Lamivudine Treatment Can Restore T Cell Responsiveness in Chronic Hepatitis B

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Abstract

High viral and/or antigen load may be an important cause of the T cell hyporesponsiveness to hepatitis B virus (HBV) antigens that is often observed in patients with chronic HBV infection. Reduction of viral and antigen load by lamivudine treatment represents an ideal model for investigating this hypothesis. HLA class II restricted T cell responses and serum levels of HBV-DNA, HBsAg, and HBeAg were studied before and during lamivudine treatment in 12 patients with hepatitis B e antigen positive chronic active hepatitis B to assess possible correlations between viral and/or antigen load and vigor of the T cell response. Cell proliferation to HBV nucleocapsid antigens and peptides and frequency of circulating HBV nucleocapsid-specific T cells were assessed to characterize CD4-mediated responses. A highly significant enhancement of the CD4-mediated response to HBV nucleocapsid antigens was already detectable in most patients 7–14 d after the start of lamivudine treatment. This effect was dramatic and persistent in 10 patients but undetectable in 2. It occurred concomitant with a rapid and marked reduction of viremia. Interestingly, lamivudine also enhanced the responses to mitogens and recall antigens, showing that its effect was not limited to HBV-specific T cells. In conclusion, an efficient antiviral T cell response can be restored by lamivudine treatment in patients with chronic hepatitis B concurrently with reduction of viremia, indicating the importance of viral load in the pathogenesis of T cell hyporesponsiveness in these patients. Since lamivudine treatment can overcome T cell hyporeactivity, combining lamivudine with treatments directed to stimulate the T cell response may represent an effective strategy to induce eradication of chronic HBV infection. (J. Clin. Invest. 1998. 102:968–975.) Key words: lamivudine • T cell response • antigen load • chronic hepatitis B • viral persistence

Introduction

The T cell response plays a crucial role in protection against viral infections, and development of an efficient antiviral T cell response is needed to avoid viral persistence and chronic evolution of virus-induced diseases (1, 2). Viral persistence is frequently associated with a state of T cell hyporesponsiveness, and recovery of the T cell reactivity is likely essential for resolution of chronic viral infections.

More than 300 million people worldwide are chronically infected with the hepatitis B virus (HBV). A defective antiviral T cell response has been documented in these patients (3–7) and may represent a critical pathogenetic determinant of viral persistence. Different mechanisms for this T cell hyporesponsiveness have been proposed (7), including a possible role of the high viral and antigen load typical of these patients, but the relevance of these factors has not been demonstrated.

Therapeutic strategies to enhance these defective responses have been proposed as possible alternatives to interferon (IFN; reference 8), the most effective drug available at present for the treatment of chronic hepatitis B (CH-B). However, all these treatments may be ineffective if T cells are non-responsive to exogenous stimuli. Therefore, understanding the mechanisms responsible for T cell hyporeactivity in CH-B may help identify rational therapeutic strategies to restore T cell responsiveness, thus allowing successful stimulation of antiviral T cell responses in vivo for obtaining long-lasting viral suppression and disease remission.

Recently, novel antiviral agents have been evaluated for their therapeutic potential for treatment of CH-B. Lamivudine, one of the most promising of such agents is a deoxycytosine analog which potently inhibits HBV DNA synthesis and rapidly suppresses serum HBV DNA levels in CH-B patients (9, 10).

The potency of lamivudine for inhibiting HBV replication provides a possible avenue for clarifying whether viral and/or antigen load may contribute to T cell hyporesponsiveness of CH-B patients. For this purpose, the T cell response was studied in a group of 12 patients with CH-B, both before and during treatment with lamivudine. Since lamivudine has no stimulatory activity on T cells (11, 12), it was anticipated that observed immunological effects in CH-B patients would be due to lamivudine-induced viral suppression rather than to direct effects of lamivudine on T cells.

