Thymosin Treatment of Chronic Hepatitis B: A Placebo-controlled Pilot Trial

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Chronic hepatitis B is a severe and frequently progressive disease. We assessed the safety and efficacy of thymosin fraction 5 and thymosin-α, in a prospective, placebo-controlled trial in 12 patients with chronic hepatitis B. All patients had histological and biochemical evidence of active liver disease for at least 6 mo before treatment and were positive for serum hepatitis B virus DNA and HBsAg. Seven patients received thymosin fraction 5 or thymosin-α, and five patients received placebo twice weekly for 6 mo. By the conclusion of the study (1 yr), serum aminotransferase levels had improved significantly in thymosin-treated patients, but not in the placebo group. Six (86%) of the thymosin treated patients and one (20%) patient given placebo cleared hepatitis B virus DNA from serum (p < 0.04, Fisher's exact test). After treatment, replicative forms of hepatitis B virus DNA were present in the liver specimens of four of five placebo-treated patients but in only one of seven thymosin-treated patients (p < 0.04, Fisher's exact test). Response to thymosin therapy was associated with significant improvements in peripheral blood lymphocyte and CD3 and CD4 counts and in in vitro production of interferon-γ over initial values. No significant side effects were observed in patients given thymosin or in placebo-treated patients. Clinical, biochemical and serological improvement in patients responding to thymosin were sustained during 26 ± 3 mo of follow-up. The results of this pilot trial suggest that thymosin therapy promotes disease remission and cessation of hepatitis B virus replication in patients with chronic viral infection. (Hepatology 1991;14:409-415.)

Chronic hepatitis B, a common form of liver disease, is associated with increased risk of cirrhosis, liver failure and HCC (1, 2). Impaired effectiveness of host cellular immune mechanisms in clearing HBV-infected hepatocytes has been proposed to explain development of chronic HBV infection (3, 4). Antiviral agents have been used to treat this disease; among them, interferon (IFN)-α has emerged as the most efficacious (5-7). IFN-α induces remission in less than 50% of patients and has significant side effects that sometimes lead to early cessation of therapy (5-8).

Clinical trials using immune modifiers in the treatment of chronic HBV infection, such as transfer factor, interleukin-2 (IL2) and levamisole have been inconclusive or have resulted in unsatisfactory responses (9-11). Another class of immune modifiers, thymosin fraction 5 (TF5) and thymosin-α, (Ta,), has been shown to trigger maturational events in lymphocytes, to augment T cell function and to promote reconstitution of immune defects (12). These thymosins may provide an alternate approach to the treatment of chronic HBV infection.

TF5, originally described by Goldstein et al. (13), is a partially purified extract of bovine thymus containing at least 40 peptide components, 20 of which have been purified to homogeneity or near-homogeneity (12). Ta1, initially isolated from TF5, has been sequenced and chemically synthesized (14). Ta1 is an acidic peptide (mol wt = 3,108) that has shown activity similar to that of TF5 in modulating the maturation of T cells (15). TF5 and Ta1 can influence immunoregulatory T cell function; promote IFN-α, IFN-γ and IL2 production by human lymphocytes; and increase lymphocyte IL2 receptor expression (16-21).

Clinical trials of TF5 and Ta1 as primary or adjuvantive therapy in patients with immunodeficiency or cancer indicate that these agents enhance immune responsiveness and augment specific lymphocyte functions (22). We describe here the results of a placebo-controlled pilot study designed to assess the potential efficacy and safety of TF5 and Ta1 in treatment of chronic hepatitis B.
MATERIALS AND METHODS

Subjects. Patients between the ages of 18 and 70 yr with chronic hepatitis B were included, based on the following criteria: documented presence of HBsAg and elevated serum ALT levels for at least 6 mo, positive serum test for HBV DNA, histological confirmation of chronic hepatitis (23) within 3 mo of randomization and evidence of mild or moderately decompensated liver disease (prolongation of prothrombin time < 4 sec over control values, serum albumin ≥ 3 g/dl and serum total bilirubin ≤ 4 mg/dl). Additional requirements included a hemoglobin level of 10 g/m, a platelet count of at least 70,000/mm³, a WBC count of at least 3,000/mm³, a polymorphonuclear cell (PMNC) count of 1,500/mm³ or less and serum creatinine level of at least 1.4 mg/dl. Patients with histories of hepatic encephalopathy, bleeding esophageal or gastric varices or previous antiviral or immunosuppressive therapy were excluded. Additional causes for exclusion included history of intravenous drug abuse, presence of hepatitis D antibody, other causes of liver disease, malignancy, pregnancy, homosexuality or a positive test for antibody to human immunodeficiency virus. Women agreed to practice birth control for the duration of the study (1 yr) but to avoid use of contraceptive medications.

