Resolution of Chronic Hepatitis B and Anti-HBs Seroconversion in Humans by Adoptive Transfer of Immunity to Hepatitis B Core Antigen

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Background & Aims: Impaired T-cell reactivity is believed to be the dominant cause of chronic hepatitis B virus (HBV) infection. We characterized HBV-specific T-cell responses in chronic hepatitis B surface antigen carriers who received bone marrow from HLA-identical donors with natural immunity to HBV and seroconverted to antibody to hepatitis B surface antigen. Methods: T-cell reactivity to HBV antigens and peptides was assessed in a proliferation assay, the frequency of HBV core- and surface-specific T cells was quantified directly by ELISPOT assays, and T-cell subsets were analyzed by flow cytometry. Results: CD4+ T-cell reactivity to HBV core was common in bone marrow donors and the corresponding recipients after hepatitis B surface antigen clearance, whereas none reacted to surface, pre-S1, or pre-S2 antigens. Furthermore, CD4+ T cells from donor/recipient pairs recognized similar epitopes on hepatitis B core antigen; using polymerase chain reaction for the Y chromosome, the recipients’ CD4+ T lymphocytes were confirmed to be of donor origin. The frequency of core-specific CD4+ and CD8+ T cells was several-fold higher than those specific for surface antigen. Conclusions: This study provides the first evidence in humans that transfer of hepatitis B core antigen-reactive T cells is associated with resolution of chronic HBV infection. Therapeutic immunization with HBV core gene or protein deserves further investigation in patients with chronic hepatitis B.

Hepatitis B virus (HBV) infection is one of the most common viral infections in humans. It is estimated that more than 2 billion persons worldwide have been exposed to the virus and approximately 350 million persons are chronically infected carriers of HBV. A large body of evidence suggests that the host immune response to HBV-encoded antigens is the main determinant of the outcome of HBV infection. In patients with self-limited acute hepatitis B, both HLA class I- and class II-restricted T-cell responses to viral antigens are strong and multispecific, whereas these responses are weak or undetectable in chronically infected patients (reviewed in Chisari and Ferrari). Moreover, in patients with spontaneous resolution of HBV infection, these strong antiviral T-cell responses are maintained for decades after clinical recovery, which keeps the virus under control.

In chronic HBV infection, persistent viral replication is associated with ongoing necroinflammation in the liver and progressive liver damage. Spontaneous remissions, with effective control of viral replication, can occur in a small proportion of patients during the natural history of chronic HBV infection. Similar remissions are the desired outcome of antiviral treatment of chronic hepatitis B and are associated with improved patient survival. However, clearance of hepatitis B surface antigen (HBsAg) in patients with chronic HBV infection is unusual (0.4%-2% per year in white patients and 0.1%-0.8% per year in Chinese patients). Even in interferon (IFN)-treated patients with chronic hepatitis B, the rate of HBsAg clearance is increased by only 6%.

It has recently been shown that adoptive transfer of immunity to HBV can be achieved after bone marrow transplantation (BMT) from donors who are immune to HBV. Interestingly, clearance of HBsAg has been observed in individual patients with chronic hepatitis B.
after BMT from immune donors\textsuperscript{10,11}; however, the mechanisms of resolution of chronic HBV infection after adoptive transfer of immunity to HBV have not been studied. The aim of this investigation was to gain information for the HBV-specific T-cell reactivity in patients who clear HBsAg after adoptive transfer of immunity to HBV, which would be of value in designing new approaches for therapeutic immunization in patients with chronic hepatitis B.

