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# A controlled study of treatment with recombinant interferon alpha in chronic hepatitis B virus infection: induction and maintenance schedules

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## Summary

To determine the antiviral effect of recombinant-interferon (rIFN)-α in hepatitis B virus (HBV) chronic infection, a controlled study was carried out. A total of 20 HBsAg chronic carriers (18 chronic active hepatitis and 2 chronic persistent hepatitis) were included. All of them had remained HBeAg, HBV-DNA and HBV-DNA polymerase (HBV-DNAp) positive at least six months before treatment. The patients were randomly assigned to two groups: control ($n$=10), and treatment ($n$=10). A dose of 5.5 megaunits of rIFN-α/m$^2$ body surface was administered every day for 21 days (induction) and twice a week for six months thereafter (maintenance). No basal differences were observed between the two groups. No case of intolerable toxicity was observed. One treated patient died in a car crash in the second month. At the end of the first week of therapy, 7/10 (70%) of the treated patients became HBV-DNAp negative. However, in the fifth month only 2 patients remained HBV-DNAp negative and also became HBV-DNA and HBeAg negative. In contrast, no changes in viral markers among control cases were observed.

In conclusion, rIFN-α has an antiviral effect on chronic HBV infection; however, the induction plus maintenance schedule is not useful to obtain a permanent effect.

HBV chronic infection; Antiviral therapy; Recombinant interferon

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## Introduction

During the last number of years, several investigators have reported promising results using recombinant human alpha-interferon (rIFN-α) on a daily, or two or three times a week schedule for 12–180 days (Dooley et al., 1986; Dusheiko et al., 1985; Mora et al., 1987; Omata et al., 1985). These studies showed that doses above 9–18 megaunits (MU) daily were poorly tolerated and did not provide a great percentage of seroconversion from HBeAg to anti-HBe.

The purpose of the present work is to evaluate the efficacy of an induction and maintenance treatment schedule, using rIFN-α for 6 months in a prospective controlled trial.

## Materials and Methods

*Patients*

The study was approved by the Ethics committee of our hospital, and signed consent was obtained from all patients.

Twenty patients (15 males and 5 females, mean age: 38.7 yr) were included in the study. All of them were HBsAg, HBeAg, HBV-DNA polymerase (HBV-DNAp) and HBV-DNA positive at least 6 months prior to the start of the study (as checked monthly). In addition, they had constant abnormal transaminase levels. All of them were antiHD and antiHIV negative.

The patients were randomly allocated to two groups. Control group: 10 patients who did not receive any treatment (8 males and 2 females, mean age: 41.5 yr); 9 with chronic active hepatitis (CAH) and 1 with chronic persistent hepatitis (CPH). The treatment group was made up of 10 patients (7 males and 3 females, mean age: 35.9 yr) 9 with CAH and 1 with CPH; they received 5.5 MU of recombinant alpha interferon (rIFN-α2C: Boehringer Ingelheim. > 98% purity) per $m^2$ body surface, i.m., daily for 21 days (induction); and afterwards the same dose, given twice weekly for up to 6 months (maintenance). No statistical difference was observed between the two groups, with respect to the basal clinical, epidemiological and analytical characteristics.

*Laboratory tests*

HBsAg, antiHBs, antiHBc, HBeAg, antiHBe and antiHD were determined by radioimmunoassay (Abbott Lab., North Chicago, IL).

HBV-DNAp was detected following the method of Robinson (1975). Serum DNAp activity was defined as HBV-specific, if an aliquot of the serum treated with swine antiHBs (Nordic Immunology, Tilburg, The Netherlands) showed at least a 50% reduction of DNAp activity as compared to the same sample treated with normal swine serum. All samples were tested in parallel. Serum HBV-DNA was tested by dot-blot hybridization (Weller et al., 1982) using the plasmid pBH20-HBV. Anti HIV antibodies were determined by enzyme immunoassay (Abbott).