Methods

Patients. 12 patients (11 males and 1 female; mean age 32 years) with hepatitis B surface antigen (HBsAg) positive chronic hepatitis were enrolled. All patients had elevated serum alanine aminotransferase

1. Abbreviations used in this paper: ALT, alanine aminotransferase; anti-HBe, anti-core hepatitis B; APC, antigen-presenting cell; CH-B, chronic hepatitis B; HBeAg, recombinant hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; SI, stimulation index.
(ALT) values for at least 1 yr, and were hepatitis B e antigen (HBeAg) and HBV-DNA positive (HBV-DNA assay; Digene, Beltsville, MD), and antibody negative to delta, hepatitis C (HCV), and human immunodeficiency (HIV) viruses. Other possible causes of chronic liver damage, such as alcohol, drugs, congestive heart failure, and autoimmune diseases were excluded. Three patients had previously been treated with INF-α (more than 1 yr before the start of the study) without clinical response.

**Study design.** The study protocol comprised 6 mo of patient monitoring before the start of treatment, followed by 12 mo of lamivudine therapy (100 mg once a day) and 6 mo of follow-up after the end of therapy.

Clinical, virological, and immunological parameters were assessed in the study patients at baseline, at monthly intervals before the start of lamivudine therapy, and at 7, 14, and 30 d, and every month thereafter during lamivudine therapy. The study was approved by the local Ethical Committee of the University Hospital of Parma. This interim report concerns the data obtained before therapy and the first 5 mo during therapy because lamivudine has a very fast antiviral activity which is already maximal after a few weeks of treatment. At each assessment, patients were evaluated for HBV DNA, HBeAg, anti-HBe, HBsAg, and anti-HBs. An adverse event inquiry was completed, and blood samples were drawn for immunosassays, blood chemistry, and hematology.

**Virological assessments.** HBsAg, anti-HBs, total and immunoglobulin M antico (anti-HBc), HBeAg, anti-HBe, antidualta, anti-HCV, and anti-HIV1 and anti-HIV2 were determined by commercial enzyme immunoassay kits (Abbott Laboratories, North Chicago, IL; Ortho Diagnostic Systems, Raritan, NJ; Sanoft Diagnostics Pasteur, Marnes-la-Coquette, France).

Quantitation of serum HBsAg and HBeAg was performed by comparing the optical densities of different serum sample dilutions with reference curves obtained from serial dilutions of recombinant HBsAg (Sorin Biomedica, Salugia, Italy) and HBeAg (Biogen, Geneva, Switzerland) of known concentration. To avoid interassay variations in individual patients, sera derived from all different time points were tested simultaneously, and the same reference curve was used for antigen quantitation of all samples from each patient.

**Antigens, mitogens, and synthetic peptides.** Recombinant hepatitis B core antigen (HBeAg) was obtained as described previously (13) and determined to be >95% pure. A HBeAg mutant lacking the carboxy-terminal 39 amino acid residues was purchased from Biogen (Sorin Biomedica, Salugia, Italy) and HBeAg (Biogen, Geneva, Switzerland) of known concentration. To avoid interassay variations in individual patients, sera derived from all different time points were tested simultaneously, and the same reference curve was used for antigen quantitation of all samples from each patient.

**Isolation of peripheral blood mononuclear cells (PBMCs) and T cell subsets.** PBMCs were isolated from fresh heparinized blood by ficoll-hypaque density gradient centrifugation. T cells and non-T cells were separated by rosetting PBMCs with 2-aminoethylisothiouronium bromide (Sigma Chemical Co., St. Louis, MO) and mouse anti-human CD3 monoclonal antibody. For cell recovery, cells in the T-cell-enriched population contained >95% CD3+ cells.

CD4+ and CD8+ T cells were purified by immunomagnetic separation using Dynabeads (Dynal A.S., Oslo, Norway). The purity of T cell preparations was tested with fluorescence isothiocyanate or phycoerythrin-conjugated mAbs (CD4/CD8, CD3/19; Becton Dickinson Monoclonal Center, Inc., Mountain View, CA) on a FACSort flow cytometer. Cell populations were resuspended at 10⁶ cells/ml in RPMI 1640 supplemented with 25 mM Hepes, 2 mM L-glutamine, 50 μg/ml gentamicin, and 8% human serum (complete medium).

