ORIGINAL ARTICLE

A Randomized, Controlled, Clinical Study of Thymosin Alpha-1 Versus Interferon-Alpha in Chinese Patients with Chronic Hepatitis B Lacking Hepatitis B Envelope Antigen

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Background: This study was designed to compare the efficacy and safety of thymosin-α1 (T-α1) with that of interferon-α (IFN-α) in patients with chronic hepatitis B who were positive for hepatitis B virus (HBV) DNA and hepatitis B envelope antibody (anti-HBe).

Methods: Fifty-six patients were randomly divided into groups A and B. Both groups were comparable ($p > 0.05$) at baseline regarding age, sex, and alanine aminotransferase (ALT) levels. Group A patients received T-α1 1.6 mg subcutaneously twice weekly, while group B patients received IFN-α 5 million IU daily for 15 days, then thrice weekly for 6 months. Results from the 2 groups were compared with data from a group of 30 patients never treated with IFN-α and who were followed-up for 12 months (historical control [HC] group); the 3 groups were comparable ($p > 0.05$).

Results: After treatment, a complete response (ALT normalization and HBV DNA loss) occurred in 8 of 26 patients in group A (30.8%) and 14 of 30 in group B (46.7%; $\chi^2 = 1.476$, $p = 0.224$). After a follow-up period of 6 months, a complete response was observed in 11 of 26 patients in group A (42.3%) and 7 of 30 in group B (23.3%; $\chi^2 = 2.299$, $p = 0.129$). The rate of complete response was significantly greater in the IFN-α than HC group at the end of therapy (46.7% vs 3.3%; $\chi^2 = 15.022$, $p = 0.0001$), and in the T-α1 than HC group at the end of follow-up (42.3% vs 3.3%; $\chi^2 = 12.566$, $p = 0.0001$). Ten of the 12 T-α1 responders (i.e. partial responders; 83.3%) experienced sustained, non-detectable HBV DNA after 6 months' treatment; 6 of the 14 T-α1 non-responders (42.9%) showed a delayed response of non-detectable HBV DNA during the follow-up period. Corresponding values for group B patients were 50% (9/18) and 0% (0/12). The rate of delayed response was significantly higher in group A than the other 2 groups ($\chi^2 = 6.686$, $p = 0.010$; $\chi^2 = 4.964$, $p = 0.038$), whereas the rate of flare was higher in group B than in the other 2 groups ($\chi^2 = 3.445$, $p = 0.063$; $\chi^2 = 7.668$, $p = 0.006$), during the follow-up period. Unlike IFN-α, T-α1 was well tolerated, i.e. no adverse effects were noted in group A.

Conclusion: These results suggest that a 6-month course of T-α1 therapy is effective and safe in patients with anti-HBe-positive chronic hepatitis B; T-α1 can reduce HBV replication in such patients. Compared with IFN-α, T-α1 is better tolerated and seems to induce a gradual and more sustained normalization of ALT and loss of HBV DNA. Combination therapy with T-α1 and IFN-α or nucleoside analogs for hepatitis B warrants further study. [*J Chin Med Assoc* 2005;68(2):65–72]

Key Words: chronic hepatitis B, hepatitis B envelope antigen, interferon-alpha, thymosin alpha-1

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Introduction

Chronic viral hepatitis is the principal cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC) worldwide and now ranks as the chief reason for liver transplantation in adults.[1–4] The World Health Organization estimates that hepatitis B virus (HBV) has infected more than 350 million people worldwide, and up to 20% of those infected will go on to become chronic carriers and be at significant risk of cirrhosis and HCC. The ultimate goals of therapy for chronic hepatitis B are to prevent progression to cirrhosis and the development of HCC. Various subgroups of hepatitis B surface antigen (HBsAg)-positive patients with chronic hepatitis have been identified. Typical HBsAg-positive patients have hepatitis B envelope antigen (HBeAg) and HBV DNA in serum during the active phase of the disease, and usually show disease remission if they seroconvert to HBeAg antibody (anti-HBe). However, a subset of HBsAg-positive patients lacks HBeAg, but instead has anti-HBe and HBV DNA present in serum. The latter form of hepatitis is characterized by a progressive and relapsing disease course with fluctuations in viral replication[5,6] and a poor response to interferon-alpha (IFN-α).[7–18]