**Materials and Methods**

**Patients**

Eight patients with chronic HBV infection (serum HBsAg positive for more than 12 months) who underwent BMT because of hematologic malignancy and received marrow from an HLA-matched sibling with natural immunity to HBV (antibody to hepatitis B surface antigen [anti-HBs] and antibody to hepatitis B core antigen [anti-HBc] positive) were studied (Table 1). Before BMT, the 3 patients with acute leukemia were in complete remission, induced by an appropriate chemotherapy regimen: cytarabine, daunorubicin, and etoposide for acute myeloid leukemia\textsuperscript{12} or vincristine, prednisolone, daunorubicin, methotrexate, and cyclophosphamide for acute lymphocytic leukemia.\textsuperscript{13} The remaining 5 patients with chronic myeloid leukemia were in the chronic phase and were receiving treatment with hydroxyurea. The preparative conditioning regimen for all patients involved either busulfan, cyclophosphamide and total-body irradiation, or cyclophosphamide and total body irradiation.\textsuperscript{14} The clinical course in 4 of these patients has been described previously.\textsuperscript{11,15} All patients were treated at the Bone Marrow Transplant Unit, Queen Mary Hospital, Hong Kong SAR, China. The donors and the recipients were negative for hepatitis C virus, hepatitis D virus, and human immunodeficiency virus infection. Routine HBV serology (including hepatitis B e antigen [HBeAg] and antibody to hepatitis B e antigen [anti-HBe], HBsAg and anti-HBs, and HBV DNA) was tested within 4 weeks before BMT and at 4 weekly intervals after transplantation. The standard immunosuppression protocol for all patients included cyclosporine alone or in combination with prednisolone for at least 6 months after BMT when the immunosuppression was withdrawn. Of the 8 patients described here, 4 underwent transplantation before 1996 and did not receive any antiviral treatment. Since 1996, all HBsAg-positive BMT recipients were given prophylactic treatment with famciclovir or lamivudine starting 2 weeks before and continuing for 6 months after BMT. In this group, 3 patients received famciclovir (500 mg 3 times a day) and 1 received lamivudine (100 mg daily) (Table 1).

**T-Cell Proliferation Assay**

Freshly isolated peripheral blood mononuclear cells (PBMCs) were used in all experiments for testing the proliferative response to recombinant HBV proteins and synthetic peptides. PBMCs were obtained at the last follow-up visit from 7 donor/recipient pairs and were tested in parallel. In 1 patient (Table 1; patient 8), the proliferative response to viral antigens was tested prospectively before BMT, during the hepatitis flare after BMT, and 52 weeks after transplantation, on all 3 occasions in parallel with the donor's PBMC. PBMCs were cultured for 6 days at 2 × 10\textsuperscript{5} cells/well in 96-well, flat-bottomed plates (Nunclon; Gibco BRL, Glasgow, Scotland) in the presence of the antigens or medium alone.\textsuperscript{16} The clinical course in 4 of these patients has been described previously.\textsuperscript{11,15} All patients were treated at the Bone Marrow Transplant Unit, Queen Mary Hospital, Hong Kong SAR, China. The donors and the recipients were negative for hepatitis C virus, hepatitis D virus, and human immunodeficiency virus infection. Routine HBV serology (including hepatitis B e antigen [HBeAg] and antibody to hepatitis B e antigen [anti-HBe], HBsAg and anti-HBs, and HBV DNA) was tested within 4 weeks before BMT and at 4 weekly intervals after transplantation. The standard immunosuppression protocol for all patients included cyclosporine alone or in combination with prednisolone for at least 6 months after BMT when the immunosuppression was withdrawn. Of the 8 patients described here, 4 underwent transplantation before 1996 and did not receive any antiviral treatment. Since 1996, all HBsAg-positive BMT recipients were given prophylactic treatment with famciclovir or lamivudine starting 2 weeks before and continuing for 6 months after BMT. In this group, 3 patients received famciclovir (500 mg 3 times a day) and 1 received lamivudine (100 mg daily) (Table 1).

**Table 1. Clinical Characteristics of BMT Recipients**

<table>
<thead>
<tr>
<th>No.</th>
<th>Age/sex</th>
<th>Hematological diagnosis</th>
<th>HBV serology before BMT</th>
<th>Hepatitis flare and HBsAg clearance after BMT</th>
<th>Serum markers at the last follow-up after BMT</th>
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<tr>
<td></td>
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<td></td>
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<td>Time of hepatitis flare (mo)</td>
<td>Peak ALT (IU/L)\textsuperscript{a}</td>
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<td>CML</td>
<td>pos neg neg</td>
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</table>

\textsuperscript{a}The peak of ALT levels after BMT occurred between 2 and 24 weeks before the loss of HBsAg.