**Proliferation assays.** Unfractionated PBMCs (2 × 10⁶/well) were incubated for 7 d at 37°C in the presence of HBV nucleocapsid antigens (0.5 μg/ml), synthetic peptides (2.5 μg/ml), or tetanus toxoid (0.5 μg/ml). In selected experiments, 10⁶ T cell subsets were cultured with autologous irradiated PBMCs (10⁴) or non-T cells (2 × 10⁴) as antigen-presenting cells (APCs) in the presence or absence of antigens. All proliferation assays were performed in triplicate in 96-well plates and [³H]-thymidine ([³H]-TdR; 0.5 μCi/well; specific activity, 2.0 Ci/mmol/liter; Amersham International, Amersham, U.K.) was added 6 h before harvesting. Results are expressed as stimulation index (SI), which represents the ratio between the mean cpm obtained in the presence and absence of antigen or mitogen. For antigenic stimulations, SI values above 4 were regarded as positive (≥2 SD above the mean SI value obtained with each individual HBV protein or peptide in normal controls).

**Limiting dilution analysis.** HBeAg-specific T cell frequency was determined by limiting dilution analysis. Varying numbers of purified T cells (1 × 10⁵, 6 × 10⁴, 3 × 10⁴, 1 × 10⁴, 3 × 10³, 1 × 10², and 1 × 10²) were cocultured in individual wells with a constant number of irradiated autologous non-T cells (2 × 10⁴) in the presence or absence of 0.5 μg/ml HBeAg. 32 replicate wells were seeded for each concentration of responder T cells. [³H]-TdR incorporation was measured after 7 d. Individual cultures stimulated with antigen, showing [³H]-TdR incorporation higher than the mean + 3 SD of that shown by cultures incubated in the absence of antigen, were scored as positive. The fraction of nonresponder cultures was calculated for each concentration of seeded T cells. T cell frequency was calculated by Poisson analysis using the ELIDA computer program (16).

**Determination of the cellular phenotype.** PBMCs were analyzed by immunofluorescence on a fluorescence-activated cell sorter after staining with fluorescein isothiocyanate, phycoerythrin, or peridinin chlorophyll protein conjugated mouse monoclonal antibodies: anti-CD4, anti-CD8, anti-DR, anti-CD45RA, anti-CD69, and anti-TCR purchased from Becton Dickinson and anti-CD45R0 purchased from Sigma.

**Statistical analysis.** Stimulation indexes derived from patients before and during lamivudine treatment were compared by the Student’s t test for paired data and by the Mann-Whitney U test. Frequencies of significant proliferative T cell responses and of circulating antigen-specific T cells were compared by χ² analysis.

**Results.**

The vigour of the T cell response to HBeAg and HBeAg is enhanced by lamivudine treatment. Taking advantage of the fact that lamivudine is a potent antiviral drug with no direct effect on the immune system (11, 12), we asked whether reduction of viral or antigen load determined by lamivudine treatment can cause a recovery of the T cell response in patients with chronic HBV infection who are generally hyporesponsive to HBV proteins.

T cell responses to HBeAg were undetectable in five consecutive determinations during the pretreatment period in 10 of the 12 patients (Fig. 1). T cell responses to HBeAg were more frequently measurable than responses to HBeAg during this period. In contrast, one patient (patient 11) displayed unusually strong basal levels of T cell proliferative response to both HBeAg and HBeAg before therapy. Responsiveness to HBV nucleocapsid antigens before treatment was unrelated to previous IFN therapy because only one of the responsive patients had previously been treated with IFN (patient 11).

Lamivudine treatment was associated with an increase in the strength of the T cell responses to HBV antigens in 10 of the 12 patients, and the difference between pretreatment and during-treatment responses was statistically significant in 8 and 9 patients for the T cell response to HBeAg and HBeAg, respectively (by the Mann-Whitney U test; Fig. 1). This result was highly significant when the patient population was analyzed as a whole.

