Impact of Complete Inhibition of Viral Replication on the Cellular Immune Response in Chronic Hepatitis B Virus Infection

GEORGE MARINOS, NIKOLAI V. NAOUMOV, AND ROGER WILLiAMS

Interferon alfa (IFN-α) treatment is effective in only a proportion of patients with chronic hepatitis B virus (HBV) infection. The mechanisms for therapeutic failure remain unknown but high levels of HBV replication are known to inhibit the immunopotentiating effects of IFN-α. In nine patients with chronic hepatitis B not responding to IFN-α monotherapy, we determined a long-lived virus-specific T-helper-cell responses during two consecutive therapeutic regimens: IFN-α alone and IFN-α in combination with a new potent inhibitor of HBV replication, lamivudine. By comparing the results obtained during the initial IFN-α monotherapy to those during the combination treatment, it was investigated whether complete inhibition of virus replication will enhance the interferon-induced immunoreactivity to HBV. Despite the rapid reduction to undetectable serum HBV DNA in all nine patients during the combination treatment, none sustained permanent hepatitis B e antigen (HBeAg) clearance during subsequent 12-month follow-up. HLA class II–restricted T-helper-cell responses to hepatitis B core antigen (HBcAg) showed no difference during IFN-α monotherapy and during the combination of lamivudine plus IFN-α. In contrast, a delayed T-cell activation occurred after a rebound in serum HBV DNA postcombination treatment, which led to increased hepatocytolysis. These findings suggest that the profound inhibition of HBV replication by a nucleoside analogue does not restore the impaired virus-specific T-cell response in chronic HBV infection.

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Interferon alfa (IFN-α) is currently the only established treatment for chronic hepatitis B virus (HBV) but it is effective in only 25%-40% of treated patients.1,2 The mechanisms responsible for IFN-α treatment failure and the optimal management of patients with chronic HBV who do not respond to an initial course of IFN-α remain unclear. IFN-α possesses immunomodulatory and antiviral properties,3 both of which contribute to sustain a permanent remission. However, HBV clearance is only dependent on establishing a long-lasting host immune control of virus replication. The immune clearance of infected hepatocytes, observed at 6-12 weeks into IFN-α treatment, is mediated by the cellular immune response and results in a hepatitis B e antigen (HBeAg) to antibody to hepatitis B e antigen (anti-HBe) seroconversion together with a sustained loss of serum HBV DNA.4 Virus-specific T-cell responses are crucial in determining the outcome after HBV infection with the HLA class II–restricted T-helper-cell response to nucleocapsid antigen shown to play a pivotal role for viral clearance.5,7

It has been suggested that in chronic HBV carriers, high levels of HBV replication will inhibit the immunopotentiating effects of IFN-α. Patients with high levels of serum HBV DNA are known to have a poor response to IFN-α therapy.8 Moreover, HBV has been shown to suppress the expression of human interferon-beta gene9,10 and to induce a cellular resistance to the action of interferons.11 Abolishing these inhibitory effects of high levels of HBV replication on the activity of interferon could lead to enhanced immune responsiveness to the virus during IFN-α treatment. Thus, a potential strategy to increase the response to IFN-α treatment is to rapidly reduce HBV replication with one of the new and very potent antiviral agents, such as the nucleoside analogue lamivudine. Lamivudine (2,3 dideoxy 3′-thiacytidine) is a second-generation, non–interferon-inducing nucleoside analogue that is well absorbed orally and results in marked inhibition of HBV replication in vitro and/or in vivo.12-15

In the investigation reported here, the effects of marked and prolonged inhibition of HBV replication on the immunomodulatory effects of IFN-α, as determined by the T-helper-cell responses to hepatitis B core antigen (HBcAg) during treatment with a combination of lamivudine plus IFN-α were compared with that observed during treatment with IFN-α alone.

PATIENTS AND METHODS

Nine chronic HBV carriers seropositive for hepatitis B surface antigen for at least 12 months (all men; aged 35.6 ± 6.8 years) who had been treated with a full course of recombinant IFN-α treatment (10 million units three times weekly for 4-8 months) but failed to seroconvert from HBeAg to anti-HBe and remained serum HBV DNA positive were enrolled to be treated with a combination of IFN-α and lamivudine (Table 1). All patients were seronegative for markers of hepatitis C virus, hepatitis delta virus, and human immunodeficiency virus. The time interval between the end of the first course of IFN-α treatment alone and the start of the combination of IFN-α and lamivudine was between 12 and 24 months, during which time the patients were not administered any antiviral or immunomodulatory treatment.