The treatment of chronic hepatitis B is directed towards inhibition of viral replication, followed by elimination of the virus. Histologic improvement of hepatic inflammation and normalization of aminotransferase levels are additional treatment goals. At present, the only treatment of proven benefit in chronic viral hepatitis is IFN-α. In chronic hepatitis B, a 4–6-month course of IFN-α is effective in inducing clearance of HBV DNA and HBeAg from serum, and in improving serum aminotransferase levels and liver histology in 25–40% of patients.[17] Chronic HBV infection is believed to result, in part, from an inadequate host immune response to the virus.[19,20] Thus, immunomodulatory drugs, including thymic humoral factor-γ2 (THF-γ2) and thymosin-α1 (T-α1) have been tested in clinical trials both as monotherapy and in combination with IFN-α.[21–30]

T-α1 is an immune modifier (a 28-amino acid peptide) that triggers maturational events in lymphocytes, augments immunoregulatory T-cell function, and promotes reconstitution of immune defects.[31,32] While not completely understood, the mechanism of T-α1 action is thought to be immune system modulation through augmentation of T-cell function.[33] *In vitro* studies resulted in T-cell differentiation and maturation, with increases in CD4+, CD8+ and CD3+ cell counts, interferon-γ, interleukin (IL)-2, and IL-3. Antigen-stimulated expression of IL-2 receptors was also observed. Immunosuppressed animals given T-α1 experienced a cytoprotective effect that led to increased survival time and numbers. T-α1 promotes disease remission and cessation of HBV replication in patients with HBeAg-positive chronic hepatitis B, and without significant adverse effects.[21–30] Moreover, clinical trials of T-α1 in patients with immunodeficiency or cancer indicate that this agent is nontoxic, enhances immune responsiveness, and augments specific lymphocyte functions, including lymphoproliferative responses to mitogens, maturation of T-cells, antibody production, and T-cell-mediated cytotoxicity.[34,35] Based on these observations, we conducted a randomized, controlled trial to compare the efficacy and safety of T-α1 with that of IFN-α in anti-HBe-positive and HBV DNA-positive chronic hepatitis B patients.

Methods

Study patients

Fifty-six Chinese patients were enrolled in the study. All patients met the following criteria: age over 18 but less than 60 years; the presence of HBsAg in serum for at least 1 year; the presence of serum anti-HBe and HBV DNA documented on at least 2 occasions, at least 3 months apart, within a period of 12 months before randomization; elevated serum alanine aminotransferase (ALT) on at least 2 occasions, at least 3 months apart, with a value of ≥ 1.5 times the upper limit of normal for at least 12 months; and liver biopsy features consistent with chronic hepatitis. Liver biopsy must have been performed in the year before screening. Eligible patients with evidence of cirrhosis were also included. Additional requirements were a hemoglobin value of ≥ 100 g/L; a platelet count of ≥ 60,000/mm^3; a white cell count of ≥ 3,000/mm^3; a polymorphonuclear leukocyte count of ≥ 1,500/mm^3; and normal renal function, with normal serum creatinine levels. Candidates were required to have compensated liver disease and no history of hepatic encephalopathy or ascites, or esophageal or gastric varices at risk of bleeding. Principal patient characteristics at study enrollment are shown in Table 1. The following patients were excluded: those treated with immunosuppressive or antiviral therapy in the year before entry; those with concurrent hepatitis C virus, hepatitis δ virus, or human immunodeficiency virus infection; those with causes of liver disease other than HBV; intravenous drug abusers; pregnant women; women unwilling to practice contraception during the study; and patients with malignancy, decompensated

Table 1. Patient characteristics at study entry*

	T-α1 group (n = 26)	IFN-α group (n = 30)	HC group (n = 30)
Male:female (n)	23:3	23:7	22:8
Age (yr)†	47 ± 12	40 ± 11	45 ± 13
Duration of infection (yr)†	9.0 ± 4.8	8.7 ± 4.2	9.5 ± 5.3
Cirrhosis at entry (n)	3	3	5
Previous IFN-α therapy (n)	0	0	0
Serum ALT (U/L)†§	188.7 ± 102.6	191.5 ± 106.5	186.9 ± 117.4
Serum AST (U/L)†§	144.5 ± 86.5	158.6 ± 81.8	146.2 ± 73.3
Albumin (g/L)†§	46 ± 8	46 ± 7	47 ± 4
Total bilirubin (μmol/L)†§	18.4 ± 9.3	15.9 ± 8.6	16.3 ± 8.4
Serum HBV DNA‡			
$< 5.0 \times 10^5$ copies/mL	7 (27)	12 (40)	11 (37)
$\geq 5.0 \times 10^5$ copies/mL	19 (73)	18 (60)	19 (63)

