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Complementarity-Determining Region 3 Size Spectratypes of T Cell Receptor β Chains in CD8$^+$ T Cells following Antiviral Treatment of Chronic Hepatitis B$^\nabla$

Shi-Wu Ma,[1,2]† Yong-Yin Li,[1]† Guang-Wen Zhang,[1,3] Xuan Huang,[1] Jian Sun,[1] Chris Li,[4] William G. H. Abbott,[1,5] and Jin-Lin Hou[1]*

Hepatology Unit and Key Lab for Organ Failure Research, Nanfang Hospital, Southern Medical University, No. 1838 North Guangzhou Avenue, Guangzhou 510515, China[1]; Department of Infectious Diseases, Kunming General Hospital of PLA, Kunming 650032, China[2]; Department of Infectious Diseases, the First Affiliated Hospital, Xinxiang Medical College, Xinxiang 453003, China[3]; MRC Human Immunology Unit, The Weatherall Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DS, United Kingdom[4]; and The New Zealand Liver Transplant Unit, Auckland City Hospital, Private Bag 92-024, Auckland, New Zealand[5]

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An increased CD8$^+$ T cell response to hepatitis B virus (HBV) peptides occurs between 12 and 24 weeks after starting antiviral therapy for chronic hepatitis B. It is not known whether these cells have antiviral function. The aim of this study was to determine whether clonal expansions of CD8$^+$ T cells at these time points predict the virological response to therapy. Peripheral blood CD8$^+$ T cells were obtained from 20 patients treated with lamivudine or telbivudine for chronic hepatitis B at baseline, 12 weeks, and 24 weeks. The CDR3 spectratype of each T cell receptor (TCR) β chain variable region (Vβ) gene family was analyzed, and the changes in the numbers of Vβ families with clonal expansions were compared in subjects with ($n = 12$) and without ($n = 8$) a virological response (52 week HBV DNA < 300 copies/ml). The number of CD8$^+$ TCR Vβ families with clonal expansions at 12 weeks relative to baseline (median [10th to 90th percentile], +2.5 [0 to +7] versus +1 [0 to +2], $P = 0.03$) and at 24 weeks relative to 12 weeks (+1 [0 to +2] versus −1 [−3 to +4], $P = 0.006$) was higher in subjects with a virological response versus subjects without a virological response, as were interleukin-2 (IL-2) but not IL-21 mRNA levels in peripheral blood mononuclear cells. The duration of new expansions at 12 weeks was higher ($P < 0.0001$) in responders. Increased numbers of CD8$^+$ T cell expansions after antiviral therapy are associated with a virological response to treatment. These CD8$^+$ T cells are a potential target for a therapeutic vaccine for chronic hepatitis B.

Chronic hepatitis B (CHB) can lead to liver cirrhosis, liver failure, and liver cancer (10). CHB is always associated with replication of the hepatitis B virus (HBV) in the liver, and suppression of this replication by antiviral therapy leads to both cessation of disease activity and a decreased risk for the serious sequelae (11). Unfortunately, the suppression of viral replication induced by current antiviral therapies is not permanent in most patients, and life-long therapy is required to maintain disease inactivity. A therapeutic vaccine that permanently enhanced the patient's own immune response to the HBV would make a valuable contribution to the management of these patients.

Long-term suppression of HBV replication in patients with an inactive, HBeAg-negative, chronic HBV infection is probably carried out by CD8$^+$ T cells responding to wild-type viral peptides presented by human leukocyte antigen (HLA) class I (13). Although CD8$^+$ T cells responding to peptides presented by the HLA-A*0201 allele have been widely studied, very little is known about the CD8$^+$ T cells that respond to peptides presented by other HLA class I alleles, which may be more relevant to the development of therapeutic vaccines in Asia. Boni et al. (4, 5) identified an opportunity to study HBV-specific CD8$^+$ T cells from subjects with a wide range of HLA class I genotypes when they found a temporary increase in CD8$^+$ T cell responses to HBV peptides at 12 and 24 weeks after beginning antiviral therapy. Whether these responses influence the outcome of antiviral therapy is unknown. Thus, there is doubt as to whether they are genuine HBV-specific responses that might be useful targets of immunotherapy, or just HLA- and HBV-nonspecific responses resulting from TCR degeneracy (8).

The complementarity-determining region 3 (CDR3) of the α and β polypeptides that make up the TCR are immediately 5′ of the variable (V) sequences that distinguish the 24 families of β chains and the 32 families of α chains. The CDR3 regions are responsible for recognition of the antigenic peptides presented to the T cell by HLA class I. Consequently there needs to be a high level of diversity in these regions, which is generated by varying both the sequence and the number of amino acids. Variation in the number of amino acids means that there is variation in the length of the CDR3 regions of the genes encoding the TCR chains expressed by different T cells. In the naive immune system, these CDR3 lengths have a Gaussian distribution within each family of V region genes. When a clone of T cells expands after recognizing a peptide antigen,

* Corresponding author. Mailing address: Hepatology Unit and Key Lab for Organ Failure Research, Nanfang Hospital, Southern Medical University, No. 1838 North Guangzhou Avenue, Guangzhou 510515, China. Phone: 86 20 61641941. Fax: 86 20 87714940. E-mail: jlhousmu@yahoo.com.cn.