Study Protocol. In this three-arm, double-blind, controlled study, patients were randomly assigned by a computer-generated program to receive TF5 (90 mg/m² body surface area), Ta, (900 μg/m² body surface area) or placebo by subcutaneous injection twice weekly for 6 mo. TF5, synthetic Ta, and placebo (1.4% sodium bicarbonate) were supplied by Alpha One Biomedicals, Inc. (Foster City, CA). Patients were instructed on self-administration of TF5, Ta, or placebo. Compliance was monitored weekly by nurse clinicians who maintained records of injection schedules. Patients were asked specifically about side effects by the nurse clinicians, who in turn transmitted this information to a physician monitor not directly involved in the clinical trial. In previous clinical trials, subcutaneous injection of TF5 has been associated with induration and pain at the injection site. Patients were instructed to inform only the nurse clinicians of local discomfort at the injection site. The physician monitor in turn made the determination to continue treatment or, if the patient was receiving TF5, to change the treatment to Ta. The patients who were experiencing local discomfort resulting from TF5 and the nurse clinicians to whom they reported could sustain such discomfort or could be reenrolled on TF5 without consent of the physician investigators, however, remained unaware of local reactions or changes in treatment. Patients were seen at 2-wk intervals for 6 mo and then monthly for an additional 6 mo. Clinical and laboratory assessments were obtained at each visit and included serum analysis for HBsAg, antibody to HBsAg, HBeAg and antibody to HBeAg, HBV DNA, ALT, AST, total bilirubin, alkaline phosphatase, BUN, creatinine, cholesterol, uric acid and total protein. Monthly determinations of serum albumin; prothrombin time; hemoglobin; WBC, PMNC, lymphocyte and platelet counts; and urine analyses were made. Immunological analyses were conducted before treatment and monthly thereafter for the entire study period (1 yr). Analysis of peripheral blood lymphocytes included determination of absolute numbers for CD3, CD4, CD8, CD11 and natural killer (NK) subsets by indirect immunofluorescence staining using a modification of a previously described method (24), by concomitant A- and phytohemagglutinin-P-induced lymphocyte transformation (25) and by peripheral blood mononuclear cell (PBMC) production of IFN-γ using solid-phase RIA (IMRIX Interferon-γ RIA; Centocor Inc., Malvern, PA). Blood samples obtained from healthy adult volunteers were included for each of the above assays and acted as a panel of normal values used in statistical analyses. These volunteers consisted of health-care providers without evidence of acute infection who were not using immunosuppressive medications and had no histories of malignancy, recent surgical intervention or blood component infusion. Percutaneous liver biopsy was repeated in all patients at 1 yr and in most patients at 6 mo. A positive response to treatment was defined at the end of 1 yr as a sustained loss of serum HBV DNA, HBeAg (if present initially) and normalization or near-normalization of ALT levels to less than 1.2 times the upper limit of normal and a value at least 75% lower than the inclusion value.

The protocol was approved by the Human Investigation Committee of Wayne State University School of Medicine, where all patients were studied. Informed, written consent was obtained from all patients.

Viral Markers. Serum HBsAg (AUSRIA II; Abbott Laboratories, North Chicago, IL) and antibody to HBsAg (AUSAB, Abbott Laboratories) were determined by RIA and both HBeAg and HBc antibody (HBeAg; Abbott Laboratories) were determined by ELISA. Antibody to the hepatitis delta virus was tested by RIA (Anti-Delta RIA; Abbott Laboratories) and antibody to hepatitis C virus was determined by ELISA (Anti-HCV Assay Kit; Ortho Diagnostics, Raritan, NJ). Viral marker studies were accomplished at the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health.