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Consistent with this enhanced T cell responsiveness, the frequency of peripheral blood HBeAg-specific T cells increased from generally undetectable levels (<1 antigen-specific T cell per 300,000 total T cells) before lamivudine therapy to >1:50,000 total T cells in most experiments, with peak levels of >1:5,000 T cells (Fig. 2). These frequencies are comparable to those detectable in patients with acute infection who recover spontaneously from disease (data not shown).

Enhanced T cell responses were evident within 1 or 2 wk after the start of lamivudine therapy in eight patients, and by wk 4 of treatment in two others. T cell responsiveness did not change in two patients after the initiation of therapy. Overall, the T cell response to HBcAg at all time points after the start of treatment differed significantly from that observed at the final time point (wk 24) preceding therapy (Fig. 3). For the T cell response to HBeAg, this comparison was significant only at wks 28, 36, and 40.

T cell proliferative responses were mostly sustained by CD4+ T cells, as shown by experiments with purified CD4 or CD8+ T cells as effectors cocultured with irradiated PBMCs as APC (data not shown). The distribution of T cell subsets and the expression of surface T cell activation markers did not differ before and during lamivudine therapy.

T cell reactivity to overlapping peptides corresponding to the core and precore region-encoded polypeptides was also enhanced by lamivudine therapy. The frequency of significant

Figure 1. Proliferative T cell response to HBcAg and HBeAg expressed by 12 patients with HBeAg+ CH-B before and during 5 mo of lamivudine treatment. The dots contained in each vertical lane represent the proliferative responses of individual patients at different time points. Values for the whole patient population before and during treatment were compared by the Student’s t test for paired data. Values for individual patients before and during treatment were compared with the Mann-Whitney U test; statistically significant differences were observed in all patients except patients 2, 3, 11, and 12 for the T cell responses to HBcAg and patients 1, 11, and 12 for the T cell responses to HBeAg.

Figure 2. Frequency of circulating HBeAg-specific T cells in 12 CH-B patients before and during lamivudine treatment. Frequencies were determined by limiting dilution analysis. Statistical comparison was made by the Chi-square test. Frequencies were determined only at those time points when a sufficient number of PBMCs were available for the assay.

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responses (SI > 4) rose sharply after the start of lamivudine therapy for peptides 91–110, 100–119, 111–125, 117–131, precore 13–29, and precore 20–2 core (Fig. 4). Multiple peptides were recognized simultaneously by individual patients, indicating that the T cell responses were generally multispecific (Fig. 5). Moreover, responses to individual peptides tended to fluctuate. Consistent responses to a given peptide throughout the treatment period were noted in relatively few instances, and the combinations of peptides recognized by patients tended to vary at the different time points.

Recovery of T cell responsiveness is temporally associated with the drop of serum HBV DNA concentrations. Serum HBV DNA dropped rapidly in all patients after the start of lamivudine treatment and became undetectable in nine of them. This decline of serum HBV DNA was associated temporally with a marked increase of circulating HBeAg-reactive T cells in 10 of 12 patients (Fig. 6).

Lamivudine treatment was also associated with a decline in viral antigenemia in most patients. The serum HBeAg concentration declined in 10 of 12 patients, and 3 patients became HBeAg negative (Table I). Four patients seroconverted to anti-HBe (one remained positive for both HBeAg and anti-HBe). Serum HBsAg concentrations declined moderately in 6 of 12 patients.

The decline of viral antigens in the serum was frequently slower and more gradual than decreases of HBV DNA. Minimal levels of antigenemia were generally reached when maximal levels of T cell responsiveness had already been restored.

The recovery of the T cell reactivity is not limited to HBV-specific responses. The T cell response to mitogens and recall antigens was studied sequentially before and during lamivudine therapy to determine whether lamivudine enhances only HBV-specific responses or the overall T cell reactivity. Responses were compared with those observed in a group of normal, uninfected subjects studied at different time points (to control for normal fluctuations of these responses over time).

T cell responses to PHA, anti-CD3, and tetanus toxoid before treatment were significantly lower than those observed in the control group (Fig. 7). Interestingly, lamivudine treatment was associated with an enhancement of all responses to a level comparable to those observed in the control group.