† S.-W.M. and Y.-Y.L. contributed equally to this study.

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TABLE 1. Patient characteristics upon entry to the study[a]

Parameter	Total	VR	NVR	CR	NCR
No. of patients	20	12	8	4	16
Mean age (yr) ± SEM	25.8 ± 1.1	26.8 ± 1.2	24.3 ± 1.9	25.0 ± 2.0	26.0 ± 1.3
Patient gender (no. male/no. female)	16/4	10/2	6/2	4/0	12/4
Mean HBV DNA ($\log_{10}$ copies/ml) ± SEM	9.3 ± 0.3	8.9 ± 0.4	9.9 ± 0.5	8.9 ± 0.6	9.3 ± 0.4
Mean ALT (× ULN) ± SEM	3.9 ± 0.8	4.1 ± 1.2	3.5 ± 0.7	4.1 ± 0.8	3.8 ± 1.0
No. of patients receiving telbivudine/lamivudine treatment	8/12	5/7	3/5	2/2	6/10

[a] VR, virological response; NVR, nonvirological response; CR, completed response; NCR, noncompleted response; ALT, alanine aminotransferase; ULN, upper limit of normal. All patients were both HCV and HIV negative.

then the Gaussian distribution is disturbed. These disturbances are large enough to be detectable by the CDR3 size spectratyping technique (12), in which the entire repertoire of CDR3 regions associated with each V gene is PCR amplified with a labeled primer and size separated on a genetic analyzer that outputs the distribution of peak areas.

We have conducted this study to test two hypotheses. The first hypothesis is that the TCR β chain CDR3 size spectratyping technique will identify new clonal expansions in peripheral blood $CD8^+$ T cells at 12 and 24 weeks that correlate with the outcome of antiviral therapy. The second hypothesis is that any changes in the number of Vβ families with clonal expansions will be associated with changes in levels of cytokines that are secreted by $CD4^+$ T helper cells that promote $CD8^+$ T cell activity. Finding data consistent with these hypotheses would increase confidence that the changes in $CD8^+$ T cell activity that occur during antiviral therapy are due to activation of $CD8^+$ T cells responding to HBV peptides presented by HLA class I.

MATERIALS AND METHODS

Patients and blood samples. Twenty subjects with HBeAg-positive CHB from Nanfang Hospital (Guangzhou, China) participated in a phase III, multicenter, double-blind clinical trial comparing telbivudine ($n = 8$) with lamivudine ($n = 12$). The clinical trial data have already been published (6). Blood samples were taken at baseline and at the follow-up visits at weeks 2, 4, 8, 12, 16, 24, 32, 40, 48, 52, 60, 68, 76, 84, 92, 100, and 104. Subjects were classified into virological response (VR; $n = 12$) or nonvirological response (NVR; $n = 8$) groups, based on whether the serum HBV DNA level was less than 300 copies/ml at week 52. This is the lower limit for detection of HBV DNA of the Roche Cobas system. The subjects had either a completed response (CR, $n = 4$) or a noncompleted response (NCR; $n = 16$) based on undergoing HBeAg seroconversion by week 52. The baseline characteristics of the patients are shown in Table 1. The study was approved by the ethics committee of Nanfang Hospital, and written informed consent was obtained from all subjects.

Preparation of $CD8^+$ T cells. Heparinized blood was collected at every follow-up visit except for weeks 2, 4, and 8. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using Nycoprep1.077 (Axis-Shield, Oslo, Norway). PBMC were suspended in 90% fetal bovine serum and 10% dimethyl sulfoxide, cooled in isopropanol at −80°C overnight, and then stored in liquid nitrogen. Thawed cells were rested for over 2 h before positive selection of $CD8^+$ T cells to more than 90% purity using magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany).

Synthesis of cDNA. RNA was extracted from either 10^6 purified $CD8^+$ T cells or the residual PBMC by using a NucleoSpin RNA II extraction kit (Macherey-Nagel, Düren, Germany). The quantity of RNA was determined on a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). RNA (0.5 μg) was reverse transcribed in quadruplicate with 250 pmol of dT18, 200 U of Moloney murine leukemia virus reverse transcriptase, and 500 μM concentrations of each deoxynucleoside triphosphate (Promega Corp., Madison, WI) in a total volume of 25 μl. The cDNA was stored at −80°C.