Anti-IFN antibodies were determined by a biological assay based on the neu-

tralization of IFN by its antibody (Kawade and Watanabe, 1984); A-549 cells were incubated with the patient's serum dilutions, or the IFN standard at a final concentration of 10 IU/ml. Then, EMC virus suspension was added and the cytopathic effect (CPE) was detected by crystal violet staining. The stained plates were read for the endpoint well showing 50% CPE as compared to the virus controls (100% CPE) and the cell controls (100% viability). The neutralization titer was defined as proposed by the WHO (Billiau, 1984).

T3, T4 and T8 lymphocytes subpopulations were tested using monoclonal antibodies (OKT3, OKT4, OKT8) (Ortho, Raritan, NJ).

Hematological and liver function tests were performed by standard methods (Coulter, SMAC Technicon, NY).

*Follow up and histological studies*

A physical examination and laboratory tests were performed weekly during the first month, every 2 wks until the end of the treatment period, and every 3 months thereafter up until 15 months. At this time a second liver biopsy was obtained in 13 patients (7 treated and 6 controls). Comparison of histological activity between the basal and final biopsies was established using the Knodell's index. Furthermore, liver HBcAg presence was detected in both biopsies by inmunoperoxidase staining (Dakopatts a/s, Glostrup, Denmark).

*Statistical analysis*

Fisher's and correlation tests were used for statistical comparisons.

## Results

All patients completed the study with the exception of one subject, belonging to the treatment group, who died in a car crash at the second month of the study.

*Assessment of antiviral activity: variations of HBV-markers*

At the end of the first week of treatment, 7 out of 10 (70%) treated patients no longer showed HBV-DNAp activity, as compared to 0/10 in the control group ($P<0.001$). This tendency was maintained with some fluctuations until the end of the induction period (Table 1). At the third week of therapy, a rebound was observed, and only 4 patients remained negative for HBV-DNAp. However, at the second, third and fourth month, significant differences in HBV-DNAp positivity were observed again between the two groups (Table 1). At the fifth month, only 2 treated patients (Fig. 1: patients B and E) remained HBV-DNAp negative with no variations until the end of the study. In contrast, none of the control patients became HBV-DNAp negative. Individual quantitative variations of HBV-DNAp in treated patients are shown in Fig. 1.

Only 2 patients (responders) belonging to the treatment group lost serum HBV-DNA at the third and sixth month, respectively, while all the controls remained positive. No changes were observed during the follow-up period.

TABLE 1

Qualitative changes in HBV-DNAp during the study.

| rIFN-Treatment | HBV-DNAp positive (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Induction | | | Maintenance | | | | | |
| | 1 wk | 2 wk | 3 wk | 4 wk | 2 mth | 3 mth | 4 mth | 5 mth | 6 mth |
| Treatment group ($n$=10) | 3 (30%) | 4 (40%) | 6 (60%) | 5 (50%) | 4 (44%) ($n$=9) | 5 (55%) | 4 (44%) | 7 (77%) | 7 (77%) |
| Statistical significance[a] | $p<0.001$ | $p<0.01$ | $p<0.05$ | $p<0.01$ | $p<0.01$ | $p<0.03$ | $p<0.01$ | N.S. | N.S. |
| Control group ($n$=10) | 10(100%) | 10(100%) | 10(100%) | 10(100%) | 10(100%) | 10(100%) | 10(100%) | 10(100%) | 10(100%) |

[a] = Fisher's exact test.
N.S. = not-significant.

