Characterization of HLA DR13-restricted CD4+ T cell epitopes of hepatitis B core antigen associated with self-limited, acute hepatitis B

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The HLA DR13 allele has been associated with a self-limited course of hepatitis B virus infection, possibly through the induction of a more vigorous hepatitis B core antigen (HBcAg) and/or hepatitis B e antigen-specific CD4+ T cell response. HBcAg-specific CD4+ T cell responses were investigated in three HLA DR13-positive subjects with self-limited, acute hepatitis B. HBcAg-specific, short-term T cell lines derived from these three subjects showed a dominant recognition of HBcAg peptides spanning aa 1–20 (P1), 11–30 (P2), 41–60 (P5), 111–131 (P12) and 141–160 (P15). In order to characterize these epitopes in more detail, CD4+ T cell clones and cell lines were generated using HBcAg. Surprisingly, 11 of 12 T cell clones examined recognized P15; one recognized P10 (aa 91–111). Of four T cell lines, two recognized P15 and two recognized P5. By peptide mapping, the minimal epitope of P15 was located to residues 147TVVRRRGRSP156.

Introduction

The clinical consequences of hepatitis B virus (HBV) infection are extremely variable. The majority of HBV-infected subjects experience a clinically apparent, acute hepatitis that recovers without sequelae and induces immunity towards the virus. In less than 1% of cases, fulminant hepatitis, which causes massive liver cell death, occurs, resulting in a mortality of over 50%. Of infected subjects, 5–10% develop a chronic infection that may lead over the years to serious complications such as liver cirrhosis, liver decompensation and hepatocellular carcinoma. There is ample evidence demonstrating that the immune response of the host to the virus largely influences both the outcome of infection and the severity of liver cell damage during chronic evolution. Both innate and adaptive immunity contribute to the clearance and/or control of HBV (Chisari & Ferrari, 1995; Guidotti & Chisari, 2001; Milich, 1997). However, which factor(s) ultimately determine the outcome of the disease remains unclear.

HLA class II molecules expressed on the cell surface membrane of antigen-presenting cells (APCs) play a key role in the regulation of humoral and cellular immune responses (Germain & Margulies, 1993; Germain, 1994). Self-limited, acute HBV infections are associated with vigorous HLA class II-restricted CD4+ T cell responses towards the hepatitis B core antigen (HBcAg), whereas weak or no responses are observed in patients with chronic hepatitis (Ferrari et al., 1991; Jung et al., 1992, 1995; Lohr et al., 1995; Marinos et al., 1995; Penna et al., 1996, 1997). These observations suggest that HBcAg-specific CD4+ T cell responses play an important role in the recovery from disease and control of the virus. The HLA system is highly polymorphic and the HLA phenotype of an individual largely determines the response to various antigens and outcome of disease. HLA DR13 has been associated with resistance to several infectious diseases, such as hepatitis C, AIDS and malaria (Hill et al., 1991; Kuzushita et al., 1996, 1998; Chen et al., 1997). Although earlier studies on the association between major histocompatibility class polymorphism and HBV infection led to conflicting results (Forzani et al., 1984; Black et al., 1986; Almarri & Batchelor, 1994; Zavaglia et al., 1996), three studies in larger cohorts of patients demonstrated independently that HLA DR13 is involved in the elimination of HBV. A first study reported that in a large study population in Gambia, the HLA allele DRB*1302 was associated with protection against persistent HBV infection in both children and adults (Thursz et al., 1995). This observation has been confirmed in Caucasians (Hohler et al., 1997) and in an Asian population (Ahn et al., 2000). Recently, it was demonstrated that the beneficial effect of the HLA DR13 alleles on the
outcome of HBV infection could be explained by a vigorous HBcAg-specific CD4+ T cell response (Diepolder et al., 1998). This effect could be due to more proficient antigen presentation by the HLA DR13 molecules themselves or a linked polymorphism in a neighbouring immunoregulatory gene. To explore further the relationship between HLA DR13 and HbcAg-specific CD4+ T cell responses in patients with acute, self-limited HBV infection, we generated HLA DR13-restricted CD4+ T cell clones and cell lines from the HLA DR13-positive subjects who recovered spontaneously from acute HBV infection. Surprisingly, most T cells generated from these donors recognized a single epitope within HBcAg, aa 147–156.

