12] reported autoimmune thyroid disease in 6 of 237 (2.5%) IFN-treated patients. Like WEBER et al.[23] we did not find any evidence of induction of autoimmune disease in our patients, although one patient experienced an exacerbation of pre-existing type 2 diabetes mellitus. Nevertheless, IFN can induce

glucose intolerance by impairing the early phase of insulin response to glucose and by reducing the sensitivity of peripheral tissues and liver to insulin[10].

The role of IFN-α in autoantibody induction and in the pathogenesis of autoimmune disease has not been clearly determined. Genetic susceptibility and/or IFN dosage are probably involved but further studies are required to establish whether a poor response to IFN-therapy is related to the development of autoantibodies during treatment.

RESUMO

Estudo dos auto-anticorpos nas Hepatites virais crônicas B e C antes, durante e após tratamento com Interferon-α

Este estudo teve como objetivo avaliar a presença de auto-anticorpos em pacientes com hepatite crônica pelos vírus B e C, antes, durante e após tratamento com interferon-α (IFN-α), assim como estudar a relação destes anticorpos com o tipo de IFN, com a dose e com a resposta terapêutica. Cinqüenta pacientes com hepatite viral crônica foram divididos em 2 grupos: grupo-controle constituído por 21 pacientes (10 hepatites B e 11 hepatites C), que foram seguidos durante 6 meses sem tratamento e grupo-IFN constituído por 29 pacientes (8 hepatites B e 21 hepatites C), que receberam IFN-α durante 6 meses. Anticorpos antinúcleo, antimúsculo liso, antimitocôndria, anticélula parietal e antitireóide foram pesquisados em amostras de soro colhidas antes do início, durante e ao final do estudo. Quatro dos 50 pacientes (8%) apresentavam auto-anticorpos no início do estudo (2 em cada grupo). Durante o estudo nenhum paciente do grupo-controle desenvolveu auto-anticorpos, enquanto que 3 (11%) pacientes do grupo-IFN passaram a apresentá-los. Os auto-anticorpos ocorreram apenas em pacientes tratados com doses mais elevadas de IFN. Verificou-se ainda tendência à resposta desfavorável ao tratamento naqueles indivíduos que apresentaram ou desenvolveram auto-anticorpos. Assim, sugere-se que mais estudos sejam realizados para determinar se existe ou não relação entre resposta desfavorável e a presença ou o desenvolvimento de auto-anticorpos.

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