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Double-Blind, Randomized Controlled Trial of Interleukin-2 Treatment of Chronic Hepatitis B

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Pilot studies have demonstrated that recombinant interleukin 2 (rIL-2) has an indirect antiviral activity against hepatitis B virus, but the minimal dose of rIL-2 for induction of this effect was not defined. The aim of the study was to ascertain the most efficient dose of rIL-2 for induction of the loss of detectable serum HBV-DNA or a 50% or greater decrease in its level. Thirty-one patients with chronic hepatitis B, hepatitis B e antigen and serum HBV-DNA positive were enrolled in this double-blind randomized controlled trial. Patients were divided: Group I (n = 8) placebo; Group II (n = 7) treated with 0.9 MU of rIL-2 subcutaneously administered daily for 8 weeks; Group III (n = 8) treated with 1.8 MU of rIL-2 under the same schedule; Group IV (n = 8) which received 3.6 MU of rIL-2 under the same conditions. At the end of treatment 25% of the patients in the placebo group, and 13% and 25% in rIL-2 groups III and IV, respectively, had a decrease in HBV-DNA higher than 50% of the basal value. None of the patients lost serum HBV-DNA. Only three patients (one from group II and two from group IV) normalized the ALT levels. Overall, during treatment, ALT levels decreased in the treated groups. This decrease occurred simultaneously with an increase in serum HBV-DNA concentration. Since the response rate in the treated groups was similar to that of the placebo group, rIL-2 is not useful as monotherapy for the treatment of chronic hepatitis B at the doses and schedules used in this study. ***J. Med. Virol. 54:167–172, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: hepatitis B virus; antiviral therapy

INTRODUCTION

The hepatitis B virus (HBV) infection is a worldwide problem, and it is estimated that 350 million people are chronically infected. The chronic hepatitis B infection may progress to liver cirrhosis and hepatocellular carcinoma. Several antiviral and immunomodulatory compounds have been used for the treatment of chronic HBV infection [Hoofnagle et al., 1984; Price et al., 1986; Mutchnick et al., 1991], but at present, only alpha interferon (alpha-IFN) has given promising results. However, only about 50% of the patients treated with alpha-IFN respond to the therapy with loss of serum HBV-DNA, anti-HBe seroconversion, and ALT normalization [Smicht et al., 1983; Scully et al., 1987; Hoofnagle et al., 1988]. There is, therefore, a need for new treatments so as to increase the number of responder patients.

The patients who develop HBV chronic infection present an inadequate immune function with respect to alpha-IFN, interleukin 2 (IL-2), major histocompatibility gene complex class I antigen display, and the 2′5′ oligoadenylate synthetase system [Poitrine et al., 1985; Ikeda et al., 1986; Chu et al., 1987; Anastassakos et al., 1988].

It has been demonstrated in several pilot studies of IL-2 therapy in patients with chronic hepatitis B that IL-2 enhances natural killer cells, induces lymphokine activated cells, and stimulates the production of tumor necrosis factor, alpha, and gamma interferon [Nishioka et al., 1987; Mizoguchi et al., 1988; Kakumu et al., 1988; Vogel et al., 1989]. In these studies, in which low doses of IL-2 (between 3×10^5 IU and 1.2×10^6 IU for up to 28 days) were administered, a decrease in viral replication was found. However, the minimal dose of IL-2 required for achieving a decrease of viral replication was not identified.

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TABLE I. Basal Characteristics of the Patients*

	I Placebo (n = 8)	II 0.9 MU (n = 6)	III 1.8 MU (n = 8)	IV 3.6 MU (n = 8)
Demographic data				
Age (years)	27 (±10)	31 (±16)	35 (±11)	24 (±5)
Height (cm)	177 (±5)	174 (±6)	174 (±6)	174 (±11)
Weight (Kg)	74 (±8)	73 (±9)4	75 (±15)3	71 (±16)
Previous treatment	4	4	3	3
Biochemistry and virology				
ALT (IU/L)	151 (±80)	95 (±41)	147 (±61)	109 (±51)
ALT (Ratio)	3.3 (±1.7)	2.2 (±1)	3.2 (±1.3)	2.6 (±1.3)
HBV-DNA (pgr/ml)	247 (±249)	264 (±150)	247 (±212)	385 (±338)
Histology				
CPH	1 (13%)	3 (50%)	1 (13%)	2 (25%)
CLH	2 (25%)	0	0	3 (38%)
CAH	5 (63%)	3 (50%)	7 (88%)	3 (38%)
Cirrhosis	0	0	2 (25%)	0

*CPH, Chronic persistent hepatitis; CLH, chronic lobulillar hepatitis; CAH, chronic active hepatitis.