Methods

Subjects and HLA typing. Three HLA DR13*-positive subjects who fully recovered from acute hepatitis B (AHB) were studied. All subjects provided written, informed consent to participate in this study, which was approved by the local Ethical Review Board of Ghent University Hospital, Ghent, Belgium. The HLA class II DR, DP and DQ haplotypes of these subjects were determined by INNO-LIPA (Innogenetics), according to the manufacturer’s guidelines and as described previously (Cao et al., 2001). HLA class II haplotypes of these subjects were as follows: (1) AHB1, DRB1*1302, DRB1*0401, DQB1*0302 and DQB1*0604; (2) AHB2, DRB1*13**, DRB1*0401, DPB1*0401, DPB1*0401, DQB1*0301 and DQB1*0003; (3) AHB3, DRB1*13**, DRB1*13*, DPB1*0201/2, DPB1*0402, DQB1*0301 and DQB1*0603.

Preparation of peripheral blood leukocytes (PBLs). PBLs were isolated from fresh heparinized blood by Ficoll-Hypaque (Nycomed) density gradient centrifugation. PBLs were washed extensively and used immediately for the generation of HBcAg-specific T cell lines or frozen in liquid nitrogen for later use.

Reagents. HBcAg is a 21 kDa protein that assembles to form a nucleocapsid particle. Recombinant HBcAg (rHBcAg, subtype ayw) expressed in Escherichia coli was purchased from DiaSorin. A total of 17 partially overlapping 20-mer peptides spanning the complete sequence of HBcAg (subtype ayw) were used in these studies (Table 1). A series of N- and C-terminally truncated peptides of HBc P15 and a panel of alanine-substituted peptides spanning HBc P15 were also employed. All peptides were synthesized with a multiple peptide synthesizer using standard Fmoc chemistry (Syro, MultiSynTech).

Recombinant gD2 (glycoprotein D2 from herpes simplex virus, expressed in mammalian cells and used as a control antigen) was a gift from GlaxoSmithKline Biologicals. Tetanus toxoid (TT) was obtained from Statens Seruminstitut, WHO (Copenhagen, Denmark). The following mouse anti-human monoclonal antibodies (mAbs) were used for cytometry analysis: anti-CD3, -CD4 and -CD8 (all from Becton Dickinson). Mouse anti-human HLA class I anti-HLA DR, DP and DQ antibodies and mouse isotopic control antibodies were from Serotec.

Generation of HBcAg-specific T cell clones and cell lines. PBLs (3×10^6 cells per well) from three AHB subjects were stimulated separately with rHBcAg (0.5 µg/ml) in 24-well plates in RPMI 1640 supplemented with 25 mM HEPES, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mM L-glutamine (all from Life Technologies), 5×10^-5 M 2-mercaptoethanol (Sigma) and 10% heat-inactivated human AB+ serum (hereafter called complete medium). After 5 days, fresh complete medium containing recombinant human interleukin-2 (IL-2, 10–50 U/ml, Eurocetus) was added to the cultures. T cell clones from subject AHB1 were generated by culturing the cells at limiting dilutions (0.3 cells per well; there is one chance in three that each well will be seeded with a cell) in 96-well, flat-bottomed plates in the presence of 0.1 µg/ml rHBcAg, 10 U/ml human rIL-2 and gamma-irradiated (2-5 Gy) autologous PBLs (3×10^6 PBLs per well). Every 2 weeks, the cultures were restimulated with rHBcAg and irradiated, autologous PBLs (2.5 Gy) as APCs. After 8 weeks, the growing T cell clones were tested for HbcAg specificity by confronting them with a series of overlapping synthetic Hbc peptides. The phenotype of Hbc-specific T cell clones was analysed by flow cytometry (FACScan, Becton Dickinson).

Because the observations made with the clones generated from subject AHB1 needed to be confirmed, HbcAg-specific T cell lines from two additional subjects who recovered from AHB (AHB2 and AHB3) were generated by culturing the cells at a density of 10 cells per well in the presence of rHBcAg (0.2 µg/ml), rL2 (25 U/ml) and irradiated (3.5 Gy), autologous or DR13-matched, allogenic PBLs as APCs.

Proliferation and restriction assay. Proliferation assays of T cell clones or lines were performed by incubating 2×10^5 T cells per well for 4 days in the presence of different antigens (rHBcAg, TT and Hbc peptides) and irradiated, autologous PBLs as APCs (5×10^6 PBLs per well). All cultures were performed at least in duplicate and in triplicate when sufficient cells were available. At 18 h before harvesting the cultures, 0.5 µCi (1.85 MBq) [3H]thymidine (Tdr) was added and the radioactivity incorporated by the cells was determined by β-counting.