Analysis of CDR3 length by spectratyping. Our method for PCR amplification of each TCR Vβ chain CDR3 region has been previously reported (22). Briefly, each Vβ family was amplified from each cDNA sample by using a Vβ family specific primer (22) and a common, 6FAM-labeled Cβ primer. A volume of 0.5 μl of fluorescent PCR products was mixed with 9 μl of formamide (Hi-Di; Life Technologies Corp., Carlsbad, CA), 0.5 μl of loading dye (25 mM EDTA, 50 ng of blue dextran/ml), and 0.5 μl of TAMRA dye-labeled size standards (GeneScan −500 LIZ, Life Technologies Corp.). The mixture was denatured at 95°C for 2 min and scanned by using an Applied Biosystems model 3730 genetic analyzer (Life Technologies). The area of each clonal peak was obtained from GeneScan software version 672 (Life Technologies). The relative fluorescence intensity (RI) for each peak was calculated as follows: %RI = 100 × each clonal peak area/the total peak area for that Vβ family. The spectratypes of seven healthy Chinese subjects were analyzed to determine the size of the peak that was most commonly dominant for each of the Vβ families, and this was assumed to be the central peak of the Gaussian distribution for each family. The spectragrams used to make this assignment for each Vβ family are shown in Fig. 1A. We then used a modification of previously reported criteria (7, 23) to determine whether each Vβ family spectragram contained a clonal expansion. These criteria were either (i) the presence of a single peak at any position in the spectragram with an RI > 35%, (ii) the presence of 2 peaks at any positions in the spectragram with an RI > 25%, or (iii) the presence of a dominant peak with an RI > 25% occurring in one of the noncentral positions. Figure 1B shows 12 spectragrams that illustrate these criteria. The spectragrams classified as showing no clonal expansion were Vβ8-baseline; Vβ6, weeks 12 and 24; and Vβ9, weeks 12 and 24. Spectragrams classified as showing a clonal expansion according to criterion 1 were Vβ6-baseline; Vβ8, weeks 12 and 24; and Vβ23, weeks 12 and 24. Vβ23-baseline was classified as showing a clonal expansion according to criterion 2. Vβ9-baseline was classified as showing a clonal expansion according to criterion 3.

Quantitative real-time PCR analysis. The mRNA expressions of interleukin-2 (IL-2), IL-21, and the internal reference GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were measured in $CD8^+$ T cells and $CD8^+$ T cell-depleted PBMC using biplexing quantitative real-time PCR on a LightCycler480 (Roche, Switzerland). cDNA was amplified by using TaqMan Universal PCR Master Mix (Invitrogen) according to the manufacturer's recommendations. The PCR conditions were as follows: 1 cycle of 50°C for 2 min; then 1 cycle of 90°C for 2 min; then 45 cycles of 95°C for 15 s, followed by 60°C for 45 s; and then 1 cycle of 40°C for 30 s. Each reaction was performed in duplicate, and the average of the two values was used for calculations. The expression levels of IL-2 and IL-21 relative to GAPDH were calculated using the formula $2-\Delta C_T$. The sequences of the primers and TaqMan probe for IL-2, IL-21, and GAPDH have been published (16, 18).

Statistical analyses. Continuous data are expressed as means ± the standard errors of the mean (SEM). Ordinal data are expressed as the median (10th to 90th percentile). Categorical data are expressed as a number (percentage). The Fisher exact test was used in comparisons of categorical data. The Wilcoxon matched-pairs *t* test was used for comparisons of paired continuous and ordinal data. The Mann-Whitney U test was used for interclass comparisons of continuous and ordinal data. The general linear model repeated measures test was used for interclass comparisons of repeated sample data. The Spearman rank order correlation coefficient was used for correlations. The statistical analysis was performed by using either GraphPad Prism 5 or SPSS 13.0 software.

RESULTS

Changes in the number of $CD8^+$ T cell clonal expansions with treatment. There was a net increase in the number of Vβ families containing clonal expansions in the 20 treated patients relative to baseline at week 12 (median number of Vβ families