\textsuperscript{b}In both patients the other HBV markers are HBeAg negative, anti-HBe positive, and HBV DNA undetectable.

\textsuperscript{c}Received famciclovir 250–500 mg 3 times daily, starting 2 weeks before and continuing for 6 months after BMT.

\textsuperscript{d}Received lamivudine 100 mg daily, starting 2 weeks before and continuing for 6 months after BMT.

NOTE. Serum HBV DNA was measured by Chiron bDNA assay. ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; BMT, bone marrow transplantation; pos, positive; neg, negative; ALT, alanine aminotransferase (upper level of normal = 53 IU/L for male and 31 IU/L for female).
2 μg/mL for recombinant HBsAg (rHBsAg), 1 μg/mL for tetanus toxoid (Connaught Int. Laboratories, Ontario, Canada), and 1 μg/mL for phytohemagglutinin. In addition, the response to synthetic peptides, corresponding to immunodominant epitopes in the pre-S1 region (amino acids 21–48) and pre-S2 region (amino acids 146–175), was analyzed in all cases. The proliferative response was evaluated by 3H-thymidine uptake and measured by a β-counter (MicroBeta Trilux Counter, Wallac, Turku, Finland) in counts per minute. A stimulation index (SI) >2.5 (which was higher than the mean plus 2 SDs in 20 HBV-negative subjects) was considered positive. By CD4+ T-cell depletion of PBMCs showing a significant SI to HBcAg, we have confirmed that the proliferative response is due to CD4+ T cells. In 4 donor/recipient pairs (patients 4, 6, 7, and 8; Table 1), epitope mapping of the T-cell proliferative response to rHBcAg was performed using 17 20-mer overlapping peptides (final concentration, 10 μg/mL), spanning the entire HBV nucleocapsid protein.

PBMCs were also obtained from 11 healthy subjects with no previous exposure to HBV (seronegative for anti-HBc) who have received HBV vaccinations (Engerix-B; Smith Kline Beecham, Welwyn Garden City, England). Serum anti-HBs titer in all these individuals was >100 U/L, and the median interval between their last immunization and the time of mononuclear cell isolation was 15 months (range, 6–60 months). Fresh PBMCs were used to test the proliferative response to rHBsAg and tetanus toxoid, whereas frozen PBMCs were used to determine the frequency of HBsAg-specific, IFN-γ-producing CD4+ T cells as outlined below.

**ELISPOT Assays for CD4+ and CD8+ T Cells**

PBMCs were cryopreserved in a freezing mixture of complete culture medium (RPMI) with 75% fetal calf serum and 10% dimethyl sulphoxide (Sigma, Poole, England). The cells were aliquoted in Nunc cryotube vials (5 x 10^6 per vial), placed in a purposefully designed Nalgene cryocountertainer (Merck/BDH, Poole, England) with isopentane, and kept at -80°C for 24 hours. The vials were subsequently transferred in liquid nitrogen for long-term storage. After thawing, the cell viability was >95%, as assessed by trypan blue exclusion. Cryopreserved PBMCs were used to determine the frequency of HBsAg-specific, IFN-γ-producing CD4+ T cells as described. Briefly, 96-well millititer plates (Millipore, Bedford, MA) were coated overnight at 4°C with a primary antibody to human IFN-γ (1-DIK; Mabtech, Nacka, Sweden) at a concentration of 17 20-mer overlapping peptides spanning the entire HBV nucleocapsid protein, (2) 2 20-mer overlapping peptides covering the immunodominant area in the pre-S1 region between amino acids 21 and 48 (amino acids 21–40, PLGFFPDHQLDPAFGANSNN; amino acids 29–48, QLDPAFGANSNPD-WDFNPD), and (3) 2 20-mer overlapping peptides covering the immunodominant area in the pre-S2 region between amino acids 146 and 174 (amino acids 146–165, SSSGTVPNAP-NIAHISISSS; amino acids 156–174, NIAHISISSSSARTGDPV). In addition, 2 peptides corresponding to known HLA-A2–restricted epitopes in the HBV core, amino acids 18–27 (FLPSDFFPSSV), and the HBV surface, amino acids 335–343 (WLSLLYPFV), were used to determine the number of HBV-specific CD8+ T cells in the ELISPOT assay.