In peptide stimulation assays performed to determine the specificity of T cell clones, autologous Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) that were treated previously with mitomycin C (50 µg/ml) were used as APCs (5×10^6 cells per well). LCLs were used as APCs rather than fresh PBMCs because the former adequately presented peptides and because we wanted to limit the number and volume of phlebotomies to a strict minimum.

In order to determine the restriction of T cell recognition, blocking assays were performed in which T cell proliferation was measured in the

Table 1. Synthetic peptides spanning the complete sequence of HBcAg (subtype ayw)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid position</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>P1</td>
<td>1–20</td>
<td>MDIDPYPKEFATVELLSFLP</td>
</tr>
<tr>
<td>P2</td>
<td>11–30</td>
<td>ASVELLFLPSDFPFSVY/RLD</td>
</tr>
<tr>
<td>P3</td>
<td>21–40</td>
<td>SDFFPSSVRDRLDTSASYRE</td>
</tr>
<tr>
<td>P4</td>
<td>31–50</td>
<td>LDTASALYREALESPEHCP</td>
</tr>
<tr>
<td>P5</td>
<td>41–60</td>
<td>ALESPEHCSHPHTHALQRAIL</td>
</tr>
<tr>
<td>P6</td>
<td>51–70</td>
<td>HHTALRQAILCWGELMTNLAT</td>
</tr>
<tr>
<td>P7</td>
<td>61–80</td>
<td>CWGELMTLATWGVNLEDPA</td>
</tr>
<tr>
<td>P8</td>
<td>71–90</td>
<td>WVGVNLLEDPAEDLVLVSYVN</td>
</tr>
<tr>
<td>P9</td>
<td>81–100</td>
<td>SRLDVVSYYNTNMGLKFQR2L</td>
</tr>
<tr>
<td>P10</td>
<td>91–110</td>
<td>TNMGLKFQR2LIVHHSCLTF</td>
</tr>
<tr>
<td>P11</td>
<td>101–120</td>
<td>LWFHHSCLTFGRETIVYELV</td>
</tr>
<tr>
<td>P12</td>
<td>111–131</td>
<td>GRETIVYELVSGVWIRTPP</td>
</tr>
<tr>
<td>P13</td>
<td>121–140</td>
<td>SGVWIRTPPAAYRNPRAPL</td>
</tr>
<tr>
<td>P14</td>
<td>131–150</td>
<td>AYPAPNAIPSTLPETTVIL</td>
</tr>
<tr>
<td>P15</td>
<td>141–160</td>
<td>STLPETTVVRRGSRPRTT</td>
</tr>
<tr>
<td>P16</td>
<td>151–170</td>
<td>RRRGSRPRTTPFSPPRRSQQS</td>
</tr>
<tr>
<td>P17</td>
<td>161–180</td>
<td>PSPPRRSOSPGRRSSQSRE</td>
</tr>
</tbody>
</table>

<ref>Diepolder et al., 1998</ref>
Fig. 1. Epitope recognition patterns of HBcAg-stimulated, polyclonal, short-term T cell lines. PBMCs (3 x 10^6 cells per well) from three DR13-positive donors were stimulated with rHBcAg (1 µg/ml) for 5 days. HBcAg-responsive T cells were expanded further with rIL-2. At days 12–14, T cell proliferative responses to a series of 17 overlapping peptides added at 10 µg/ml (see Table 1) spanning the complete sequence of HBcAg and presented by irradiated (2–5 Gy), autologous PBLs were examined. All assays were performed in triplicate. [3H]TdR (0.5 µCi per well) was added 18 h before harvesting. The cultures were harvested on day 4 and assayed for [3H]TdR incorporation by liquid scintillation counting in an LKB-Wallac 8100 counter (LKB). Data are expressed as the mean value of triplicate wells.
presence or absence of mouse anti-human HLA class I, class II (DR, DP and DQ) and isotypic control antibodies (1 µg/ml). In confirmatory assays, T cell proliferation was measured following antigen presentation by HLA-typed allogeneic PBLs displaying different haplotypes.