**No significant differences were observed between groups; †data are expressed as mean ± standard deviation; ‡data are expressed as number (%); §normal values: ALT < 40 IU/L; AST < 40 IU/L; albumin 35–55 g/L; total bilirubin < 17 μmol/L.*
ALT = alanine aminotransferase; AST = aspartate aminotransferase; HBV = hepatitis B virus; HC = historical control; IFN-α = interferon-α; T-α1 = thymosin-α1.

liver disease, chronic renal failure, or other serious medical illnesses or psychiatric problems that might interfere with the trial.

Thirty patients with the same virologic and clinical characteristics, and who had never been treated with IFN-α, were followed-up for ≥ 12 months and served as a historical control (HC) group.

Study protocol

The 56 patients were randomly divided into 2 groups to receive a 6-month course of either T-α1 (Zadaxin®, supplied by SciClone Pharmaceuticals Inc, San Mateo, CA, USA) 1.6 mg subcutaneously twice weekly (n = 26), or IFN-α (SINOGEN®, supplied by Shenzhen Kexing Biotech Co Ltd, Shenzhen, China) 5 million IU subcutaneously daily for 15 days and then thrice weekly (n = 30); patients in the HC group were followed-up without specific treatment. All patients were assessed every 2 weeks for the first 2 months, and then monthly, for a total study duration of 12 months. After the initial study period, patients were enrolled for long-term follow-up, during which they were seen at irregular intervals.

At each visit, patients were examined, and blood samples were taken for biochemical and hematologic analyses, and for HBV markers. Clinical and laboratory assessments comprised a detailed history, including post-injection symptoms, physical examination, routine serum biochemical tests (serum ALT, aspartate aminotransferase [AST], r-glutamine transpeptidase [r-GT], alkaline phosphatase [AKP], albumin, globulin, bilirubin), complete cell count, markers of HBV replication, and urinalysis. All biochemical and hematologic tests were performed with routine automated techniques. HBV markers (HBsAg, anti-HBsAg antibody, HBeAg, anti-HBe antibody, anti-hepatitis B core [HBc] antibody, and logarithm of anti-HBc antibody) were detected by enzyme-linked immunosorbent assay (ELISA) methods. Serum HBV DNA levels were measured by polymerase chain reaction (PCR) assay: this assay has a sensitivity of 5.0×10^2 copies/mL, and its linear quantification ranges between 5.0×10^3 and 5.0×10^7 copies/mL.

At a long-term follow-up visit, blood samples were taken for analysis of HBV DNA and ALT levels. Patients were monitored for compliance with the study protocol by injection-vial counts, telephone communication, and attendance at scheduled outpatient appointments.

Clinical responses

Clinical responses were evaluated, both at the end of treatment and follow-up. A virologic response was defined as sustained disappearance of serum HBV DNA, and a biochemical response as sustained normalization of serum ALT (on at least 2 consecutive occasions, at least 3 months apart). At the end of treatment and follow-up, a complete response (CR) was defined as sustained serum HBV DNA-negative status, and normalization of ALT activity. A delayed response (DR) was defined as non-detectable HBV DNA during the follow-up period. Relapse was assessed on the basis of ALT flare and/or HBV DNA reappearance during follow-up.