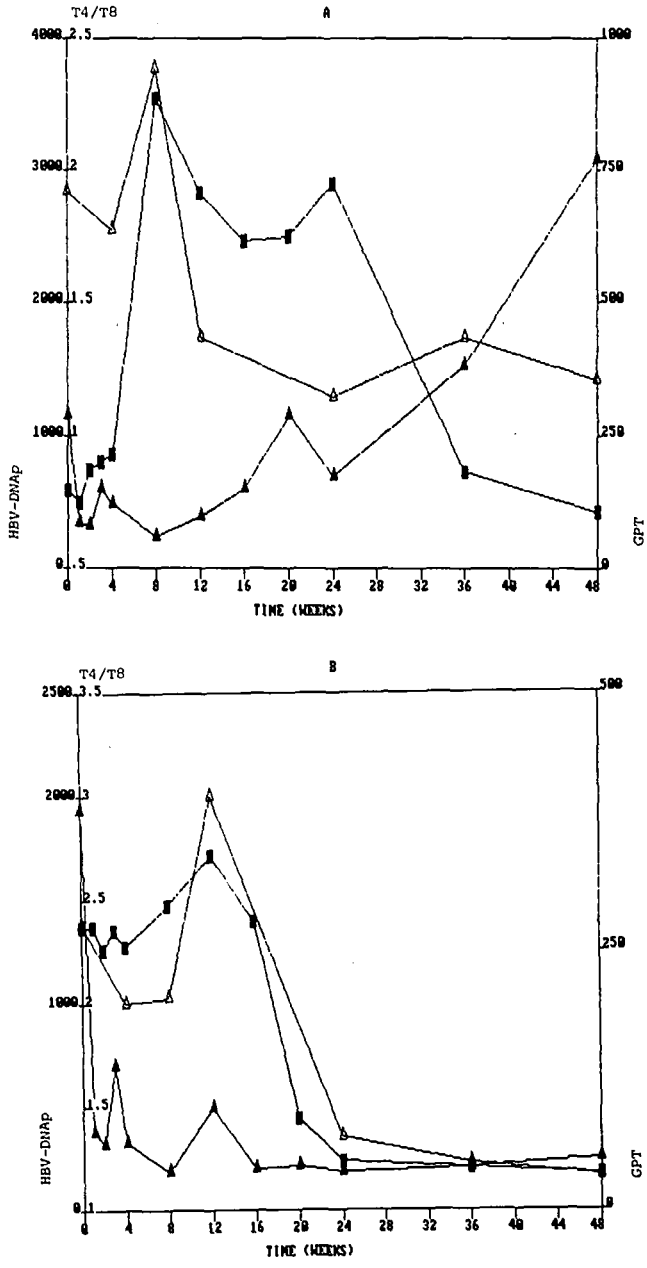
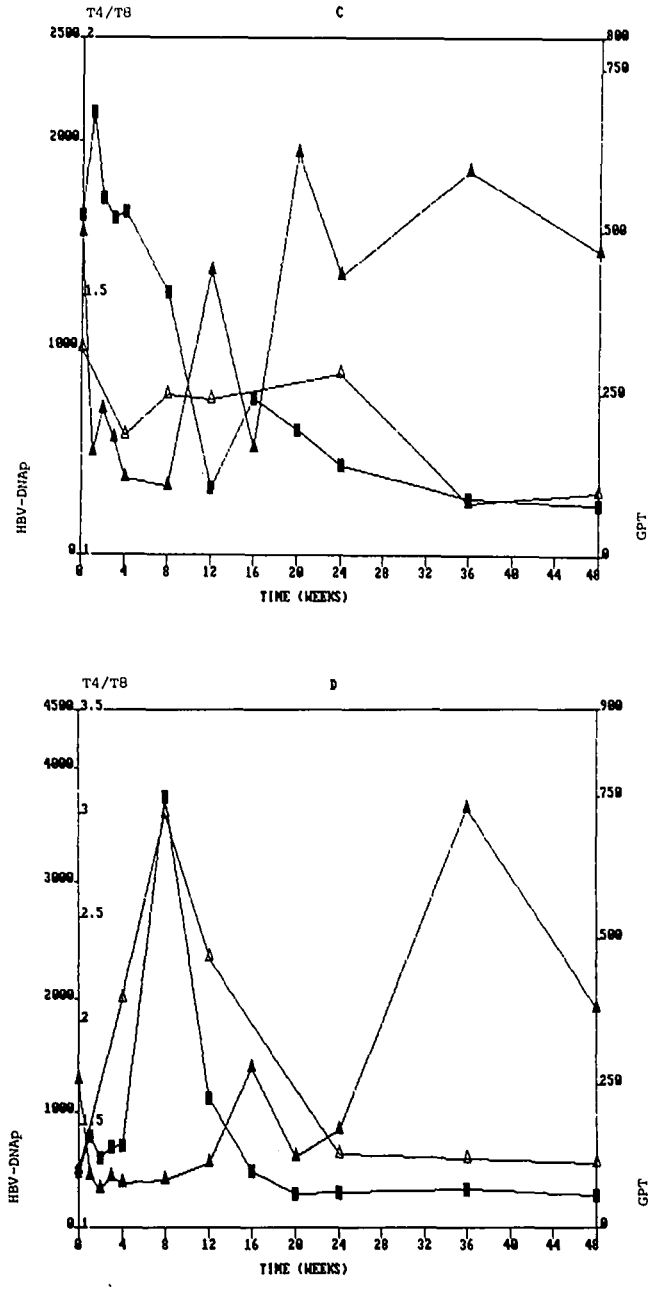