**Results**

**Generation of short-term HBcAg-specific T cell lines and analysis of epitope recognition patterns**

Three HLA DR13-positive donors (AHB1, AHB2 and AHB3) who spontaneously recovered from AHB were examined in this study. PBMCs (3 × 10⁶ cells per well) of these donors were stimulated with rHBcAg (0·5 or 1 µg/ml) for 5 days and HBcAg-responsive T cells were expanded further with human rIL-2 for another week. The fine specificities of the HBcAg-stimulated short-term T cell lines from these three subjects were determined on days 12–14 with 17 synthetic peptides spanning the complete sequence of HBcAg (subtype ayw) (Table 1). An overview of this analysis is shown in Fig. 1. Core peptides P4, P6, P10, P13 and P14 were recognized by only one of the three polyclonal T cell lines induced by HBcAg.

Fig. 2. HBcAg-specific clones recognize HBcAg P15. The fine specificity of 10 HBcAg-specific T cell clones was examined in a series of proliferation assays using peptide-loaded (10 µg/ml), mitomycin C-treated, autologous, EBV-transformed LCLs as APCs. [³H]Tdr incorporation of T cells was counted, as described, and the data shown are expressed as stimulation indexes. All 10 HBcAg-specific T cell clones examined recognized the same region of HBcAg, aa 141–160. The responses of clones A2, A4 and A5 are shown.
HBcAg-specific DR13-restricted T cell response

stimulation. P2, P5 and P12 were recognized by two of three T cell lines and the HBcAg peptides that were recognized by all three short-term T cell lines were P1 and P15.

Generation of long-term HBcAg-specific T cell clones

A total of 30 HBcAg-specific T cell clones were generated from donor AHB1 by culturing the cells at limiting dilutions (0–3 cells per well; there is one chance in three that each well be seeded with a cell). When these clones were stimulated with different antigens (HBcAg, TT and gD2) in the presence of irradiated, autologous PBMCs, only HBcAg induced a proliferative response, which demonstrated the core specificity of these clones (data not shown). The fine specificity of 10 HBcAg-specific T cell clones was determined using our series of synthetic HBcAg peptides and all 10 recognized P15. These HBcAg P15-specific T cell clones were designated clones A1–A10. The results obtained with three representative clones are shown in Fig. 2. We generated additional HBcAg-specific T cell clones from the same donor and, of the two HBcAg-specific T cell clones tested, one clone (A11) recognized HBcAg P15, while the other clone (A12) recognized HBcAg P10 (data not shown). Additionally, when HBcAg-specific T cell lines from the two other DR13-positive subjects who had recovered from AHB were obtained (by stimulating their PBLs with HBcAg and seeding these stimulated cells at 10 cells per well for further expansion in IL-2-containing media), two of four HBcAg-specific T cell lines (B1 and C1) recognized HBcAg P15. The other two T cell lines (B2 and C2) responded to P5 (data not shown). The phenotype of these HBcAg-specific T cell clones and cell lines were analysed by flow cytometry. All T cell clones and cell lines examined were CD3+CD4+CD8− (data not shown).

HLA restriction of the HBcAg P15-specific T cell clones or cell lines

To define the restriction element involved in the presentation of P15 to the P15-specific T cell clones, we stimulated these clones with HBcAg in the presence or absence of anti-DR, -DP, -DQ, anti-class I and isotypic control antibodies. Six HBcAg T cell clones (clones A1–A6) specific for aa 141–160 from donor AHB1 and one cell line (B1) from donor AHB2 were examined. All were predominantly DR-restricted (80–95%). Results obtained from T cell clones A1, A2 and A3 are shown in Fig. 3. Some of these clones were also stimulated with P15 in the presence of anti-DR and these responses were similarly inhibited by this antibody (data not shown).

The HLA restrictions of clones A1–A6 and cell line B1 were examined further in a series of experiments studying the presentation of HBcAg by seven or eight HLA-typed, allogeneic PBMCs displaying different haplotypes. All T cell clones (A1–A6) and T cell line B1 responded when HBcAg was presented by DR13-expressing APCs. No proliferation of the T cell clones or the T cell line occurred upon stimulation with allogeneic PBMCs that were partially matched with those of the donor but lacked DR1301 or DR1302 (Fig. 4). These experiments, however, could not rule out that HBcAg was presented by DQ06*. We were unable to find APCs that
Fig. 4. HLA class II restriction of HBcAg P15-specific T cell clones defined by antigen stimulation with haploidentical allogeneic PBLs. Six HBcAg P15-specific DR-restricted T cell clones from donor AHB1 and one cell line from donor AHB2 were stimulated with HBcAg (0.2 µg/ml) in the presence of a series of HLA-typed, irradiated (3.5 Gy) PBLs that partially matched the haplotype of the responder. [3H]Thymidine uptake of responsive T cells was counted. Data obtained from two representative clones and one cell line are shown.
HBcAg-specific DR13-restricted T cell response