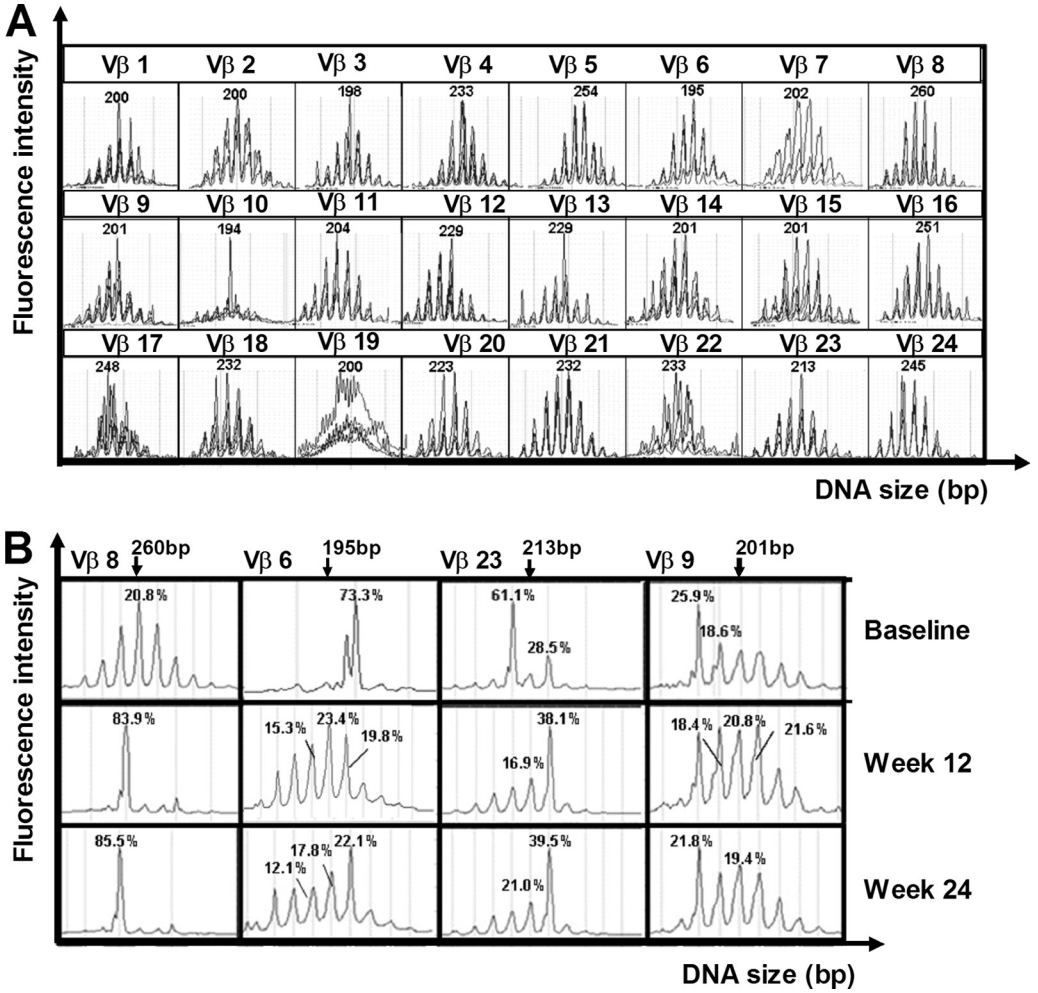

FIG. 1. Criteria for classifying a Vβ family spectragram as containing a clonal expansion. The *y* axis shows the fluorescence intensity of the peaks, and the *x* axis shows the PCR product length. (A) Overlaying the spectragrams from seven healthy subjects shows the size in base pairs of the peak that most commonly had the highest peak area for each Vβ family. This was taken as the central peak of the Gaussian distribution for that Vβ family. (B) Twelve spectragrams from one subject demonstrating the criteria for classifying a Vβ spectragram as containing a clonal expansion.

containing clonal expansions [10th to 90th percentile]: 8 [4 to 14] versus 6.5 [3 to 11], $P < 0.001$), and this difference was still present at week 24 (8.5 [4 to 15] versus 6.5 [3 to 11], $P = 0.003$; Fig. 2A). This net figure takes account of both new expansions that appeared between 0 and 12 weeks ($n = 63$) and between 12 and 24 weeks ($n = 29$) and expansions that were present at baseline and lost between either 0 and 12 weeks ($n = 18$) or between 12 and 24 weeks ($n = 19$). This increase in expansions was associated with decreases in the serum HBV DNA and alanine aminotransferase (ALT) levels (Fig. 2A). There was no correlation between the number of Vβ families containing expansions and either the baseline ALT level ($r = 0.17$, $P = 0.48$) or the baseline HBV DNA level ($r = 0.06$, $P = 0.80$). The numbers of Vβ families containing expansions were similar in the groups treated with either lamivudine or telbivudine at baseline ($P = 1.00$), 12 weeks ($P = 0.89$), and 24 weeks ($P = 0.37$) (data not shown).

Comparison of VR and NVR groups. The number of Vβ families containing clonal expansions at baseline was similar ($P = 0.10$) in the VR groups (5.5 [2 to 10]) and NVR groups (7 [4 to 16]) (Fig. 2B). In the VR group, the number of Vβ families containing expansions was significantly higher than baseline at both 12 weeks (8 [3 to 14] versus 5.5 [2 to 10], $P = 0.001$) and 24 weeks (9.5 [4 to 15] versus 5.5 [2 to 10], $P = 0.001$) (Fig. 2B). In the NVR group, the number of Vβ families containing expansions was significantly higher than the baseline number at 12 weeks (8.5 [4 to 17] versus 7 [4 to 16], $P = 0.007$) but was similar to the baseline at 24 weeks (7 [4 to 15] versus 7 [4 to 16], $P = 0.79$) (Fig. 2B). There was a larger increase in the number of Vβ families containing expansions from baseline to week 12 ($P = 0.03$, Fig. 2C) and from week 12 to week 24 ($P = 0.006$, Fig. 2D) in the VR than the NVR groups. The change in the number of Vβ families containing expansions at week 24 relative to baseline was negatively correlated with the HBV DNA levels at week 24 ($r = -0.67$, $P = 0.001$, Fig. 2E).