The aim of this study was to define the most effective dose of IL-2, administered subcutaneously for 8 weeks, which induces a 50% or greater decrease in serum HBV-DNA compared to the base line values.

PATIENTS AND METHODS

The study was designed as a double-blind, randomized, placebo-controlled trial using three doses of r-IL2 (EuroCetus, Amsterdam, The Netherlands), administered subcutaneously. The sample size was calculated assuming a 20% response rate in the placebo group and 80% response in the most active dose. According to these parameters, 16 evaluable patients were needed in each group in order to detect a significant difference at the 5% level, with a power of 90%. Considering a possible drop-out rate of above 12%, 18 patients per group were required.

The trial began with the first 40 consecutive patients enrolled in the different hospitals participating in the study between March and August, 1992. However, as the preliminary results were not as expected, due to side effects, no further patients were included. A total of 31 patients were evaluable according to protocol. All 31 patients had a histologically confirmed chronic liver disease and none had received antiviral or immunosuppressive therapy during the year preceding study initiation.

The patients had serum HBsAg documented for at least 6 months, as well as HBeAg and HBV-DNA and abnormal ALT within 6 weeks of the first injection. Other causes of liver disease were excluded, and the patients were anti-HDV, anti-HCV, and anti-HIV negative. The base line features of the patients are summarized in Table I.

Two months before study entry, serial determinations of serum HBV-DNA concentration were performed in each patient in order to avoid inclusion of patients showing fluctuations higher than 50% in HBV-DNA concentration. Patients were allocated randomly into four groups: Group I (n = 8), placebo; Group II (n = 7), treated with 0.9 MU of rIL-2 daily for 8 weeks; Group III (n = 8), treated with 1.8 MU of rIL-2 under the same schedule, and Group IV (n = 8) which received 3.6 MU of rIL-2 under the same schedule as for groups II and III.

The patients were studied every 4 weeks during treatment and post-treatment follow-up (12 additional weeks after the end of therapy). A clinical examination was undertaken during each visit, and blood samples were taken for blood cell counts, biochemical liver function tests and HBV markers. The trial was approved by the Ethical Committees of the participating hospitals, and a written, informed consent was obtained from each patient.

LABORATORY TESTS

Serum HBV-DNA concentration was determined prospectively by a liquid phase hybridization test (Abbott Laboratories, North Chicago, IL). Patients who had a decrease in serum HBV-DNA concentration of 50% or more, or who had undetectable HBV-DNA at the end of treatment, were considered as responders. HBeAg and anti-HDV were determined by commercial immunoassays (Abbott Laboratories). Anti-HCV was tested by ELISA (Ortho Diagnostic System, San Diego, CA) and anti-HIV by ELISA (Abbott Laboratories). Liver function and haematological tests were carried out by standard methods.

STATISTICAL ANALYSIS

The normal distribution of the sample was analyzed by the Kolmogorov-Smirnof test. The Student's *t*-test, the ANOVA test, the Snedecor and Cochran test, and, the X^2 test were used when applicable.

RESULTS

All but one patient from group II finished the treatment. This patient was removed from the efficacy analysis so only six patients from group II were considered for that purpose.

At the end of the treatment period (8 weeks), no differences were observed in the frequence of decrease of serum HBV-DNA between the placebo and patients treated with rIL-2. Thus, 2/8 (25%) in the placebo