Fig. 5. Responses of HBcAg P15-specific T cell clones to the N- and C-terminally truncated peptides spanning HBcAg aa 141–160. Four HBcAg P15-specific T cell clones were stimulated with mitomycin C-treated, autologous EBV-transformed EBV-LCLs loaded with different N- and C-terminally truncated HBcAg peptides. Uptake of [%H] Thymidine by stimulated T cells was quantitated. Results shown are the means of triplicate wells. Data obtained from four T cell clones are shown.
Fig. 6. Effects of single alanine substitutions in HBcAg P15 on the responses of HBcAg P15-specific T cell clones. Four HBcAg P15-specific T cell clones were stimulated with mitomycin C-treated, autologous, EBV-transformed LCLs that were loaded with different alanine-substituted peptides (10 µg/ml). Uptake of [3H]TdR by responsive T cells was counted. Mean values of triplicate cultures are shown. Data obtained from four T cell clones are shown.
expressed DR13 in the absence of DQ06, probably because these alleles are in linkage disequilibria. However, based on the blocking experiments, we conclude that these P15-specific T cell clones or lines are DR13-restricted. The P10-specific T cell clone (A12) from donor AHB1 was also examined and was also DR13-restricted (data not shown). Because of poor cell expansion, we were unable to examine the HLA-restriction of cell line C1 from donor AHB3.

**Determination of the minimal epitope within HBcAg P15**

Four P15-specific T cell clones (A1–A4) from donor AHB1 were selected to map the minimal T cell epitope. T cell clones were stimulated with a series of overlapping C- and N-terminally truncated peptides spanning aa 141–160 presented by mitomycin C-treated, autologous, EBV-transformed LCLs. The minimal epitope shared by all four P15-specific T cell clones was the sequence represented by aa 147–156 (TVVRR-RGRSP). The four T cell clones displayed slight differences in their sensitivities to the removal of V148 and S155 (Fig. 5).

**Determination of the critical residues involved in antigen presentation and/or T cell recognition of epitope HBcAg (aa 141–160)**

To define the critical residues within aa 141–160 of HBcAg that interact with the HLA DR13* molecules or that are recognized by the T cell receptor of the HBcAg P15-specific T cell clones, a series of peptides were synthesized that contained single alanine substitutions from position 141 to 160. The results obtained with four T cell clones are shown in Fig. 6. The four clones did not display exactly the same response pattern to these alanine-substituted peptides but they did recognize the same minimal peptide. Alanine substitution at position R151 abrogated the proliferation of all T cell clones. Clones A1 and A2 were also sensitive to alanine substitution at positions R150, R152, C153 and P156. Clone A1 was sensitive to further changes at positions R154 and S155. However, clones A3 and A4 appeared more tolerant to alanine substitutions than clones A1 and A2, at least when peptides were used at a concentration of 10 µg/ml. In clone A3, alanine substitutions at positions R150, R152, C153, R154, S155 and P156 reduced but did not abrogate the responses of the clone when the peptides were used at a concentration of 10 µg/ml. In clone A4, alanine substitutions did not reduce the immunogenicity of the peptides when these were used at a concentration of 10 µg/ml, except for the substitution at position R151. However, both clones A3 and A4 were sensitive to alanine substitutions at positions 150–156 when used at a concentration of 1 µg/ml (data not shown).

All four clones tolerated alanine substitutions at positions S141, T142, L143, P144, E145, T146, T147, V148, V149, R157, R158, R159 and T160 (Fig. 6). Collectively, the distinct recognition patterns of the four clones confirmed that these were distinct clones and showed the polyclonality of the response to a dominant epitope.

**Discussion**

Recent studies have shown that in Africans (Thursz et al., 1995), Caucasians (Hohler et al., 1997) and Asians (Ahn et al., 2000), HLA DR13 is associated with a self-limited course of HBV infection and thus seems to convey protection against chronicity. A strong HLA class II-restricted HBcAg-specific CD4+ T cell response has also been associated with acute, self-limited disease. Diepolder et al. (1998) suggested that the HLA DR13 allele might have a beneficial effect on the outcome of HBV infection through the induction of a more vigorous HBcAg-specific CD4+ T cell response, as compared to DR13-negative subjects. In the present study, we further explored the relation between HLA DR13 and the HBcAg-specific CD4+ T cell response.