**Discussion**

Patients with chronic HBV infection are generally hyporesponsive to HBV proteins, and the level of T cell reactivity at this stage of infection is significantly weaker than in acute self-limited hepatitis B. This different vigor of the T cell response has been proposed to represent an important determinant of the final outcome of infection (7).

Little is known about the mechanisms responsible for the T cell hyporeactivity of patients with chronic HBV infection. Among different possible explanations, it is widely suspected that the high viral and antigen loads typical of these patients represent an important cause of this hyporesponsiveness, but direct evidence for this has never been provided.

The present study investigated whether decreases of viral or antigen load induced by lamivudine are associated with improvement of T cell responsiveness and whether T cell reactivity is enhanced generally or restricted to the virus-specific component of the immune response. The HBV-specific T cell response was studied with HBV nucleocapsid antigens (HBcAg and HBeAg) because they represent the most powerful immunogens for HLA class II restricted, CD4+ T cells in HBV infection (3, 4). All study patients were positive for serum HBsAg and HBV-DNA and exhibited persistent ALT elevations. As expected, lamivudine treatment led to a rapid drop of viremia in all patients and a slower, more variable decrease of serum HBeAg concentrations. Most patients experienced improvements in serum ALT values after initiation of lamivudine therapy. Effects on HBsAg concentration were minimal during this short-duration therapy.

In most patients, treatment was followed rapidly by a marked increase of T cell responses to HBcAg and HBeAg. There was a clear temporal association between the decline of viremia and recovery of the T cell reactivity to HBV antigens (Fig. 6). Both enhanced proliferative responses to HBV proteins or synthetic peptides, and increased frequencies of circulating HBeAg-reactive T cells were generally preceded by, or temporally associated with, the decline of serum HBV DNA (Fig. 6). In a few patients, the frequency of HBeAg-reactive T cells was already measurable before the start of therapy (pa-
Figure 4. (A) Percentage of study patients responsive to HBcAg synthetic peptides before and during 5 mo of lamivudine treatment. (B) Frequency of significant proliferative T cell responses to HBcAg synthetic peptides expressed by 12 CH-B patients before and during 5 mo of lamivudine treatment. Data before and during treatment were compared by the Chi-square test. *P = 0.01–0.05; **P < 0.01.

Table 1. Behavior of Serum ALT, HBsAg, and HBeAg Values under Lamivudine Treatment Compared with Pretreatment Levels (Wk 24)

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ND = not determined; NEG = negative.
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Figure 5. Longitudinal analysis of the T cell proliferative response to HBV core peptides by PBMCs of 12 patients with CH-B. The figure illustrates the response at different time points to only those peptides to which individual patients gave at least one significant response. Responses were tested only when a sufficient number of PBMCs were available; for this reason, some time points are missing.

Patients 1, 4, 7, and 11), but T cell frequencies never exceeded 1 antigen-specific T cell per 50,000 total T cells (Fig. 6).

Overall, the strength of the T cell response to HBeAg and HBcAg was significantly greater during than before lamivudine treatment. Only two patients (patients 11 and 12) did not show significant enhancements of T cell responsiveness despite a rapid drop of HBV-DNA caused by lamivudine treatment. Patient 12, the only Oriental patient in the study, was believed to be infected perinatally. Genetic factors and/or the route of infection may have influenced her different immunological response. Patient 11 exhibited elevated ALT levels and T cell reponsiveness to HBV proteins before lamivudine treatment. He had previously been treated with INF-α 10 yr before entering the present study. These factors suggest a reactivation of liver disease which can be associated with increased T cell reactivity to HBV nucleocapsid antigen (17, 18) and often precedes spontaneous HBV clearance and resolution of the disease process.