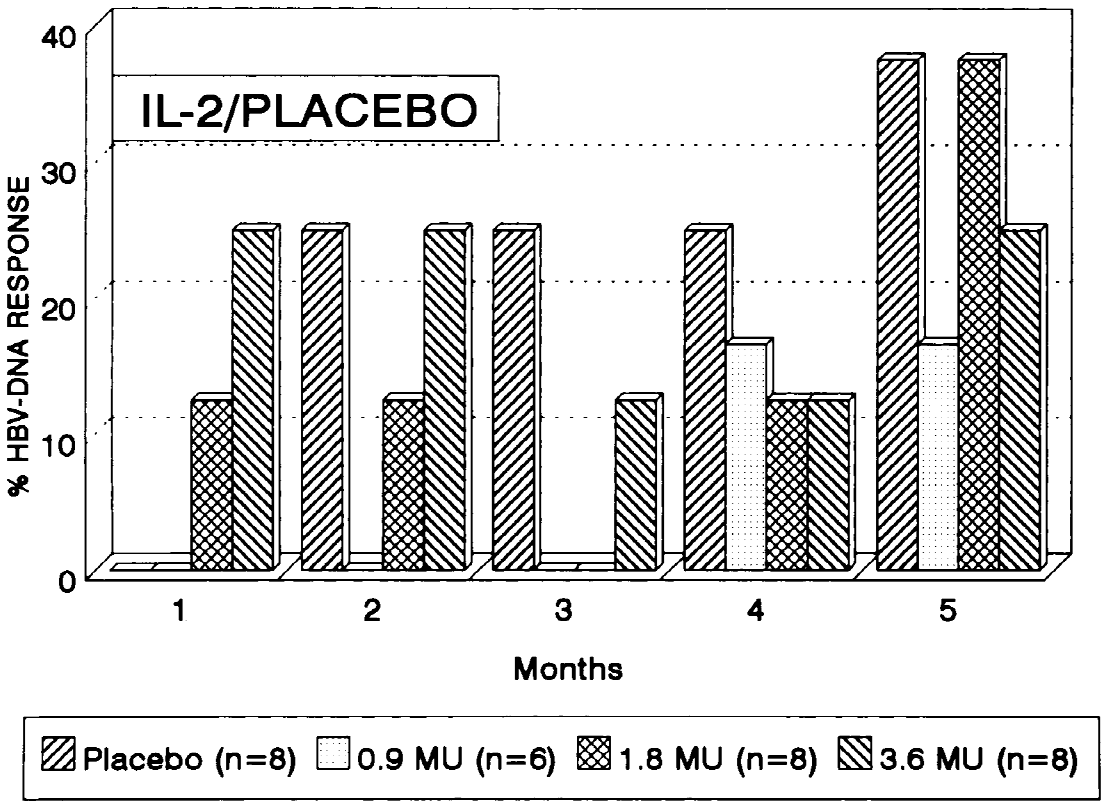


Fig. 1. Percentage of HBV-DNA response in each group during rIL-2 treatment and follow-up.

group, and 1/8 (13%) and 2/8 (25%) in IL-2 groups III and IV, respectively, had a decrease in serum HBV-DNA higher than 50% of the base line value. A similar trend was observed at the end of posttreatment follow-up (placebo 3/8 [35%] and groups II 1/6 [17%], III 3/8 [38%], and IV 2/8 [25%]; Fig. 1). Furthermore, no differences with respect to the frequency of HBV-DNA clearance were observed between the placebo and treatment groups, either at the end of treatment (0% in each group), or at the end of follow-up (1/8, 13% in the placebo group and 1/6, 17% and 1/8, 13% in groups II and IV, respectively).

None of the patients lost HBeAg during the treatment period. However, at the end of the posttreatment follow-up, 2/8 (25%) patients from the placebo group (group I) and 1/8 (13%) from group III were HBeAg negative. Only one of these three patients (from the placebo group) developed anti-HBe.

ALT values during treatment were normal in only three patients (1/6 [17%], group II and 2/8 [25%], group IV). At the end of follow-up, ALT levels were normal in 2/8 (25%) patients from group I, 1/6 (14%) from group II, and 2/8 (25%) from group IV. Overall, the ALT values decreased during treatment in the three rIL-2 treated groups of patients, without change in the placebo group. This decrease in ALT values in the patients treated with IL-2 occurred simultaneously with an increase in serum HBV-DNA concentration (Fig. 2).

With respect to the predictive response factors, defined the response as loss of HBV-DNA, or a >50% decrease in its concentration, responder patients to the rIL-2 therapy had higher ALT levels (135 ± 72 IU/l) than the nonresponders (113 ± 49 IU/l), although the differences did not reach statistical significance. In contrast, the responder patients had statistically lower HBV-DNA levels (91 ± 47 pg/ml) than the nonresponders (390 ± 264 pg/ml; $P < 0.05$).

Of the patients included in the study, 14 had been nonresponders to a previous IFN therapy carried out more than 1 year before the rIL-2 treatment and of these, only one (from group IV) responded to the rIL-2 therapy.

Side effects were a moderate local reaction at the site of injection and a flu-like syndrome occurred in most of the patients. One patient (from group II) suffered an allergic reaction and was dropped from the study after 2 weeks of therapy (not included in the analysis).