Fig. 1. Changes in HBV-DNAp (▲), GPT (■) and T4/T8 ratio (△) levels in rIFN-treated patients during the study. Results in HBV-DNAp detection are expressed as $^{3}$H-dpm incorporation. Cutoff value: mean dpm of the negative controls + 4 SD. In this assay the cutoff value was 500 dpm. GPT levels are expressed in IU/ml (normal range: 0–45 IU/ml) Only in two cases, a significant correlation between GPT levels and T4/T8 ratio was detected (patient B: $r$=0.945, $P$<0.001; patient D: $r$=0.896, $P$<0.006).
Fig. 1. cont.

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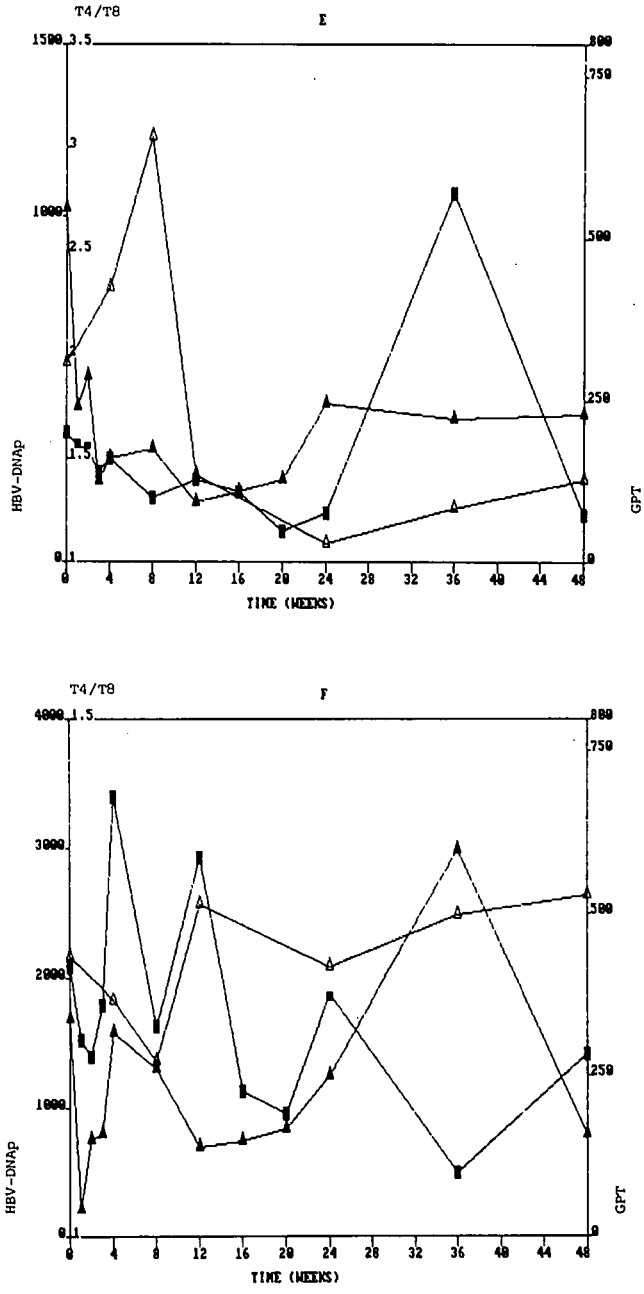