Histological Assessment. All liver biopsy specimens stained with hematoxylin and eosin and with trichrome were analyzed under code by a single observer (H.D.A.) according to a system devised for asymptomatic CAH (Knodell index; 23). Specimens were also stained for HBcAg using the peroxidase-antiperoxidase techniques (26). When possible, portions of liver biopsy tissue were frozen in liquid nitrogen and analyzed by hybridization for the presence of HBV DNA molecular forms.

HBV Hybridization Studies. Serum was analyzed for the presence of HBV DNA sequences by spot hybridization as previously reported (27), except that the proteinase K digestion step was not included. This increased the sensitivity of the assay by a factor of 10, with little increase in background radioactivity obtained with negative control serum. This assay detected as little as 0.05 to 0.10 pg purified HBV DNA applied as a simple spot in a 5-μl aliquot. Sera negative by direct spot hybridization were confirmed as negative by extracting total viral DNA (25) and applying the total extract as a single spot (27). For these studies, it was critical to use a highly purified HBV DNA probe that did not produce a positive hybridization signal with 0.1 μg control plasmid DNA (pBR322) applied to the filter.

Total DNA was isolated from liver biopsy tissues, subjected to electrophoresis and hybridized as previously reported (28). Purified HBV DNA (3,200 bp) was labeled with 32P phosphorus to high specific activity (2 to 8 × 10⁶ cpm/μg DNA) by random primer extension (29).

For patients in whom frozen pretreatment liver biopsy tissue was not available, the presence of HBV DNA in the liver was determined by in situ hybridization of formalin-fixed and paraffin-embedded tissue used for histological analysis. In situ hybridization was performed by a modification of the method of Prosser et al. (30) using a sulfurlabeled plus (+) strand HBV RNA probe. The probe included a portion of the HBsAg coding sequence (map position 180 to 490 bp from the EcoRI site); it was prepared from a recombinant pGem3Z plasmid containing this sequence and linearized with restriction enzyme EcoRI, followed by RNA synthesis under direction of SP6 polymerase and the SP6 promoter.
The biological effects of TF5 and Tal are similar (22, and combined. The final study groups consisted of seven patients. The response to treatment were similar for the TF5 and Tal treatment arms, the two thymosin groups were combined. The completion of the trial (12 mo)

and biochemical and serological parameters (Table 1)

were comparable with respect to sex, age

receiving placebo. At inclusion, the thymosin and

receiving TF5 or Tal (thymosin group) and five patients

were randomized to receive T(x,. The known

levels at 1 yr; these were significantly lower than corresponding values in patients treated with placebo (169 ± 68, p < 0.05 and 172 ± 53, p < 0.02, respectively). However, transient ALT elevations (two to six times higher than preinclusion values) were observed in five of the six responders to thymosin. The duration of these ALT flares was 4.6 ± 0.6 wk (n = 5), and they preceded clearance of HBV DNA in each case (Fig. 1).

Long-term follow-up of the thymosin group (27 ± 3 mo) revealed persistently negative tests for serum HBV DNA in the six responders and normalization of ALT levels in all seven patients (29 ± 5 IU/L). After 14 mo of follow-up, two thymosin responders (29%) had cleared HBsAg and exhibited HBs antibody.

Before randomization, the 12 patients had significantly decreased peripheral blood lymphocyte (p < 0.01), CD3 (p < 0.02), CD4 (p < 0.05) and CD11 (p < 0.05) counts compared with healthy volunteers (n = 67). No differences were noted in CD8 and NK counts or in CD4/CD8 ratios between the patients and healthy volunteers. No significant differences were observed in lymphocyte, T cell subset or NK counts between the thymosin and placebo groups at inclusion. Figure 2 shows that within 1 mo of beginning treatment, the thymosin group exhibited higher lymphocyte and CD4 count (p < 0.02, respectively). These increases were usually sustained during the 6-mo follow-up. No significant changes in these parameters were noted in the placebo group. The lymphocyte and CD3 values for the thymosin group achieved significantly higher values than the placebo group at the 4-mo interval (p < 0.05) and at both the 4- and 5-mo intervals for the CD4 count (p < 0.05).