DISCUSSION

Several antiviral and immunomodulatory agents have been used for the treatment of the chronic hepatitis B virus infection [Hoofnagle et al., 1984; Price et al., 1986; Mutchnick et al., 1991]. At present, only alpha interferon has given promising results [Smicht et al., 1983; Scully et al., 1987]. However, approximately 50% of the patients treated do not respond to the therapy. It is therefore necessary to search for new antiviral strategies. Taking into account the pathologi-

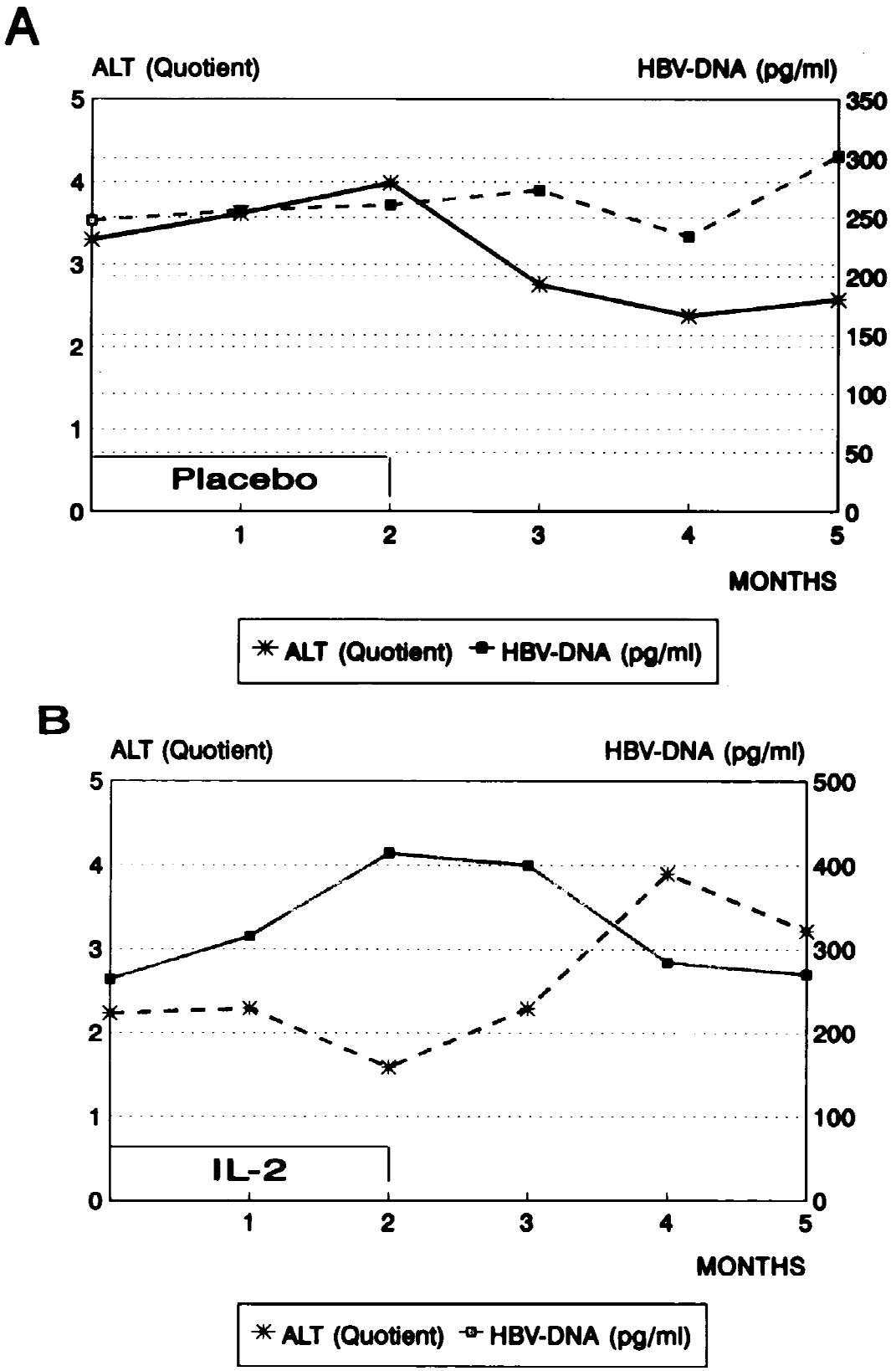
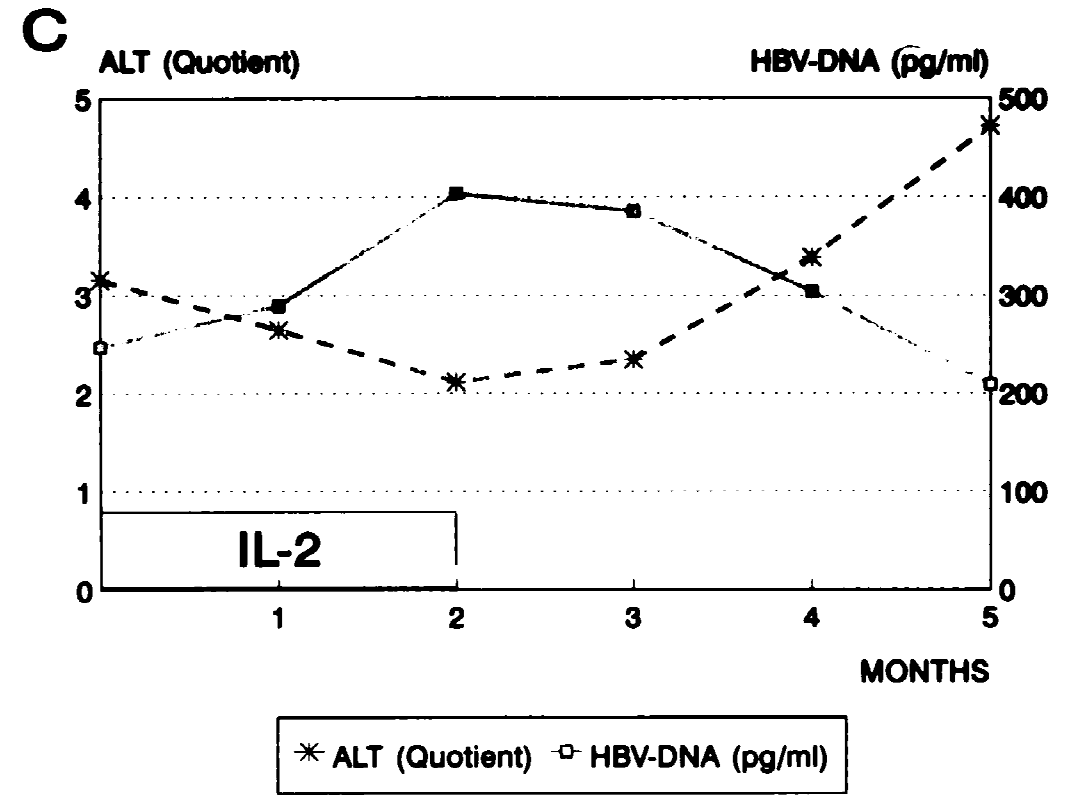