Table 2. Responses to treatment [number (%) of patients] in patients with chronic hepatitis B

	T-α1 group (n = 26)	IFN-α group (n = 30)	HC group (n = 30)
	After 6 months' treatment		After 6 months' follow-up
ALT normalization	10 (38.5)*	15 (50)†	3 (10)
HBV DNA negative	12 (46.2)†	18 (60)†	2 (6.7)
ALT normalization and HBV DNA negative	8 (30.8)†	14 (46.7)†	1 (3.3)
	After 6 months' follow-up		After 12 months' follow-up
ALT normalization	17 (65.4)†§	10 (33.3)†	2 (6.7)
HBV DNA negative	16 (61.5)†§	9 (30.0)*	2 (6.7)
ALT normalization and HBV DNA negative	11 (42.3)†	7 (23.3)*	1 (3.3)

*$p < 0.05$ vs HC; †$p < 0.01$ vs HC; ‡$p = 0.0001$ vs HC; §$p < 0.05$ vs IFN-α.
ALT = alanine aminotransferase; HBV = hepatitis B virus; HC = historical control; IFN-α = interferon-α; T-α1 = thymosin-α1.

Statistical analysis

A Chi-squared test was used to analyze data, with a p value of less than 0.05 considered statistically significant.

Results

No patients withdrew from the study, and all completed the 6-month follow-up period. At study entry, the 3 groups were not significantly different regarding age, sex, biochemical, histologic and serologic parameters, and the number of patients with histologic evidence of cirrhosis (Table 1).

Biochemical and virologic changes at the end of treatment and follow-up are shown in Table 2. In the group receiving T-α1, serum HBV DNA was negative in 12 of 26 patients (46.2%) at the end of treatment. During follow-up, 6 other patients showed HBV DNA loss (at the second [n = 1], third [n = 1], fifth [n = 2] and sixth months [n = 2]), whereas HBV DNA reappeared in 2 patients (at the second [n = 1] and third months [n = 1]). In the group receiving IFN-α, 18 of 30 patients (60%) showed HBV DNA loss at the end of treatment. However, during 6 months' follow-up, HBV DNA reappeared in 9 patients (at the first [n = 2], second [n = 5] and third months [n = 2]), while no one lost HBV DNA. In the HC group, HBV DNA became negative in 3 of 30 patients (10%; at the sixth [n = 2] and twelfth months [n = 1]), whereas HBV DNA reappeared in 1 patient (at the eighth month).

The numbers of patients with a CR, DR or flare are shown in Table 3. Ten of the 12 T-α1 responders (83.3%) experienced sustained non-detectable HBV DNA after the 6-month treatment period. Six of the 14 T-α1 non-responders (42.9%) showed a DR of non-detectable HBV DNA during the follow-up period; corresponding values for the IFN-α group were 50% (9/18) and 0% (0/12). The DR rate was significantly greater in the T-α1 group than in the other two groups (χ^2 = 6.686, p = 0.010; χ^2 = 4.964, p = 0.038), and the rate of flare was higher in the IFN-α group than in the other 2 groups (χ^2 = 3.445, p = 0.063; χ^2 = 7.668, p = 0.006), during the follow-up period. A significantly greater proportion of patients in the T-α1 and IFN-α groups than the HC group had HBV DNA loss at the end of therapy (χ^2 = 11.58 [p = 0.001] and χ^2 = 19.20 [p = 0.0001], respectively) and follow-up period (χ^2 = 19.23 [p = 0.0001] and χ^2 = 5.46 [p = 0.020], respectively).

Table 3. Responses [number (%) of patients] to treatment at the end of the study

	CR	DR	FN
T-α1	11 (42.3)*	6 (23.1)†	2 (7.7)‡
IFN-α	7 (23.3)†	0	9 (30)§
HC	1 (3.3)	1 (3.3)	1 (3.3)

*$p = 0.0001$ vs HC; †$p < 0.05$ vs HC; ‡$p < 0.05$ vs IFN-α; §$p < 0.01$ vs HC. CR = complete response; DR = delayed response; FN = flare number; HC = historical control; IFN-α = interferon-α; T-α1 = thymosin-α1.

Serum ALT levels were normalized in 10 of 26 patients in the T-α1 group (38.5%), and in 15 of 30 patients in the IFN-α group (50.0%), at the end of treatment, and in 3 of 30 patients in the HC group (10.0%) after 6 months' follow-up. During follow-up, 8 patients receiving T-α1 had ALT levels normalized and 1 patient had ALT flare, whereas no patients receiving IFN-α had ALT levels normalized and 5 patients had ALT flare. In the HC group, 2 patients had ALT levels normalized between the sixth and twelfth months of follow-up; ALT flare occurred in the 3 patients with ALT normalization during the first 6 months of follow-up. At study completion, a

CR was observed in 11 of 26 patients treated with T-α1 (42.3%), in 7 of 30 receiving IFN-α (23.3%), and in 1 of 30 in the HC group (3.3%) (T-α1 vs IFN-α: $\chi^2 = 2.30$, $p = 0.129$; and T-α1 vs HC: $\chi^2 = 12.57$, $p = 0.0001$).