**Fluorescein-Activated Cell Sorter Analysis**

The phenotypic marker expression was analyzed by flow cytometry on cryopreserved PBMCs after dual staining with phycoerythrin (PE) and fluorescein isothiocyanate (FITC)–conjugated mouse monoclonal antibodies: anti-CD4-PE/anti-CD8-FITC, anti-CD4-FITC/anti-CD25-PE, anti-CD4-FITC/anti-HLA DR-PE, anti-CD4-FITC/anti-CD45RO-PE, and anti-CD4-FITC/anti-CD45RA-PE (all from BD Biosciences, Oxford, England). Briefly, 1 x 10^6 PBMCs were incubated with the respective combination of PE- and FITC-labeled antibodies at a concentration of 1:20 for 30 minutes on ice.
Subsequently, the cells were washed twice in phosphate-buffered saline with 1% fetal calf serum. The acquisition and analysis were performed by FACScan (Becton Dickinson, Oxford, England) and CELLQuest (version 1.0, Becton Dickinson), respectively.

**Polymerease Chain Reaction for the Y Chromosome**

Fresh PBMCs collected at the last follow-up from 5 male patients who received bone marrow from a female donor were analyzed. CD4-positive T cells were purified by immunomagnetic separation using Dynabeads (Dynal, Oslo, Norway) according to the manufacturer’s instructions. The purity of the CD4-positive T cells was confirmed to be >90% by fluorescein-activated cell sorter analysis using a PE-labeled anti-CD4 antibody. DNA was extracted from the purified CD4+ T cells, and microsatellite polymerase chain reaction (PCR) for detection of the Y chromosome was performed using 6 different sets of primers. The 6 Y-chromosome microsatellite loci (DYS19, DYS388, DYS390, DYS391, DYS392, and DYS393) were amplified in single 10-μL multiplex PCR reactions using 1 μL of DNA template as previously described.18 One primer in each primer pair was labeled with 1 of 3 ABI dyes (HEX, TET, and 6-FAM), and PCR products were run on an ABI-310 genetic analyzer (Applied Biosystems, Warrington, England) using GeneScan using POP-4 polymer and a 36-cm POP-4 capillary. The size of the PCR products ranged from 123 to 208 base pairs, and individual microsatellites were identified by a combination of size range and dye label.

**Hepatitis Serology and HBV-DNA Quantitation**

Serum HBV markers, including HBsAg, anti-HBs, HBeAg, and anti-HBe, were tested by commercially available enzyme immunoassays (Abbott Laboratories, Chicago, IL). The presence of anti-HBc was tested by radioimmunoassay (Corab; Abbott Laboratories). Commercial enzyme immunoassays (Abbott Laboratories) were used to test serum samples from all donors and recipients for antibody to hepatitis C virus, antibody to hepatitis D virus, and antibody to human immunodeficiency virus. Serum HBV-DNA level was quantified by the signal amplification assay (Quantiplex bDNA assay; Chiron Corp., Emerville, CA). Using this assay, the threshold for HBV-DNA detection in serum is 0.7 × 10^6 viral copies/mL.19

**Statistics**

Wilcoxon rank sum test was used to analyze paired values in the same patients at different time points. SPSS (SPSS Inc., Chicago, IL) was used.