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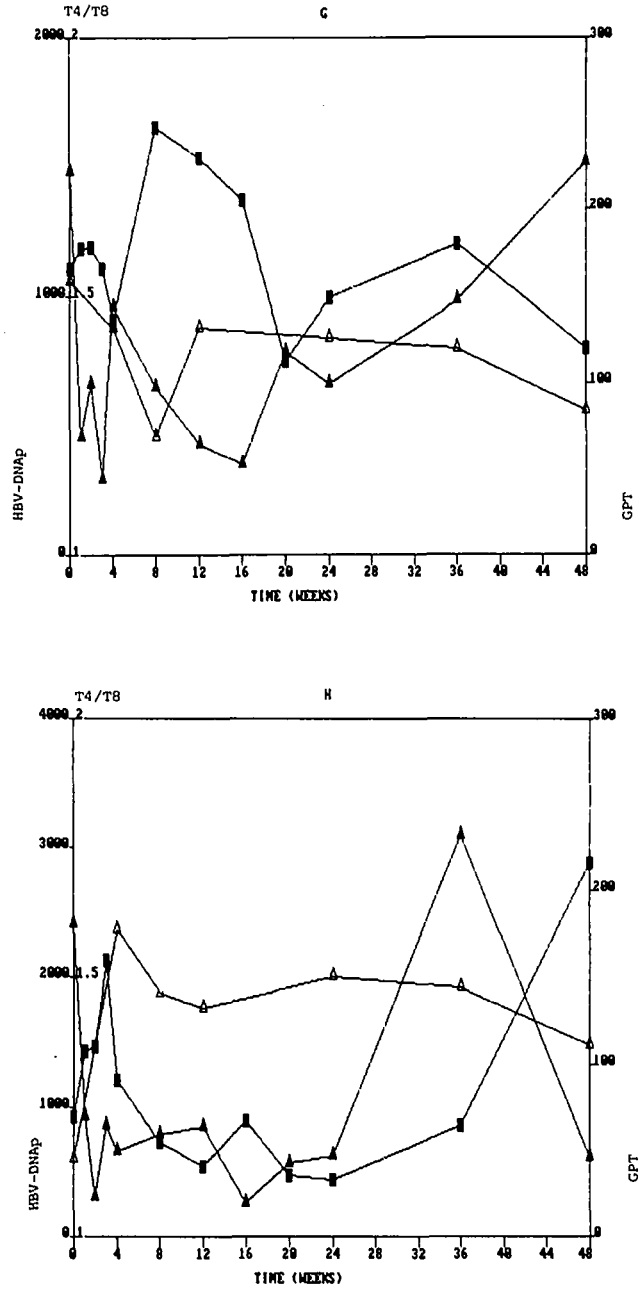
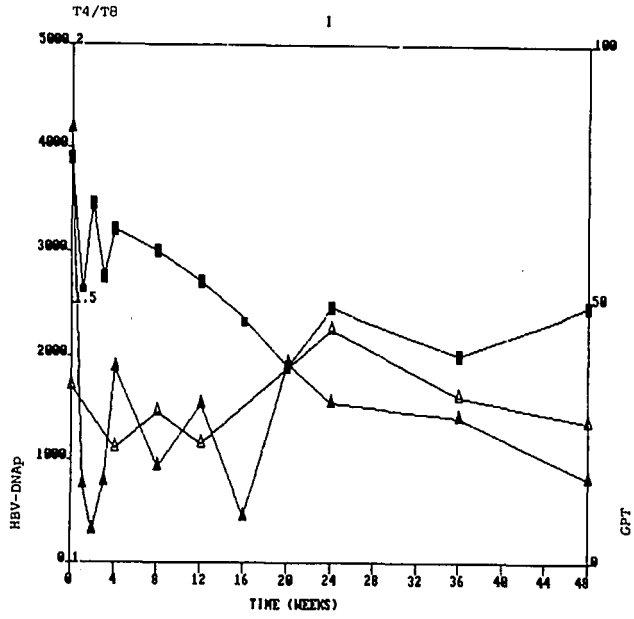



Fig. 1. cont.