Our analysis of the T cell recognition of HBcAg by three HLA DR13-positive subjects who recovered from an acute HBV infection shows that all three subjects display a significant response to recombinant HBcAg and that each individual recognizes at least four different HBcAg-derived peptides. This confirms the observations of Diepolder et al. (1998) who analysed eight DR13-positive AHB patients. The frequency of peptide recognition by our patients displayed the following hierarchy: P1 and P15 (aa 1–20 and 141–160) were recognized by three of three subjects; P2, P5 and P12 (aa 11–30, 41–60 and 111–131) were recognized by two of three subjects and P4, P6, P10, P13 and P14 (aa 31–50, 51–70, 91–110, 121–140 and 131–150) were recognized by one of three subjects. This differs somewhat from the frequencies reported by other investigators (Ferrari et al., 1991; Jung et al., 1995; Penna et al., 1997; Diepolder et al., 1998) who found peptide spanning aa 50–69 to be the most immunodominant one (it was recognized by seven of eight subjects) and further disclosed the following hierarchy of recognition: of eight subjects, peptide 61–85 was recognized by six, peptide 81–105 was recognized by five, peptide 141–165 was recognized by four, peptide 101–125 was recognized by three, peptides 1–25, 21–45, 121–145 and 161–183 were recognized by two and peptide 41–65 was recognized by one. The difference in the hierarchy observed may be due to differences in the patient populations examined, differences in the qualities of the peptides employed and differences in the culture methods used. However, when the peptide recognition pattern of DR13-positive and -negative subjects was compared, it was observed that the three immunodominant peptides were the same in both groups of patients and that only one peptide, namely peptide 141–165, was preferentially recognized by HLA DR13-positive individuals (P<0.001). The investigators were, however, unable to demonstrate that this peptide (aa 141–165) was presented to the T cells by the DR13 molecule. Our study provides ample evidence that this is indeed the case. Using blocking experi-
ments with mAbs directed against DR and lymphoproliferation assays wherein HBcAg or peptide 141–165 were presented to P15-specific T lymphocytes by haploidentical PBLs, we demonstrated that six clones and one T cell line specific for P15 recognized this peptide in the context of the HLA DR13 molecule.

To exclude the occurrence of some inexplicable or accidental phenomenon causing all T cell clones generated from subject AHB1 to recognize the same peptide P15 (aa 141–160), we started a new cloning procedure with cells from subject AHB1 and, in addition, we produced HBCAg-specific T cell lines from PBLs of two other AHB patients (AHB2 and AHB3). Each experiment led to the production of P15-specific T lymphocytes, making the possibility that results were experimental artefacts very unlikely and suggesting that P15-specific T cells are consistently induced during an acute HBV infection in DR13-positive patients.

In an attempt to understand the molecular basis of the preferential presentation of P15 by the HLA DR13 molecule, we analysed the recognition characteristics of four T cell clones using a series of N- and C-terminally truncated and single alanine-substituted peptides spanning HBCAg aa 141–160. All four T cell clones recognized the minimal epitope HBCAg 141TVVRRGRSP158. Our analysis further revealed the critical role of certain (V148, V149 and R151), and the less important role of other (R156, R151, G153 and P158), amino acids, but the information gathered did not allow us to define which amino acids might be involved in the binding to the DR13 molecule and which are recognized by the T cell receptor. Importantly, this analysis showed the polyclonal nature of the response to this dominant epitope within HBCAg.

In the past, therapeutic immunizations with HBV envelope proteins have produced disappointing results (Couillin et al., 1999; Pol et al., 2001). Recent studies have shown that transfer of immunity to HBV can be achieved by bone marrow transplantation from immune donors (Ilan et al., 1993; Shouval et al., 1993; Shouval & Ilan, 1995; Lau et al., 1997). Recently it was demonstrated that this resolution of HBV infection was associated with transfer of HBCAg-specific T cells present in the marrow graft (Lau et al., 2002). Interestingly, in this study, freshly isolated PBMCs from four immune donors responded to HBCAg P1, P2, P5, P6, P7, P8, P15 and P16 (Lau et al., 2002). Similar responses were observed with freshly isolated PBMCs from recipients. Moreover, the responding T cells found in the recipients were shown to be of donor origin. These results, together with previous observations (Diepolder et al., 1998) and our results, strongly indicate that recognition of the T cell epitope at aa 141–160 can be correlated with virus clearance in HLA DR13 subjects.

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References


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