The typical adverse effects of IFN-α therapy, such as flu-like syndrome, fatigue, irritability, and headache, were seen in most IFN-α recipients; however, no serious or long-term adverse effects were noted, and no patients discontinued treatment. Treatment with T-α1 was not associated with significant adverse effects: only 1 patient reported local discomfort at injection sites; no systemic or constitutional symptoms were observed; and no dosage adjustment was required.

Discussion

Anti-HBe-positive patients respond less often to IFN-α therapy than do HBeAg-positive patients and often relapse when treatment is stopped.[7–18,36–39] Clinical studies suggest that IFN-α 3–9 million IU thrice weekly for 6 months can suppress HBV replication in more than 50% of treated patients, but the relapse rate after treatment withdrawal is high.[7–18] In our study, a CR was seen in 46.7% of patients at the end of treatment, and in 23.3% at the end of follow-up.

New therapies are needed for hepatitis B, especially for HBeAg-negative hepatitis B, because the overall rate of beneficial response to IFN-α is not satisfactory and re-treatment is rarely helpful.[17] Agents evaluated for use against hepatitis B include prednisone, interferon-γ, thymosin, vaccination with antigenic HBV epitope, adoptive transfer of immunity, and several new nucleoside analogs, such as lamivudine, famciclovir, lobucavir, and adefovir dipivoxil.[17] Trials of long-term therapy, and of combination therapy with IFN-α, are now under way.

The present randomized, controlled trial shows that T-α1 1.6 mg subcutaneously twice weekly for 6 months is effective and safe in anti-HBe-positive and HBV DNA-positive chronic hepatitis B, because 42.3% of treated patients became HBV DNA-negative 6 months after the end of treatment. This response rate is not only significantly higher than the spontaneous seroconversion rate (3.3% in this study), but also higher than the response to IFN-α therapy alone (23.3%), assessed 6 months after the end of therapy. No significant differences were identified between the T-α1 and IFN-α groups at the end of treatment regarding rates of ALT normalization ($\chi^2 = 0.75$; $p = 0.386$) or HBV DNA loss ($\chi^2 = 1.07$; $p = 0.3$). However, significant differences were evident between the 2 groups regarding response rates at the end of follow-up ($\chi^2 = 5.73$, $p = 0.017$ and $\chi^2 = 5.61$, $p = 0.018$, respectively). The rate of CR was not significantly different between the 2 groups at the end of treatment ($\chi^2 = 1.48$; $p = 0.224$) and follow-up ($\chi^2 = 2.30$; $p = 0.129$). Normalization of serum ALT and loss of HBV DNA were observed more frequently in the IFN-α group at the end of therapy and in the T-α1 group at the end of follow-up. Furthermore, in the T-α1 but not the IFN-α group, an additional response to treatment was also observed during the follow-up period.

Based on these results, and considering that ALT normalization and HBV DNA loss may occur spontaneously in untreated patients infected by pre-core mutant virus, we retrospectively compared the 2 treated groups with a group of untreated patients followed-up for at least 12 months. The results showed a significantly greater CR rate in the IFN-α group at the end of treatment, and in the T-α1 group at the end of follow-up, compared with the HC group. The outcome of this study contrasts with other clinical trials in patients with chronic hepatitis B, where T-α1 was shown to be both effective and safe when used as monotherapy or in combination with IFN.[21–23,25,27–30]

It was reported in a multicenter, American trial that 5 of 12 responders to T-α1 therapy had a DR.[29] This is in contrast to IFN-α, to which responses usually occur during the first 4 months of treatment. These contrasting patterns of response were best demonstrated in a recent Italian study involving HBeAg-negative, HBV DNA-positive, interferon-naive patients with high ALT levels (181 ± 159 U/L), in which the CR rate increased gradually from 29.4% at the end of T-α1 treatment to 41.2% 6 months later. In that study, the response to IFN-α decreased from 43.8% at the end of treatment to 25% 6 months later.[30] This trend towards a delayed effect for T-α1 in patients with chronic hepatitis B was also recently reported by other investigators.[21–23,25,28]