**Results**

After engraftment of the bone marrow, all 8 HBsAg-positive recipients developed a hepatitis flare (defined as elevation of serum alanine aminotransferase level >3-fold above the upper level of normal) at a median of 3.3 months (range, 1.6–13.3 months). Between 0.5 and 6 months later, all 8 BMT recipients cleared serum HBsAg (Table 1). In 6 cases, the seroconversion to anti-HBs was sustained at the last follow-up visit (between 1.7 and 7.9 years after BMT) (Table 1). The other 2 patients lost HBsAg and seroconverted to anti-HBs for a period of 3 and 6 months, respectively, but became HBsAg positive again. Despite this, in one of the latter 2 cases, a partial response with sustained control of HBV replication was maintained because the patient cleared serum HBeAg and HBV DNA (Table 1).

**Proliferative T-Cell Response to HBV in Donor and Recipients**

Initially, we determined the HBV-specific proliferative responses of PBMCs taken at the last follow-up visit from 7 donor/recipient pairs (Table 1; patients 1–7) using rHBCag, rHBsAg, and pre-S1 and pre-S2 peptides. Significant CD4-positive T-cell proliferation to HBcAg was found in 6 of 7 donors (86%), whereas there was no response to HBsAg and pre-S1 or pre-S2 peptides. Proliferative response to HBV nucleocapsid protein was also detected in 4 of 7 recipients (57%), while none showed reactivity to the envelope antigens. A significant proliferative response to rHBsAg was present in 4 of 11 healthy HBV vaccine recipients with an SI varying between 3.3 and 12.2. The response to tetanus toxoid, which was used as a positive control, was similar in the group of 7 BMT recipients and in the group of healthy individuals; the SI (mean ± SD) was 28.9 ± 23.8 and 36.4 ± 29.7, respectively.

Next, we analyzed the specificity of T-cell reactivity to the nucleocapsid protein in 3 donor/recipient pairs (patients 4, 6, and 7; Table 1) with a significant proliferative response to HBCag. The epitope mapping was performed with a panel of 17 synthetic 20-amino acid peptides, spanning the entire nucleocapsid protein (Figure 1A). The results showed similarity in the fine specificity of the CD4-positive T cells from the donor and the recipient, because within each pair they recognized 1 or 2 identical epitopes of HBcAg within 3 immunodominant regions between amino acids 1 and 30, 61 and 90, and 141 and 170.

**CD4-Positive T Cells in the Recipients Are of Donor Origin**

To confirm that the CD4-positive T lymphocytes in the recipients are of donor origin, we used the sex mismatch in 5 of these 7 pairs, in which the donor was female and the recipient was male. CD4-positive T cells were isolated from the recipients’ PBMCs, taken at the
last follow-up visit, and multiplex PCR was performed using 6 sets of Y chromosome–specific primers (Figure 1B). The CD4-positive T cells showed no positive signal for the Y chromosome with any of the primers, thus confirming that they were of donor origin.

**Direct Enumeration of HBV-Specific T Cells**

To further characterize the T-cell reactivity to HBV, we determined the frequency of IFN-γ-producing CD4-positive and CD8-positive T cells in response to HBCAg and HBsAg using ELISPOT assays. PBMCs obtained at the last follow-up from 2 donor/recipient pairs with an HLA-A2 haplotype were used for this analysis. The number of IFN-γ–producing CD4-positive T cells was determined in response to recombinant nucleocapsid (rHBCAg) and envelope (rHBsAg) proteins, whereas synthetic peptides corresponding to 2 HLA-A2–restricted epitopes (core, amino acids 18–27; surface, amino acids 335–343) were used to define the number of IFN-γ–producing CD8-positive T cells (Figure 1C). In both the donors and recipients, the number of CD4-positive T cells specific for HBCAg was substantially greater than those specific for HBsAg. Similarly, the frequency of CD8+ T cells recognizing the HBV core peptide in the 2 BMT recipients was 22 and 27 SFCs/10^6 PBMCs, which was considerably higher than the frequency of the CD8+ T cells recognizing the envelope peptide (7 and 3 SFCs/10^6 PBMCs, respectively) (Figure 1C). In the group of HBV-vaccine recipients (n = 11), the mean frequency of IFN-γ–producing CD4+ T lymphocytes in response to rHBsAg was 137.2 specific SFCs/10^6 PBMCs (range, 35–258).