T4/T8
I
HBV-DNAp
GPT
TIME (WEEKS)
0
4
8
12
16
20
24
28
32
36
40
44
48

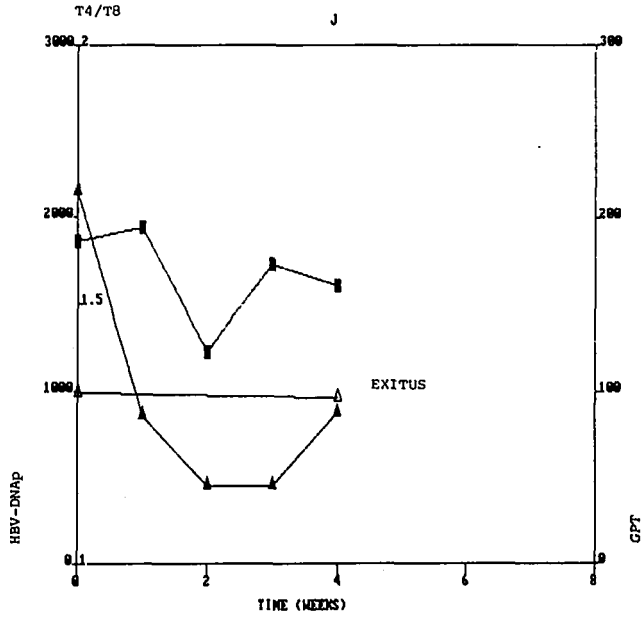

T4/T8
J
EXITUS
HBV-DNAp
GPT
TIME (WEEKS)
0
2
4
6
8

Fig. 1. cont.

With respect to the 'e' system, one patient became HBeAg negative by the third month of therapy (treatment was stopped then), and another, at the sixth month. In addition, both patients developed anti-HBe. No such changes were observed in the control group. This trend remained like that until the 15th month when the study was stopped. None of the patients in either group lost circulating HBsAg.

*Changes in T lymphocyte subpopulations*

No significant variations with respect to T3 lymphocytes were observed (data not shown). In relation to the T4/T8 ratio, at the second month, a significant increase was detected among treated patients as compared to the controls (mean ± SD: 1.88 ± 0.72 vs 1.34 ± 0.09, $P < 0.05$). This rise was due to a decrease in T8 lymphocytes, while T4 did not change at that time. Afterwards, the T4/T8 ratio decreased in treated patients, due to an increase of their T8 lymphocytes. Furthermore, at the sixth month, the T4/T8 ratio in treated patients was significantly lower than the basal one (mean ± SD: 1.58 ± 0.39 vs 1.29 ± 0.16, $P<0.05$). No changes were observed among the control group.

*Changes in liver function tests*

Among treated patients, a progressive increase in the GPT level during treatment was observed. During the follow-up a slight decrease in GPT levels was detected, reaching normal values in the two patients who seroconverted to anti-HBe (Fig. 1: patients B and E). No significant variations were observed in the other treated patients or the control group, until the end of the study.

No significant changes were observed with respect to the other liver function tests (Gamma glutamyl transpeptidase, total bilirubine, alkaline phosphatase, albumin, gammaglobulin, etc.)

*Correlation between T4/T8 ratio, HBV-DNAp and GPT levels*

In the treatment group a significant correlation ($r= 0.945$, $P<0.001$) was found between the T4/T8 ratio and the GPT levels during therapy. Thus, the maximum elevation of both values occurred at the second month, and both T4/T8 and GPT decreased afterwards. With respect to the relation between these parameters and the changes in HBV-DNAp positivity, 55% of the treated patients were HBV-DNAp negative by the second month. Then, a rebound in HBV-DNAp was detected as T4/T8 and GPT levels decreased. Individual changes of HBV-DNAp, GPT and T4/T8 levels in treated patients are shown in Fig. 1.

*Histological evaluation*

When comparing the basal and final liver biopsies, a decrease in the Knodell's index was observed in treated (10.2 ± 5.6 vs 8.6 ± 4.1) and control patients (13.2 ± 4.4 vs 9.3 ± 3.4). HBcAg became undetectable only in the two responder patients. In addition, an amelioration of liver histology was observed in one responder (Knodell's index, basal vs final: 17 vs 2), while the other presented a cirrhotic evolution (13 vs 14).