Prediction of the virological response to antiviral therapy. The patients were classified into groups with an increasing

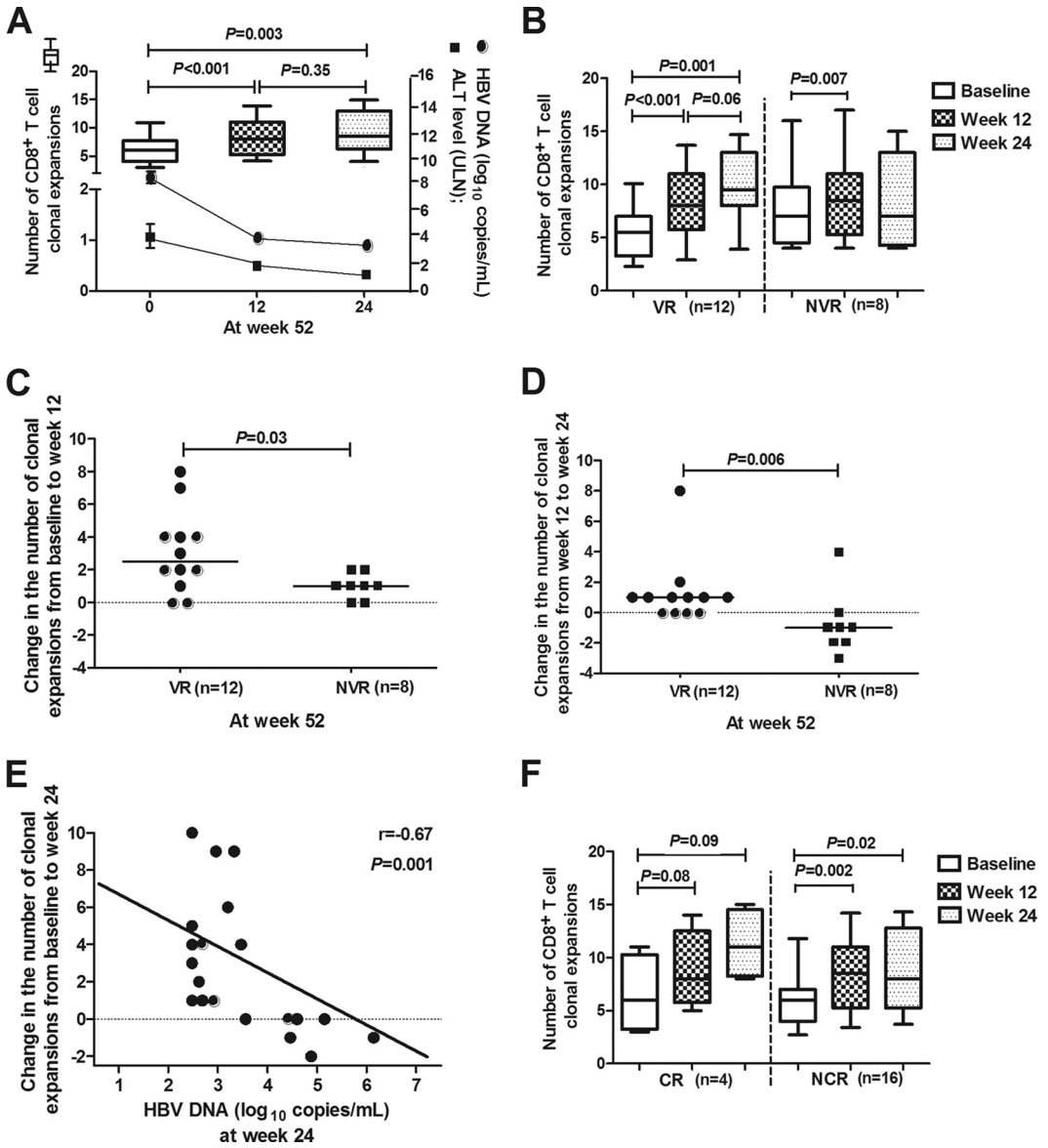

FIG. 2. Net changes in the number of $CD8^+$ T cell clonal expansions in the 24 Vβ families during antiviral therapy. (A) Box-and-whisker plot showing the median (10th to 90th percentile [range]) net number of $CD8^+$ T cell clonal expansions per patient and the HBV DNA (log_{10} copies/ml, mean ± the SEM) and ALT (× ULN, mean ± the SEM) levels at baseline, 12 weeks and 24 weeks. *P* values show the significance of changes in the number of net clonal expansions. (B) Box-and-whisker plot (median, 10th to 90th percentile [range]) comparing the changes in net number of clonal expansions within the VR and NVR groups. (C) Comparison of the change in the net number of clonal expansions from baseline to 12 weeks in the VR and NVR groups. (D) Comparison of the change in the net number of clonal expansions from 12 weeks to 24 weeks in the VR and NVR groups. (E) Negative correlation between the increase in the net number of clonal expansions from baseline to 24 weeks and the HBV DNA levels at 24 weeks. (F) Box-and-whisker plot (median, 10th to 90th percentile [range]) comparing the changes in the net number of clonal expansions within the CR and NCR groups.