**CD4-Positive T Cells Are Activated During Hepatic Flare**

We next studied the changes in CD4+ T-cell activation and the proportions of naive and memory CD4+ T cells in these 7 recipients after the engraftment of bone marrow with immunity to HBV. For this purpose, we analyzed PBMCs obtained at 2 time points for each patient; the first sample was taken at the time when the recipients had a hepatitis flare, and the second sample was taken at the last follow-up visit. Cryopreserved PBMCs from these 2 time points were analyzed simultaneously by dual flow cytometry to determine the expression of early (CD25) and late (HLA-DR) T-cell activation markers as well as markers for naive (CD45RA) and memory (CD45RO) phenotype on CD4+ T lymphocytes (Figure 1D). The percentage of CD25-positive/CD4-positive T cells was significantly higher at the time of hepatitis flare than at the end of follow-up (mean ± SEM, 22.1% ± 9.9% vs. 6.6% ± 4.5%, respectively; P = 0.04, Wilcoxon test). The activation of CD4-positive T cells during the hepatitis flare was also shown by the higher expression of HLA-DR at this time point compared with the last follow-up visit (36.7% ± 8.6% vs. 14.4% ± 7.8%; P = 0.04). There was a significant increase in the proportion of memory CD4-positive T cells during the time of hepatitis flare; the percentage of cells expressing CD45RO was 96.1% ± 1.6% versus 83.6% ± 2.9% at the last follow-up (P = 0.04).

**Prospective Analysis of HBsAg Clearance After BMT**

We further investigated the virus-specific T-cell reactivity after adoptive transfer of immunity to HBV in a prospectively studied patient who was HBeAg and HBV DNA positive (patient 8; Table 1). Histologic analysis of the liver before BMT showed mild/moderate inflammatory infiltrates in the portal tracts with focal interface hepatitis. Immunostaining for HBCAg and HBsAg was positive in 5% and 10% of hepatocytes, respectively. The patient received bone marrow from an HLA-identical (HLA-A2 positive) male donor and developed hepatitis flare 24 weeks after BMT (peak alanine aminotransferase level, 461 IU/L) (Figure 2A). HBeAg to anti-HBe seroconversion occurred at the time of peak levels of alanine aminotransferase, with loss of HBsAg 4 weeks later, followed by seroconversion to anti-HBs.