Our data suggest that lamivudine can break the state of T cell nonresponsiveness in a large proportion of HBeAg positive patients. Although HBeAg clearance was observed in a few patients, the increased T cell reactivity induced by lamivudine was not sufficient to cause extensive anti-HBV seroconversion. Thus, combining lamivudine therapy with other agents able to stimulate the antiviral T cell response directly and specifically may be a rational strategy for treating chronic HBV infection. The observation that enhanced T cell responses occurred in 10 of 12 patients by wk 4 of lamivudine therapy may be relevant to the issue of when to initiate adjunctive immunomodulatory therapies. This may be particularly important when lamivudine is combined with a T cell growth-inhibitory drug such as IFN-α. Indeed, the antiproliferative
action of IFN-α on T cells requires the recruitment by the IFN-α receptor signaling complex of components needed for T cell receptor (TCR) engagement (19). If recovery of T cell reactivity by lamivudine is ultimately mediated by effector mechanisms able to restore signal transduction through the TCR, one could speculate that IFN can prevent lamivudine effect on T cells by perturbing TCR signaling when both drugs are administered simultaneously. If this is the case, lamivudine and IFN should be given sequentially rather than simultaneously. For this reason, Marinos et al. may not have found significant effects on helper T cell responses to HBeAg in their population of patients treated with a combination of lamivudine and IFN (20). Alternatively, their different results may be related to different patient selection, most of the patients being naive in our study instead of IFN nonresponders as in Marinos’s report.

While enhancement of T cell responses was clearly associated temporally with decreases in viremia (serum HBV DNA) in 10 of the 12 patients studied, the relationship between reduction of the serum HBeAg concentration and recovery of T cell responses was less clear. A slight decrease of HBeAg concentration preceded increased T cell reactivity in approximately half of the patients, but in all patients the reduction of antigen load was gradual, and antigenemia continued to decline after T cell reactivity had reached its maximum. Thus, it is unlikely that recovery of T cell reactivity is related to the decline of serum HBeAg, but this possibility cannot be excluded since it is totally unknown to what extent antigenemia must decline in vivo to allow T cells to restore their reactivity.

One possible mechanism for the T cell hyporesponsiveness observed in patients with chronic hepatitis B is deletion of immunodominant T cell populations by exhaustion (21–23), possibly caused by high serum antigen concentrations. However, the T cell response to synthetic peptides seems to rule out this possibility. Comparison of the present results with those obtained previously in patients with selflimited acute HBV infection (24) shows that the immunodominant amino acid sequences for induction of T cell responses are the same at both stages of infection, although some differences in the frequency of responses to individual peptides are obviously observed as a likely consequence of the heterogeneous genetic background of the two groups of patients.

The results obtained with T cell mitogens and tetanus toxoid show that the overall T cell pool was influenced by lamivudine treatment, not only the reactivity of virus-specific T cells. This finding, coupled with the observation that T cell responses generally increased before disease improved (ALT reduction), suggests that enhanced peripheral blood T cell responsiveness associated with lamivudine treatment is not due to the migration of HBV-specific T cells from the liver to the blood, subsequent to decreased liver inflammation.

Overall, this study suggests that the basic T cell defect in patients with chronic HBV infection is sustained by antigen nonspecific mechanisms, which are able to act on all T cells.
without regard to their antigen specificity. With respect to this interpretation, it will be useful to study the prevalent cytokine pattern of HBeAg+ patients with chronic hepatitis and possible modifications that are induced by treatment.

Moreover, this study suggests that HBV, or specific HBV-encoded amino acid sequences (including HBeAg and perhaps other proteins), can interfere with general functions or effector molecules of the immune system. This may lead to T cell hyporesponsiveness, as described for other viruses that can interfere with the biological activity of cytokines or their receptors (25–28) or inhibit signal transduction via protein kinase C, other proteins), can interfere with general functions or effector molecules of the immune system. This may lead to T cell hyporesponsiveness, as described for other viruses that can interfere with the biological activity of cytokines or their receptors (25–28) or inhibit signal transduction via protein kinase C, thereby affecting the overall T cell reactivity.

These results, which suggest that viral load contributes to persistent T cell hyporeactivity in chronic HBV infection, also have practical implications for treatment of chronic hepatitis B. Inhibition of viral replication with agents such as lamivudine may enhance the likelihood that therapeutic stimulation of the T cell response will induce HBV antigen seroconversion, ultimately leading to recovery from disease. Further clinical studies are needed to explore this possibility.

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References