*Side effects*

No case of intolerable toxicity was observed, and all patients completed the study. A flu-like syndrome was present in all treated patients during the induction period, increasing at the beginning of the maintenance dose and diminishing thereafter. Seven patients lost an average of 10% weight in relation to the pretreatment weight. In addition, 5 patients experienced moderate hair loss during the treatment. All side effects disappeared at the end of IFN therapy. None of the patients developed anti-IFN antibodies. Slight decreases in white blood cells (mean ± SD: 6889 ± 2044 vs 3322 ± 959, $P<0.01$) and platelets (215444 ± 46207 vs 131000 ± 30690, $P<0.05$) were seen after 3 wks of treatment in all patients, reaching the basal values thereafter.

## Discussion

In the last years, IFN has been given at different doses (10-58 MU) and generally 2 or 3 times a week for periods up to 6 months (total doses: 342-2800 MU) (Dooley et al., 1986; Dusheiko et al., 1985; Mora et al., 1987; Omata et al., 1985). In our study, using a total dose of 620 MU, 2/9 patients (22%) responded to the therapy. They became HBV-DNAp, HBV-DNA and HBeAg negative: in addition, they lost liver HBcAg with a regression of the hepatic damage in one case and a cirrhotic evolution in the other. It should be remarked that none of the controls or non-responder patients became negative for HBV replication markers either in serum or liver. The reasons for the relatively poorer results obtained in this controlled trial in contrast to other reports (Dooley et al., 1986; Dusheiko et al., 1985; Mora et al., 1987; Omata et al., 1985), may be several. Our report is the first in which an induction plus maintenance schedule is used. It could be argued that the dose of rIFN we used during the maintenance period, was too low (11 MU/$m^2$ body/surface/week); however, lower doses (7.5 MU/$m^2$ body surface/week) have yielded better results (personal observation, unpublished data). Another argument would be that the 3 times weekly administration could give better results than those we reached (Hess and Meyer zum Büschenfelde 1986), although even with 2 times weekly a higher response rate has been reported (Mora et al., 1987). Our results do not exclude the possibility that a similar schedule (2 or 3 times a wk) and dosage applied up to no more than 4 months may give better results in future trials, since in our hands this schedule used for more than 4 months did not give any additional benefit.

The rIFN-α that we used had an antiviral effect, since 7 treated patients became negative to HBV-DNAp during the first week of therapy, and the difference with respect to HBV-DNAp positivity between control and treated patients remained significant up to the fourth month. After the 3 wk-induction period, a rebound in HBV-DNAp levels was observed. The explanation for this rebound is not clear. An immune depletion induced by the daily administration of rIFN could be the cause, although this should be proven by further studies with closer monitoring of the immunological changes. After 2 months of rIFN-α therapy, an increase in the

T4/T8 ratio and GPT levels were observed. This tendency could be related to the immunological changes that have been previously reported (Horning et al., 1982). Thus, the expression of target antigens (HBcAg, HLA) and the increase in T4/T8 ratio, both facilitated by IFN (Basham and Merigan, 1983; Horning et al., 1982; Mondelli et al., 1982) could lead to hepatic necrosis and negation of HBV-DNAp as demonstrated in our study.

All the patients completed the 6 month therapy period. The adverse effects were similar to those reported in other studies (Dooley et al., 1986; Dusheiko et al., 1985; Mora et al., 1987; Omata et al., 1985), and their severity was lower during the first 21 days than afterwards. This could be due to the fact that when more distance between the IFN administrations is allowed, the intensity of the secondary effects increases (Lok et al., 1984). The changes in white blood cells and platelets were higher during the induction period, as could be expected, since the depression in bone marrow seems to be dose dependent (Borden, 1984).

In summary, although an antiviral effect of rIFN-α was demonstrated in our study, the schedule and dosage selected did not give convincing results. The use of rIFN two or three times a week directly from the beginning, as has been demonstrated in other reports (Dooley et al., 1986; Dusheiko et al., 1985; Mora et al., 1987; Omata et al., 1985) might be a better approach.

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