The nine patients, part of a multicenter trial of a combination of IFN-α and lamivudine, were treated with 16 weeks of 10 megaunits of recombinant IFN-α 2b (Intron-A; Schering-Plough, Welwyn Garden City, England) subcutaneously three times a week and 100 mg of lamivudine (2,3 dideoxy 3′-thiacytidine; 3-TC; Glaxo Wellcome, Middlesex, England) orally once a day. Successful response was defined as a sustained loss of HBV DNA and a HBsAg to anti-HBe seroconversion within 6 months of completing treatment. Each patient underwent a percutaneous liver biopsy within 3 months of starting both treatment courses. A histological activity...
The PBMCs were resuspended at 2×10^6/mL concentration did not decrease significantly during the course of standard density gradient centrifugation with Lymphoprep (Nygaard, Oslo, Norway). The pretreatment histological activity and serum HBV DNA were not significantly different before starting either therapeutic regimen (Table 1). PBMC Preparation and Proliferation Assay. To analyze the time course of the HBcAg-specific T helper cell–response in relation to both therapeutic regimens we prospectively studied the proliferative response of peripheral blood mononuclear cells (PBMCs) to recombinant HBcAg on a monthly basis before, during, and after the two therapeutic regimens. PBMCs were separated from fresh, heparinized venous blood by standard density gradient centrifugation with Lymphoprep (Nygaard, Oslo, Norway). The interphase layer of cells was washed three times in buffered RPMI 1640 medium (GIBCO BRL, Paisley, Scotland) supplemented with 2 mmol/L of L-glutamine, 100 U/mL of penicillin, and 2 μg/mL of amphotericin B. The PBMCs were resuspended at 2×10^6 cells/mL in supplemented RPMI 1640 with 10% (vol/vol) heat-inactivated fetal calf serum (GIBCO BRL).

Freshly isolated PBMCs (2×10^6 cells/well) were cultured in 96-well, flat-bottomed sterile microtiter plates (Nunclon; GIBCO BRL) for 5 days at 37°C in a humidified atmosphere of 5% CO_2 in air in the presence or absence of recombinant HBcAg at a final concentration of 1 μg/mL in complete medium (RPMI with 10% fetal calf serum). Each well was pulsed with 0.5 μCi of tritiated thymidine (Amersham, Buckinghamshire, England) 16 hours before harvesting. The cells were washed and harvested onto glass fiber filters (Packard Instruments) using an automated cell harvester. The amount of the radiolabeled thymidine incorporated into DNA was measured by a direct measurement assay. A stimulation index (SI) was calculated by dividing the mean counts per minute (CPM) minus the mean CPM for the 12 replicates. The stimulation assay was performed in 12 replicates, and the results were expressed as the mean CPM per well for the 12 replicates. The stimulation index was calculated by grading portal tract inflammation, periportal piecemeal necrosis, and lobular inflammation each on a 0–3 scale with a maximum value of 9. There was no difference in the mean histological activity index before the two treatment courses. Hepatitis B surface antigen, HBcAg, anti-HBe, antibody to hepatitis delta virus, antibody to hepatitis C virus, and antibody to human immunodeficiency virus were tested using commercially available assays (Abbott Diagnostics, Maidenhead, England; Sorin Biomedica, Saluggia, Italy; and UBI HCV EIA; United Biochemical, Inc., Lake Success, NY). Serum HBV DNA was measured using a solution hybridization assay (Genostics; Abbott Diagnostics). Serum HBcAg concentration was assessed semiquantitatively by an automated enzyme immunoassay, using a 12-point standard curve created by serial dilution of the Paul Ehrlich Institute standard (Kodak Amerlite; Kodak Clinical Diagnostics, Amersham, England). The pretreatment histological activity and serum HBV DNA were not significantly different before starting either therapeutic regimen (Table 1).

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RESULTS

At the end of the combination treatment with IFN-α and lamivudine, two of the nine patients transiently seroconverted to anti-HBe but became HBcAg and serum HBV DNA positive again within 3 months of the end of treatment. The other seven patients remained HBcAg and serum HBV DNA positive, and none of the nine patients had sustained a permanent HBcAg to anti-HBe seroconversion at the 12-month follow-up visit.

The reduction of serum HBV DNA was significantly greater during the combination therapy in comparison to IFN-α monotherapy (Table 1; mean percentage decrease in serum HBV DNA during these treatments was 100% and 81%, respectively; P < 0.05, Wilcoxon’s matched pairs test). Serum HBV DNA became undetectable (<3 pg/mL) within 1 week of beginning the lamivudine treatment in all nine patients and remained undetectable until the end of treatment. In contrast, during IFN-α monotherapy, serum HBV DNA gradually decreased, and only one patient became serum HBV DNA negative at the end of treatment (P < 0.01, Fisher’s exact test). Measurement of serum HBcAg concentration was available only during the combination treatment of IFN-α plus lamivudine. Unlike serum HBV DNA, the HBcAg concentration did not decrease significantly during the course of the combination treatment (mean HBcAg level at the beginning and at the end of treatment, 5.75 ± 0.84 PEU/mL; range,
The PBMC proliferative response to recombinant HBCAg was significantly inhibited only by the anti-HLA DR antibody in a concentration-dependent manner (>90% inhibition at a 1:6 dilution), thus defining the PBMC proliferative response as HLA class II-restricted CD4+ T helper cell-response (Fig. 1).