PBMCs taken from the recipient before starting the pretransplant conditioning regimen showed no proliferative response to any HBV antigen (SI to rHBCAg, 1.2), whereas PBMCs from the donor showed significant proliferation to HBCAg (SI, 4.6). Epitope mapping with 17 core peptides showed consistent proliferative responses to 3 peptides, corresponding to amino acids 41–60, 51–70, and 141–160 of the HBV nucleocapsid protein. ELISPOT assays with PBMCs from the donor also showed predominance of CD4-positive and CD8-positive T cells specific for the HBV nucleocapsid compared with the envelope antigen (for IFN-γ–producing CD4-positive T cells, 168 SFCs/10^6 PBMCs with HBCAg and 25 SFCs/10^6 PBMCs with HBsAg; for IFN-γ–producing CD8-positive T cells, 13 SFCs/10^6 PBMCs with core peptide [amino acids 18–27] and 3 SFCs/10^6 PBMCs with surface peptide amino acids 335–343 [Figure 2B]). At the time of hepatitis flare, there was a significant proliferative response only to HBCAg (SI, 2.6) with activation of CD4-positive T cells and an increased proportion of CD45RO-positive T cells (Figure 2C). At 52 weeks after BMT, the recipient maintained HBCAg-specific T-cell proliferation (SI, 2.6) and the numbers of IFN-γ–producing T cells in response to HBV antigens have increased significantly (for IFN-γ–producing CD4-
Figure 1. (continued on next page).
Figure 1. Characterization of T lymphocytes in BMT recipients and their corresponding donors. (A) Fine specificity of CD4-positive T-cell reactivity in 3 donor/recipient pairs who have shown a significant proliferative response (SI, >2.5) to recombinant HBcAg using a panel of 20-mer overlapping, synthetic core peptides. D, donor; R, recipient. The numbers assigned to the pairs correspond to those in Table 1. (B) Multiplex PCR for the detection of Y chromosome in the recipients' CD4-positive T lymphocytes. Using 6 sets of Y chromosome-specific primers, male CD4-positive T cells (a) show the presence of 6 positive signals (**) between 123 and 208 base pairs. The standard size markers are shown in red. Amplification of DNA from purified CD4-positive T cells of male recipients who have received bone marrow from a female donor (b, c, and d) show no signal for the Y chromosome, thus confirming the donor origin of their CD4-positive T cells. (C) Direct ex vivo analysis (IFN-γ ELISPOT assays) of the number of HBV-specific CD4-positive and CD8-positive T lymphocytes in PBMCs from 2 HLA-A2-positive donor/recipient pairs (D1/R1 and D2/R2). D, donor; R, recipient. Recombinant (■) HBcAg and (□) rHBsAg were used as antigens to determine the frequency of HBV-specific CD4-positive T lymphocytes, whereas core (amino acids 18–27 [■]) and surface (amino acids 335–343 [□]) peptides were used to determine the frequency of HBV-specific CD8-positive T cells. SFC, specific spot-forming cells per 1 × 10⁶ PBMCs. (D) Changes in the proportion of CD4-positive T lymphocytes, which express activation markers (CD25 and HLA-DR) and memory phenotype (CD45RO), at the time of hepatitis flare (■) and at the last follow-up (□) in 7 BMT recipients. The bars show the mean ± SEM.
Figure 2. Prospective analysis of patient 8 (Table 1) after adoptive transfer of immunity. (A) Time course of serum alanine aminotransferase levels (ALT, normal level <53 IU/L) and serum HBV markers after BMT. The hepatitis flare occurred 24 weeks after BMT, and serum HBsAg was cleared 8 weeks later. (B) Analysis (by IFN-γ ELISPOT assays) of the number of HBV-specific CD4-positive and CD8-positive T lymphocytes in the donor (D); the recipient at the time of hepatitis flare (R fl), and the recipient 52 weeks after BMT (R FU). The (■) nucleocapsid and (□) surface antigens used for CD4-positive and CD8-positive T lymphocytes, respectively, are the same, as shown in Figure 1C.

Discussion

The phenomenon of HBsAg clearance and seroconversion to anti-HBs after adoptive transfer of immunity in patients with chronic hepatitis B provides a unique model to understand virus-specific T-cell reactivity associated with resolution of chronic HBV infection. By studying the largest series of patients, who cleared HBsAg after the engraftment of HLA-identical bone marrow from a donor with past exposure to HBV, we found that resolution of chronic HBV infection is associated with a transfer of CD4+ T-lymphocyte reactivity to HBcAg rather than to HBV envelope proteins. The present study also shows that the CD4+ T cells are of donor origin and that activation of the memory subset, CD45RO+ T cells, occurs during the hepatitis flare, which precedes the seroconversion to anti-HBs. These results explain our earlier clinical observation that HBsAg clearance occurs only after adoptive transfer of naturally acquired immunity to HBV (anti-HBs- and anti-HBc-positive donors) and not in patients who received marrow with a vaccine-induced immunity (anti-HBs alone).15

The ability of HBcAg-primed T-helper cells to provide functional T-cell help and to elicit anti-envelope antibody production when challenged with HBV has been shown in mice.20 In the present study, HBcAg-specific CD4+ T cells were found in 7 of 8 donors, and the transfer of such primed T-helper cells seems to have a pivotal role for seroconversion to anti-HBs after the adoptive transfer of immunity because they would provide intermolecular T-cell help on challenge with HBV in the recipients. Further examples of such functional
Figure 2 (Cont'd.) (C) Comparison of the proportions of activated CD4-positive T lymphocytes (CD25 or HLA-DR positive) and the memory subset (CD45RO positive) in the donor, the recipient at the time of hepatitis flare, and the recipient 52 weeks after BMT.