At inclusion, no differences were found in in vitro IFN-γ production or in concanavalin A and phytohemagglutinin-P lymphocyte proliferation assays between the study groups or between either study group and healthy volunteers. After inclusion, PBMC synthesis of IFN-γ in the thymosin group rose to levels

Statistics. Group means were compared by Student’s two-tailed t test. Changes in measurements between the inclusion values and subsequent time points were compared by Student’s two-tailed paired t test.

RESULTS

Twelve patients (11 white and 1 black) were assessed in this pilot study. Four patients were randomized to receive TF5, three to receive Tal, and five were randomized to receive placebo. Two patients assigned to the TF5 arm experienced local discomfort at the injection sites and after 2 wk were treated with Tal. The known biological effects of TF5 and Tal are similar (22), and since analysis of the pretreatment characteristics and response to treatment were similar for the TF5 and Tal treatment arms, the two thymosin groups were combined. The final study groups consisted of seven patients receiving TF5 or Tal (thymosin group) and five patients receiving placebo. At inclusion, the thymosin and placebo groups were comparable with respect to sex, age and biochemical and serological parameters (Table 1).

None of the patients tested positive for antibody to hepatitis delta virus, and only one patient (who responded to Tal, treatment) was positive for antibody to hepatitis C virus.

Clearance rates for HBV DNA, HBeAg and HBsAg at completion of the trial (12 mo) are shown in Table 2 and indicate a significantly higher HBV DNA clearance rate in the thymosin group compared with the placebo group (86% vs. 20%, respectively; p < 0.04 by Fisher’s exact test). Serum HBV DNA levels decreased in six of the seven patients given thymosin during the 6-mo treatment period. Serum HBV DNA disappeared during treatment in four of these six patients and at 2 and 6 mo after treatment in the remaining two patients.

The thymosin group had normal or near-normal ALT (42 ± 5 IU/L, S.E.M.) and AST (43 ± 7 IU/L, S.E.M.) levels at 1 yr; these were significantly lower than

### Table 1. Characteristics of study groups at inclusion

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treated group (TF5/Ta)</th>
<th>Control group (placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>M:F</td>
<td>5:2</td>
<td>3:2</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>47 ± 15</td>
<td>48 ± 17</td>
</tr>
<tr>
<td>Duration of HBsAg presence (yr)</td>
<td>2.6 ± 2.3</td>
<td>2.1 ± 2.2</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>196 ± 164</td>
<td>116 ± 41</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>160 ± 151</td>
<td>176 ± 78</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.7 ± 0.2</td>
<td>1.3 ± 1.2</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>4.1 ± 0.5</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Prothrombin time (sec)</td>
<td>13.1 ± 0.7</td>
<td>12.9 ± 0.9</td>
</tr>
<tr>
<td>HBV DNA (0-5)</td>
<td>2.1 (0.5-3.0)</td>
<td>1.6 (0.5-2.5)</td>
</tr>
</tbody>
</table>

*Values are mean ± S.D. Differences between groups were not statistically significant.

**Normal values: ALT < 40 IU/L, AST < 45 IU/L, bilirubin < 1.5 mg/dl; albumin 3.5-5.2 gm/dl.

*Geometric mean (range).

### Table 2. HBV marker seropositivity at inclusion and at 12 mo

<table>
<thead>
<tr>
<th>HBV marker</th>
<th>Thymosin-treated (n=7)</th>
<th>Placebo (n=5)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Initial</td>
<td>7 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td></td>
<td>12 mo</td>
<td>1 (14%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>HBeAg</td>
<td>Initial</td>
<td>6 (86%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td></td>
<td>12 mo</td>
<td>1 (14%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Initial</td>
<td>7 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td></td>
<td>12 mo</td>
<td>6 (86%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>HBeAg (liver)</td>
<td>Initial</td>
<td>7 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td></td>
<td>12 mo</td>
<td>1 (14%)</td>
<td>4 (80%)</td>
</tr>
</tbody>
</table>

NS = not significant.

*By Fisher’s exact test.
higher than those seen with the healthy volunteers, whereas values in the placebo group were lower (Fig. 3).