The longitudinal analysis of the CD4+ T-cell proliferative response showed no difference in the immune responsiveness to HBCAg during IFN-α monotherapy and during the combination therapy. Four of the nine patients (44%) showed a significant increase in the T helper cell–proliferative response to recombinant HBCAg (SI ≥ 2.5) during both treatment courses (Fig. 2). Four of the five patients who did not have a significant proliferative response to recombinant HBCAg during IFN-α monotherapy also showed no response during the combination treatment, despite profound inhibition of HBV replication (Fig. 3).

A significant difference in the pattern of the T helper cell–response was observed after the end of the two treatment regimens. Five of the nine patients (56%) showed a significant increase in the SI to recombinant HBCAg at 2-3 months after cessation of lamivudine plus IFN-α therapy (Fig. 4). In contrast, none of these nine patients had an elevation of the SI after IFN-α monotherapy (P < 0.05, Fisher’s exact test). This delayed activation of the HBCAg-specific T helper cell–response followed the rapid increase of serum HBV DNA by approximately 1 month. The serum HBV DNA levels increased from undetectable (<3 pg/mL) to 259 pg/mL (median; range, 13-385 pg/mL) after stopping the combination treatment, and these levels are on average 100% of the pretreatment serum HBV DNA (Table 1). In contrast, after IFN-α monotherapy, the increase of serum HBV DNA was much less: from 26 pg/mL (median; range, <3-114 pg/mL) at the end of treatment to 83 pg/mL (median; range, 3-250 pg/mL, on average 50% of the pretreatment HBV DNA level) by 1-3 months. In association with this enhancement of the HBCAg-specific T helper cell response, serum alanine aminotransferase (ALT) levels increased by an average of 378% (±373% at 3 months after combination treatment, whereas the ALT levels showed no significant changes after the end of IFN-α monotherapy.

**DISCUSSION**

This study examined the effect of complete inhibition of HBV replication on the immunomodulatory effects of IFN-α that are ultimately required to bring about viral clearance. The combination of a powerful nucleoside analogue with IFN-α did not alter the HBCAg-specific T helper-cell response in patients who did not previously respond to IFN-α monotherapy, which may explain the lack of sustained seroconversion to anti-HBe in these patients after the second course of treatment. Interestingly, on stopping the combined treatment of IFN-α plus lamivudine, there was a significant augmentation of the T helper cell–response to HBCAg, which followed the rebound in serum HBV DNA and resulted in an increase in
The longitudinal analysis shows that the enhanced T-helper-cell response to HBeAg follows the rapid reemergence of serum HBV DNA posttreatment. Previous studies have shown that the HLA class II–restricted nucleoside analogue-specific T-helper-cell responses are accentuated during exacerbations of hepatitis, which are preceded by an increase in serum HBV DNA and HBeAg concentrations. This is compatible with the hypothesis that, in patients with chronic HBV, the immune response to HBeAg/HBeAg is antigen dose dependent, and the immune-mediated cell damage requires a threshold concentration of antigen. The sudden increase in viral replication is associated with an enhanced expression of viral antigens (including HBeAg), providing more targets for the virus-specific T-cell responses. In keeping with this is a recent case report of a chronic HBV carrier being treated with a 6-month course of lamivudine alone, which describes a rapid resurgence of active viral replication in this patient after the end of treatment that was associated with a short-term increase of ALT and bilirubin levels.

Unlike most other viral infections, e.g., herpes simplex, herpes zoster, cytomegalovirus, and human immunodeficiency virus, in which nucleoside analogues are the mainstay of therapy, in chronic HBV infection they have not yet been proven more effective than IFN-α in inducing long-term remission. The results of this study emphasize the important role of the host immune response in achieving HBV clearance. Thus, at the present time with the availability of several inhibitors of HBV replication such as lamivudine, famciclovir, and ganciclovir, our data provide some insight for their optimal usage, in particular in combination with immunopotentiating agents.

Optimal management of patients who failed previous IFN-α treatment has not been defined, and repeated courses of IFN-α are rarely beneficial and not recommended. The complete inhibition of HBV replication with a nucleoside analogue for a period of 4 months does not appear to be enough to induce antigen-specific T-cell activation that is necessary for viral clearance, and alternative approaches to enhance the cellular immune responses should be explored.

REFERENCES