($n = 9$), an unchanged ($n = 5$), or a decreasing ($n = 6$) pattern of response, based on the direction of the change in number of Vβ families containing clonal expansions between weeks 12 and 24 (Fig. 3). All patients with a decreasing pattern were in the NVR group (Fig. 4A), and the pattern of Vβ response to treatment predicted the influence of antiviral therapy on HBV DNA levels ($P < 0.001$, Fig. 4C) but not ALT levels ($P = 0.17$, Fig. 4D).

Increases in clonal expansions within specific Vβ families. Figure 5 compares the repertoire of Vβ families that contained new clonal expansions at 12 and 24 weeks compared to baseline in the VR and NVR groups. Increases in the number of expansions were found in 22 of the 24 Vβ families. In the VR group, there were 46 new expansions relative to baseline at 12 weeks, and 42 of these were still present at 24 weeks, with 21 additional expansions. In the NVR group, there were 17 new expansions relative to the baseline at 12 weeks, and 8 of these were still present at 24 weeks, with 8 additional expansions. The percentage of new clonal expansions at 12 weeks that lasted until 24 weeks was higher ($P < 0.0001$) in VR than in

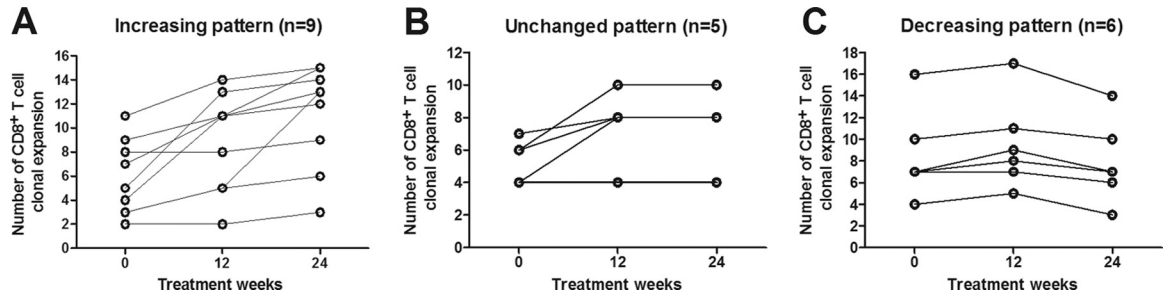

FIG. 3. Changes in the net number of $CD8^+$ T cell clonal expansions in subjects classified as having either an increasing (A), unchanged (B) or decreasing (C) pattern of behavior, based on the change in number of Vβ families with clonal expansions between weeks 12 and 24.

NVR. There was no evidence that new clonal expansions occurred in a restricted number of Vβ families or that increases in a restricted number of Vβ families might predict the outcome of antiviral therapy.

Comparison of CR and NCR groups. There was also a larger increase in the number of Vβ families containing clonal expansions in the CR group compared to the NCR group at both 12 and 24 weeks (Fig. 2F), and subjects with the decreasing pattern of Vβ response during this time interval were all in the NCR group (Fig. 4B). However, there were only four subjects in the CR group, and these differences did not reach statistical significance.

IL-2 and IL-21 mRNA expression in $CD8^+$ T-cell-depleted PBMC. IL-2 and IL-21 are produced by $CD4^+$ helper T cells and regulate the proliferation of antigen-specific $CD8^+$ T cells (15, 19). Figure 6A shows that the level of IL-2 mRNA in $CD8^+$ T-cell-depleted PBMC was increased after 12 weeks of antiviral therapy in the VR group ($P = 0.02$) but not in the NVR group ($P = 0.95$). The IL-2 mRNA level was similar to baseline in both groups after 24 weeks of therapy. Figure 6B shows that IL-21 mRNA levels were similar at each time point in the VR and NVR groups, with a decrease between weeks 12 and 24 in both groups. There were no correlations between changes in IL-2 or IL-21 mRNA levels and changes in number of Vβ families containing clonal expansions (data not shown).

DISCUSSION

It is nearly 20 years since $CD8^+$ T cells responding to the HBV core18-27 peptide presented by HLA-A*02 were associated with a successful immune response to the HBV (2). This finding has allowed detailed studies of the immune response to the HBV to be carried out in HLA-A*02-positive subjects with genotype A and D viruses. However, no other consistent $CD8^+$ T cell responses to peptides presented by other HLA class I alleles have been identified in the intervening time, although