Donor

Recipient flare

Recipient follow up

Studies of patients with acute self-limited hepatitis B also emphasize the notion that activation of HBcAg-specific CD4⁺ T lymphocytes is a prerequisite for resolution of HBV infection. The HBV nucleocapsid protein has been shown to be the strongest immunogen for HLA class II–restricted T-cell responses during acute infection, with the peak of T-cell proliferation to HBcAg usually coinciding with the loss of serum HBeAg and HBsAg. These T-cell responses to HBV nucleocapsid are long-lasting and sustained by CD45RO⁺ cells. Instead, the T-cell proliferative response to HBV envelope antigens is very weak or undetectable in acute HBV infection. A recent analysis of circulating HBV-specific CD8⁺ T cells in patients with acute hepatitis B using HLA-A2/peptide tetramers showed different frequencies of core, polymerase, and envelope-specific CD8⁺ cells. The frequency of core epitope 18–27-specific CD8⁺ T cells was considerably higher than the proportion of cells specific for the envelope epitope 335–343, which supports the bias toward HBcAg seen in CD4⁺ T-helper cell reactivity during acute self-limited hepatitis B.

The reasons for the weak virus-specific T-cell reactivity in patients with chronic HBV infection are not fully understood. Induction of HBcAg-specific T-cell reactivity in patients with chronic hepatitis B has been shown...
in both spontaneous or IFN treatment-induced seroconversion to anti-HBe with an effective control of HBV replication.28,29 The present study further indicates that long-lasting reconstitution of T-cell reactivity to the HBV nucleocapsid protein in such patients results in resolution of chronic hepatitis B and development of natural immunity to HBV, an immunologic profile analogous to cases with previous exposure and spontaneous viral clearance. So far, the attempts to achieve this through therapeutic immunization have produced disappointing results. Immunotherapy using recombinant preS2/S protein has elicited CD4+ T-cell proliferation to HBV envelope proteins in 7 of 27 immunized patients with chronic hepatitis B, and some cases showed a reduction in serum HBV-DNA levels.30 However, this protocol did not increase the rate of HBcAg/anti-HBe seroconversion, and serum HBsAg clearance did not occur in any of the immunized patients. These results were confirmed in a multicenter controlled trial involving 118 patients.31 Another approach using a vaccine which involves a CTL epitope (the core peptide amino acids 18–27) has induced only a low level of CTL activity, and there was no resolution of HBV infection and chronic hepatitis B in any of the patients studied.32

Recent experimental data showed the essential role of the CD4+ T-cell help for effective function of CTL and viral elimination.33 The practical implication of the present findings of successful HBsAg clearance after adoptive transfer of immunity to HBcAg is that therapeutic immunization of patients with chronic HBV infection should include the HBV nucleocapsid protein (or the core gene for DNA immunization) and aim to induce both HBcAg-specific CD4+ and CD8+ T-cell responses. Apart from priming the T-helper cells, which will then provide intermolecular help for anti-envelope antibody production, HBcAg is approximately 100-fold more efficient than HBsAg in its ability to activate T cells.34 Th2 predominance and low CTL activity are characteristic for patients with chronic hepatitis B,35 and a potent immunogen, like particulate HBcAg, may help to revert this in favor of Th1, break the tolerance at the CTL level, and hence result in resolution of chronic HBV infection. The success of the adoptive transfer of immunity to HBV nucleocapsid protein in the present series of Chinese patients with chronic HBV infection, in whom the annual rate of HBsAg clearance is as low as 0.1%–0.8%,6 shows its potential power for resolution of chronic HBV infection.

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