Results of liver biopsy histological activity scores are presented in Table 3. During the 12-mo study period, histological activity scores in six of the seven thymosin-treated patients decreased; they rose slightly in the seventh patient. The histological score increased in four placebo patients and decreased in the fifth. All 12 chronic hepatitis B patients had cirrhosis at the time of the initial biopsy, and since cirrhosis was still demonstrable in the last biopsy specimen from six of the seven thymosin-treated patients and in four of the five placebo-treated patients, the changes in score were due almost entirely to changes in inflammatory activity and lobular damage. A p value <0.01 was considered significant (Table 3). At 12 mo, HBcAg was not detected by peroxidase-antiperoxidase staining in liver biopsy specimens from the six responders to thymosin and in the one patient given placebo, who experienced spontaneous remission. However, HBcAg was identified in the single nonresponder to thymosin treatment and in the four remaining placebo patients (Table 2).

HBV DNA molecular forms in liver were examined in three thymosin-treated patients for whom pretreatment and 6-mo and 12-mo tissue specimens were available. One patient (the single nonresponder to thymosin treatment) had replicative forms of HBV DNA in all three samples. In the remaining two patients, treatment with thymosin was associated with elimination of HBV replicative forms seen in the initial specimens. In one of these patients, no HBV DNA molecular forms were identified in the final specimen, whereas in the other supercoiled and relaxed circular genomes remained in the final 12-mo specimen (Fig. 4, responder). Two other patients who responded to thymosin showed supercoiled HBV DNA but no replicative intermediates in the 6-mo and 12-mo specimens, respectively. In two patients in whom pretreatment frozen liver was not available, cessation of HBV DNA replication during thymosin treatment was demonstrated by in situ hybridization of paraffin-embedded tissues used for histological studies.
**FIG. 3.** Serial analysis of IFN-γ production by PBMCs obtained from the TF5/Ta₁ group (○) and the placebo group (●). Comparisons were made between the patient groups and with healthy volunteers (n = 67) with a 95% confidence limit (demarcated by parallel lines). *p < 0.05 when value was compared with inclusion value by Student's paired t test. ▲ = Time at which treatment was initiated; ▼ = treatment was completed.

**TABLE 3.** Histological grading of liver biopsy specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Thymosin-treated (n = 7)</th>
<th>Placebo (n = 5)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion</td>
<td>11.1 ± 2.0</td>
<td>9.8 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Excluding</td>
<td>7.1 ± 2.0</td>
<td>5.8 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final specimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12 mo)</td>
<td>Total score</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.4 ± 1.3</td>
<td>11.6 ± 1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Excluding</td>
<td>4.8 ± 1.3</td>
<td>7.8 ± 1.9</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = not significant.
*p values were determined using Student's t test.

Values are expressed as mean ± S.D.

Therefore, objective evidence of cessation of HBV replication in liver tissue by Southern-blot or in situ hybridization analysis was obtained in all patients who responded serologically and histopathologically to thymosin treatment. Four of five placebo-treated patients had replicative forms of HBV DNA in both the pretreatment and 12-mo biopsy specimens (Fig. 4, nonresponder). The remaining placebo patient (who had spontaneous remission) was negative by Southern blotting for HBV DNA molecular forms in the 12-mo specimen.

Therapy with TF5 and Ta₁ was not associated with significant side effects. Three patients reported local discomfort at TF5 injection sites during the first 2 wk. Two of these patients were changed to Ta₁ without further difficulty, but the third insisted on continuing TF5 treatment. In the latter patient, local discomfort gradually disappeared without subsequent complications. No local, systemic or constitutional symptoms were observed with Ta₁ administration. Throughout treatment and follow-up, no alterations were observed in hematological status, biochemical parameters or renal function (data not shown). Seven patients (five treated, two placebo) complained of mild to moderate fatigue before randomization. At the conclusion of the study (1 yr), one treated patient (nonresponder) and one placebo patient still experienced fatigue.

**DISCUSSION**

Thymic extracts or thymus-derived peptides have previously been used in clinical trials in the treatment of chronic hepatitis B. In one study (31), thymostimulin (an extract of calf thymus) resulted in clinical, biochemical and histological improvement; however, treatment effect on HBV markers was not reported.