Fig. 2. ALT and HBV-DNA levels during treatment and follow-up. **A:** Placebo; **B:** group II (0.9 MU of rIL-2); **C:** group III (1.8 MU of rIL-2); **D:** group IV (3.6 MU of rIL-2).



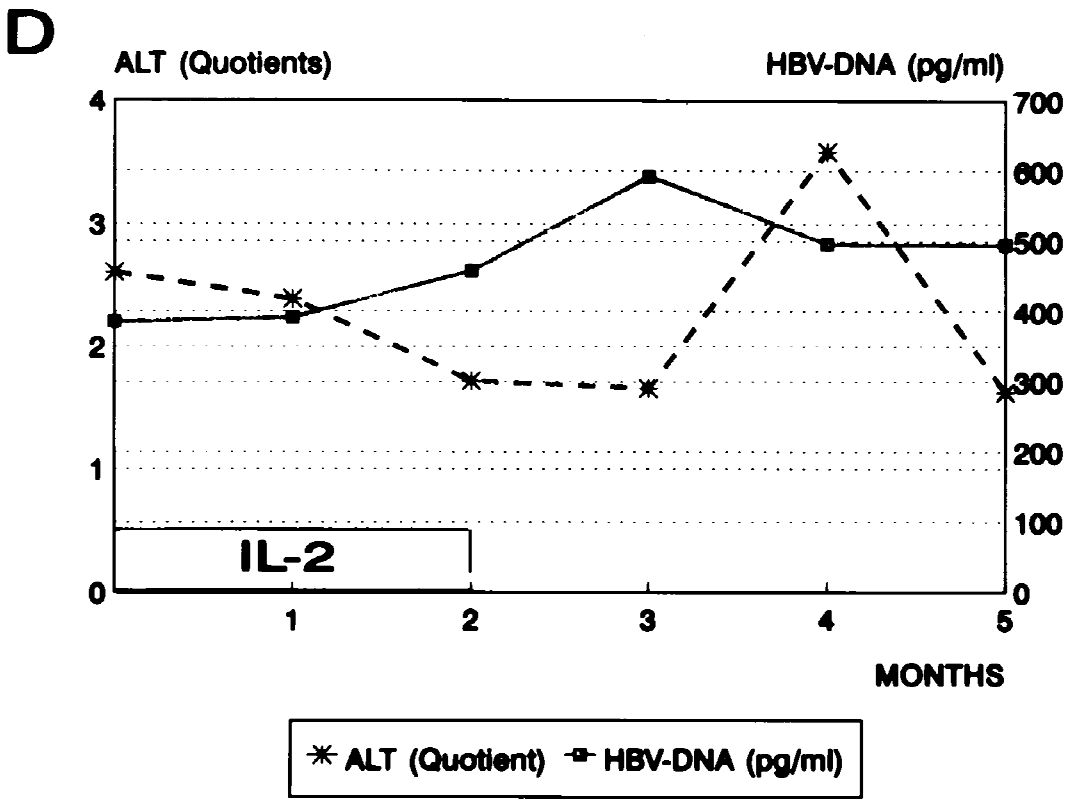


Fig. 2. Continued.

cal mechanism of liver damage in chronic hepatitis B, mediated by T cytotoxic lymphocytes, IL-2 may be useful as a treatment for these patients [Nishioka et al., 1987]. Thus, IL-2 increases IFN-gamma production and stimulates CD4 helper cells; and in consequence, enhancement of the T cytotoxic lymphocytes may occur.

For the above reasons, a double-blind placebo-controlled trial was undertaken, using three different doses of rIL-2, administered subcutaneously to patients with chronic hepatitis B. We did not obtain an antiviral effect of rIL-2 in these patients, as the decrease in serum HBV-DNA concentration and the frequency of HBV-DNA clearance were similar in the treated and placebo patients. Moreover, no differences in the frequency of loss of HBeAg were observed between treated and placebo groups.

Our results differ from pilot studies published previously. In this context several investigators [Nishioka et al., 1987; Kakumu et al., 1988; Vogel et al., 1989] observed HBV-DNA polymerase and serum HBeAg clearance in as many as 40% of patients with chronic hepatitis B treated with IL-2. Several explanations may exist for the discrepancies between our results and those published previously. All previous studies concerning IL-2 were pilot studies, without a control group, and it cannot thus be discounted that the antiviral effect in those studies was due to spontaneous seroconversion [Realdi et al., 1980]. We administered rIL-2 subcutaneously, while in other studies administration was intravenously and this may influence the results. Finally, the doses that we used in two groups (1.8–3.6 MU) were substantially higher than those used by other investigators (0.3–0.9 MU); although we did not obtain an antiviral effect with the dose of 0.9 MU. Moreover, all our patients were Caucasian; in the other studies, the patients were of Oriental origin, and racial differences may influence the response to treatment in chronic hepatitis B. In contrast, our results are in agreement with those obtained by Bruch et al [1993], who found that in Caucasian patients, the combination of alpha interferon and IL-2 did not improve the response rate obtained with interferon alone. Several of the patients who were included in the study, and treated with rIL-2, had not responded to a previous cycle of IFN alpha therapy, and also failed to respond to rIL-2. This finding is not surprising, as the predictive response factors to rIL-2 that we found (ALT levels and HBV-DNA concentration) are identical to those of alpha-IFN [Brook et al., 1989]. Furthermore, we observed that during rIL-2 treatment, there is an inverse correlation between the ALT and HBV-DNA levels. Thus, the HBV-DNA levels increased during treatment, while the ALT values decreased. In this sense, although IL-2 induces T cell proliferation it has been shown that it also induces apoptosis of activated T cells [Oda et al., 1997]. This mechanism may be responsible of the effect observed in ALT and HBV-DNA.

In conclusion, this study has demonstrated that IL-2 is not useful in the treatment of chronic hepatitis B at the doses and schedules used in this study.

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