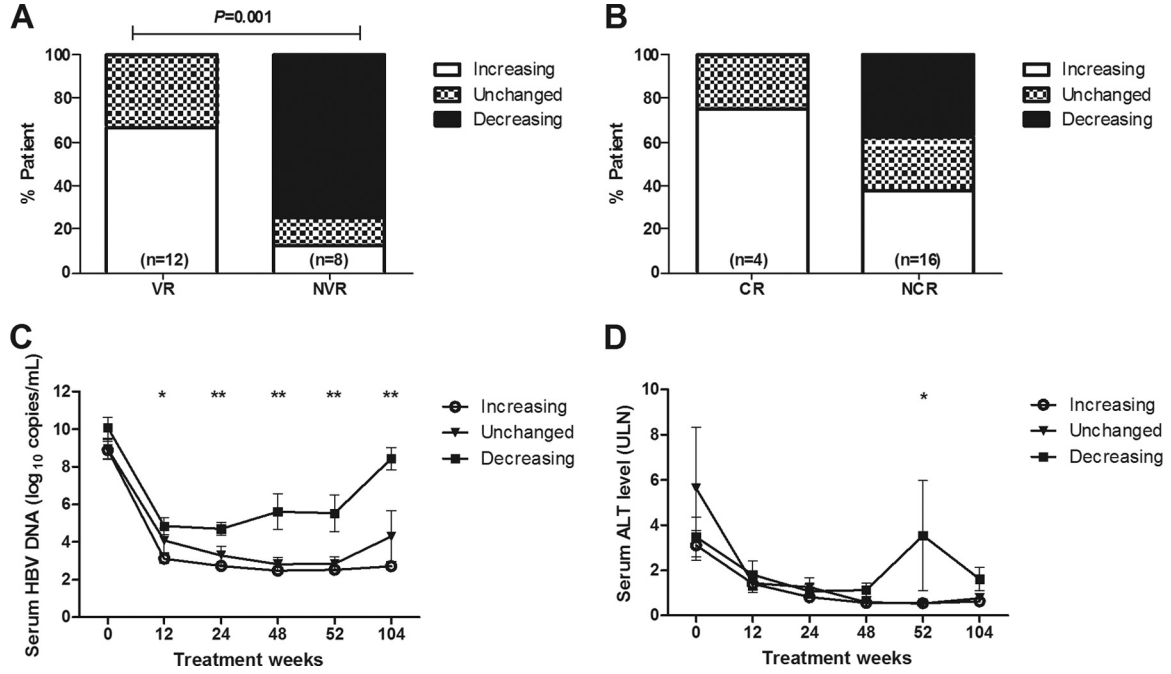

FIG. 4. Influence of the pattern of changes in the net number of Vβ families with clonal expansions on the virological response to treatment ($P = 0.001$) (A), the HBeAg seroconversion on treatment ($P = 0.27$) (B), HBV DNA levels (mean ± the SEM, $P = 0.001$) (C), and ALT levels (mean ± the SEM, $P = 0.17$) (D). *, $P < 0.05$; **, $P < 0.01$.

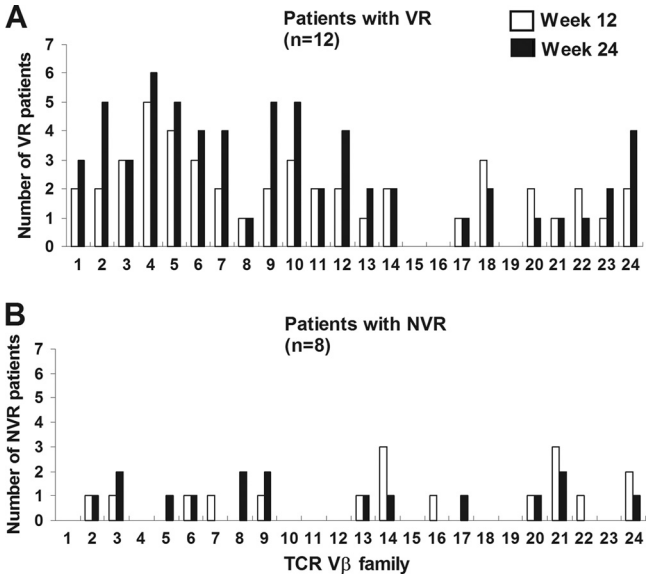

FIG. 5. Comparison of the number of patients in the VR (A) and NVR (B) groups with a new expansion relative to baseline in each Vβ family at 12 and 24 weeks.

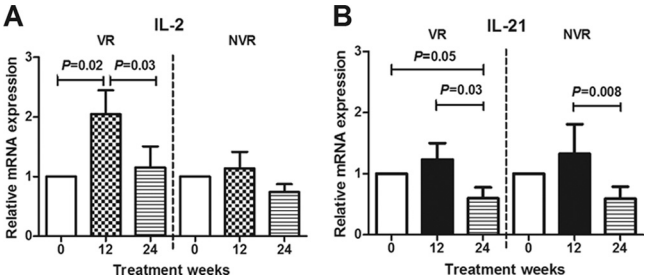

FIG. 6. Levels of IL-2 (A) and IL-21 (B) mRNA expression in $CD8^+$ T-cell-depleted PBMC at baseline, 12 weeks, and 24 weeks (mean ± the SEM).

there is some evidence they exist (1). Consequently, studies of HBV-specific $CD8^+$ T cells in subjects with HLA alleles and HBV genotypes that are common in Asia need to be carried out with other techniques.