**FIG. 4.** Results of HBV DNA hybridization studies of liver biopsy specimens in a nonresponder given placebo and in a responder treated with thymosin. Southern-blot analysis of 10 μg DNA for each specimen examined revealed that in lanes A, B and C from the patient treated with placebo, all the expected forms of HBV DNA replicative intermediates are present in high amounts and were unchanged over the 12-mo study period (A, inclusion; B, 6 mo; C, 12 mo). Similar replicative forms were present at inclusion (lane D) in the patient given Ta₁, but only supercoiled and relaxed circular free genomes were present at 6 mo (lane E) or 12 mo (lane F).
Similar responses have been reported in other studies using thymopeptide (32), porcine thymic extract (33) and thymic factor x (34). In a recent preliminary investigation (35), six chronic woodchuck hepatitis virus (WHV) carrier woodchucks were given twice-weekly subcutaneous injections of Ta, for 28 wk. At the conclusion of treatment, serum WHV DNA levels were undetectable in four of the treated animals and were depressed 100-fold in the two remaining animals. No changes were identified in serum WHV DNA levels in any of the six untreated control animals.

This study was performed to assess the safety and potential efficacy of TF5 and Ta, in treatment of chronic hepatitis B. In previous studies, thymosin was shown in vitro to decrease spontaneous cell-mediated cytotoxicity in patients with chronic hepatitis B (36) and to enhance concanavalin A-induced suppressor cell function in PBMCs from these patients (37).

TF5 and Ta, are not known to possess antiviral properties (38). The results of this trial suggest that the salutary responses to these agents may be derived from the modulation of immune responses by these peptides. The decreased peripheral blood lymphocyte count observed in the 12 patients with chronic hepatitis B before treatment has not, to our knowledge, been reported previously. The decrease in total T cells (CD3, CD11) and the CD4 subset are findings consistent with previous studies (39).

The significant increases in lymphocyte and CD3 and CD4 counts observed in the thymosin-treated patients parallel the in vitro effects of thymosin as reported previously (20). IFN-γ production by PBMCs from patients with chronic hepatitis B at entry into the study did not differ from healthy control subject values, as reported previously (40, 41). Therefore enhanced production of IFN-γ by PBM from thymosin-treated (but not placebo-treated) patients, observed during the 12-mo study (Fig. 3), may represent an in vivo thymosin effect on immunoregulatory function (17, 19, 20).

Although the mechanism(s) by which the thymosin mediates their effects is unknown, evidence suggests that Ta, may function in a manner similar to that of IFN-α. The C-terminal sequence of IFN-α shares homology (36%) with prothymosin-α, the precursor form of Ta, (38). Unlike the N-terminal domain of IFN-α, which may direct antiviral activity, the C-terminal domain may be responsible for IFN-α immunomodulatory activity. Furthermore, the octapeptide corresponding to the region of highest homology between IFN-2α and Ta, competes for the same receptor on thymocytes responsible for induction of proliferation in the presence of concanavalin A (38).

For the most part, resolution of disease and loss of HBV replication occurred gradually. HBV DNA did not redevelop in any of the six patients who responded to thymosin (26 ± 3 mo), and all six patients have normal ALT values (26 ± 5 IU/L) at this writing. Histological improvement in the 12-mo liver biopsy specimens from treated patients suggests decreased inflammation, hepatocyte damage and necrosis (Table 3). Hybridization studies showed either no HBV DNA molecular forms or residual free genomes (but not replicative intermediates) in the final liver biopsy specimens (12 mo) of respondents to thymosin therapy. In placebo-treated patients, however, replicative HBV DNA persisted in the liver tissue, except for the single patient with spontaneous remission. The patient illustrated in Fig. 4 who responded to Ta, (lanes D, E and F) became serum HBsAg- and HBV DNA−negative during the 6 mo of Ta, treatment and remained negative for the 6 mo after therapy, at which time antibody to HBs developed. This suggests that although HBV DNA persisted in the liver tissue in free genome form, the HBV infection had become latent because neither viral protein production (HBsAg) nor active virus replication (HBV DNA) was detected.

The patients in this study all had histological evidence of active cirrhosis. Our results suggest that TF5 and Ta, are nontoxic in the dosages used and may promote resolution of disease activity in patients with chronic hepatitis B. Furthermore, improvement in clinical, immunological and histological parameters is associated with cessation of HBV replication and either elimination of HBV DNA from liver tissue or conversion from replicative forms to free genomes, with transition to a latent form of infection. A randomized, controlled, multicenter study will be required to establish the efficacy of thymosin in treatment of chronic hepatitis B.

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REFERENCES