The characteristic delayed response to T-α1 noted in other trials[21–23,25,28–30] was also seen in the present study. Ten of the 12 T-α1 responders (83.3%) in the current study experienced sustained non-detectable HBV DNA after 6 months' treatment. Six of the 14 T-α1 non-responders (42.9%) showed a DR of non-detectable HBV DNA during follow-up. T-α1 dosage, injection schedules and duration of treatment in the present study were similar to those used in other clinical trials.[21–23,25,28–30] Furthermore, the efficacy of T-α1 in hepatitis B was recently evaluated in 353 patients in a meta-analysis of 5 trials.[23] The results showed that there was an increasing trend towards

virologic response with time since the cessation of thymosin treatment ($p = 0.02$). Thymosin effectively suppresses viral replication in chronic HBV infection, and has a cumulative effect after treatment cessation on viral clearance. Indeed, in the meta-analysis, as long as 12 months were required after treatment cessation for antiviral efficacy to become apparent. It is likely that the results reflected the response of HBeAg-positive patients, as such patients were studied in 4 of the 5 trials included (3 trials included HBeAg-positive patients and 1 included both HBeAg-positive and HBeAg-negative patients). Only 2 trials included HBeAg-negative patients (1 trial included HBeAg-negative patients and 1 included both HBeAg-positive and HBeAg-negative patients).[23] Thus, whether the efficacy of thymosin in HBeAg-negative patients differs from that in HBeAg-positive patients requires further research, and reasons for the above-mentioned, delayed effect of T-α1 are not yet clear.

Patients treated with T-α1 have an increased peripheral blood helper T-cell count (CD4), and IFN-r production by peripheral blood mononuclear cells, during and after the end of treatment.[27] T-α1 has been used to treat patients with cancer or immunodeficiency, resulting in up-regulation of lymphocyte function to include mitogen responsiveness, T-cell maturation, enhanced T-lymphocyte cytotoxicity, and increased B-lymphocyte antibody production.[32,40] Furthermore, T-α1 is found in, and secreted by, lymphocytes, justifiably characterizing it as a cytokine.[41] Other thymus-derived compounds have been used in clinical trials. A synthetic octapeptide, THF-γ2, was studied in 9 patients who had previously failed IFN-α2b treatment,[26] and appeared to potentiate the suppressive effect of IFN-α2b on HBV replication. In view of the immune mechanisms involved in the pathogenesis of liver injuries in chronic HBV infection, it is possible that T-α1 may activate viral-specific helper T-cells and amplify the humoral immune response to viral proteins, and the induction of viral antigen-specific cytotoxic T-lymphocytes, by secreting endogenous IFN-α, IFN-γ, IL-2, and tumor necrosis factor; it may also increase lymphocyte IL-2 receptor expression.[42–51] Moreover, T-α1 can act synergistically with endogenous IFN-α and IFN-β to stimulate natural killer activity.[52]

Although T-α1 is not known to possess antiviral properties, a preliminary report showed that it inhibited woodchuck hepatitis virus replication.[53] Hence, the delayed effect after T-α1 therapy in the present study was possibly due to immunomodulation and the induction of persistently higher levels of helper T-cell function. Because noncytolytic inhibition of HBV RNA, nucleocapsid particles, and replicative DNA intermediates by cytotoxic T lymphocytes have been described in the transgenic mouse model,[54,55] it is also possible that viral clearance after T-α1 therapy, particularly that without a preceding ALT flare, may also be mediated by the noncytolytic antiviral effects of cytotoxic T lymphocytes. Clearly, further studies are needed to elucidate the possible mechanisms of such viral clearance.

In conclusion, results from this trial indicate that, at the dosage tested, T-α1 is of potential interest in patients with anti-HBe-positive and HBV DNA-positive chronic hepatitis B. However, a response rate of 42.3% is still less than ideal. A potentially more effective therapeutic approach, such as combination therapy using the immunomodulating effect of T-α1 and the antiviral activity of interferon or nucleoside analogs (e.g. lamivudine, famciclovir), warrants further study.

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