A study using pools of HBV peptides to stimulate $CD8^+$ T cells in a cohort containing HLA-A*02-negative subjects (3) confirmed previous data (2, 17) showing that subjects with acute hepatitis B have markedly higher antiviral $CD8^+$ T cell responses than subjects with a chronic HBV infection. This has been taken as evidence that responses stimulated by peptide pools are due to HLA-restricted, HBV peptide-specific, $CD8^+$ T cells. Stimulation of PBMC by peptide pools has been used to show that the treatment of chronic hepatitis B with antiviral drugs results in a temporary increase in $CD8^+$ T cell responsiveness at about 12 weeks after beginning therapy (4). This is a potentially significant discovery, since a therapeutic vaccine could strengthen this response and permanently suppress HBV replication. It would be useful to know whether this change in $CD8^+$ T cell responsiveness has any influence on the outcome of therapy and whether it is associated with any other immune characteristics of an antiviral $CD8^+$ T cell response, such as T helper cytokine production. Measurement of TCR β chain CDR3 size spectratypes has the potential to help with these questions.

The increase in the number of $CD8^+$ T cell Vβ families that lost the Gaussian distribution of CDR3 lengths due to clonal expansions at 12 and 24 weeks after beginning antiviral therapy supports the finding of Boni et al. (4), in that it shows evidence of increased activity in the $CD8^+$ T cell compartment of the immune system at this time. There was evidence of a clonal expansion in a median of two Vβ families at 12 weeks, with an expansion in a median of one more Vβ family at 24 weeks. A permanent $CD8^+$ T cell response to dominant HBV antigens might be expected to produce expansions in more Vβ families than this (14). However, given that this is a weak, temporary immune response, an expansion in only 3Vβ families seems realistic. The possibility that the immune response might be to subdominant viral antigens (14) is also worth noting.

The most important finding of the present study is that patients who had a virological response to treatment had more clonal expansions that were of longer duration. The finding of a significant negative correlation between the increase in number of clonal expansions and the HBV DNA level at 24 weeks provides additional support for the hypothesis that these $CD8^+$ T cells have some clinical effect. Although this is consistent with the possibility that they have some role in suppressing viral replication, it is unclear how an immune response that is believed to be temporary (4) could have long-term effects. It is possible that $CD8^+$ effector cells could still be present but not detectable by a technique that relies on *in vitro* expansion to get data of adequate sensitivity (4). Collection of CDR3 spectratype data over a full course of therapy might resolve this difficulty.

There is still doubt about whether the immune responses detected here and in the study of Boni et al. (4) are the result of $CD8^+$ T cells responding to HBV peptides presented by HLA class I or whether they are a nonspecific epiphenomenon that may be a marker for a separate immune response that has an effector role. It is even possible that this is a temporary autoimmune response that can occur as part of a primary immune response to viral antigens (9). To partly address this issue, we looked for associations with T helper cytokines that might reasonably be expected to drive an HLA-restricted, peptide-specific, $CD8^+$ T cell response. The significant increase in IL-2 mRNA in the VR group is consistent with the possibility that $CD4^+$ T cells contribute to the activation of $CD8^+$ T cell memory in this group. This would fit with the observation of Boni et al. (4) that $CD4^+$ T cell expansion precedes the detection of increased $CD8^+$ T cell activity after antiviral therapy. Our data do not support a role for IL-21 in activating $CD8^+$ T cells in this situation. However, this has not been excluded, since samples may not have been obtained at the optimal time points for detecting this association.

It is also noteworthy that the increase in $CD8^+$ T cell clonal expansions coincided with a time when ALT levels were decreasing. This is important from the point of view of therapeutic vaccine development, since there is a concern that severe, life-threatening flares of chronic hepatitis B may be caused by $CD8^+$ T cells responding to viral peptides. A recent study by Tan et al. (20) found no evidence to suggest that $CD8^+$ re-

sponses to wild-type peptides cause these flares, but the possibility that $CD8^+$ T cell responses to mutated peptides cause these flares has still not been excluded (21). Thus, a therapeutic vaccine based on amplifying the $CD8^+$ T cell response to wild-type peptides that appears at 12 to 24 weeks after starting antiviral therapy is still a reasonable goal.

In summary, our findings support the possibility that clonal expansions of $CD8^+$ T cells occur at ~12 weeks after starting antiviral therapy for chronic hepatitis B. A successful virological response to therapy was associated with both the size and the duration of these expansions. However, there is still a need for data showing that this effect is mediated by $CD8^+$ T cells responding to HBV peptides presented by HLA class I. The present study did not have enough statistical power to test the hypothesis that these clonal expansions are associated with HBeAg seroconversion. A larger study that replicated the current findings, looked for an association with HBeAg seroconversion, and looked for associations between common HLA class I molecules and Vβ-specific clonal expansions would thus be valuable.

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