Hepatitis B synthetic immunogen comprised of nucleocapsid T-cell sites and an envelope B-cell epitope

DAVID R. MILICH*,†, JANICE L. HUGHES*, ALAN MCLACHLAN‡, GEORGE B. THORNTON§, AND ANN MORTARY§

Departments of *Molecular Biology and of †Basic and Clinical Research, Research Institute of Scripps Clinic, La Jolla, CA 92037; and §Johnson and Johnson Biotechnology Center, Inc., San Diego, CA 92121

Communicated by Frank J. Dixon, October 29, 1987

ABSTRACT Previous studies located T-cell recognition of the nucleocapsid of the hepatitis B virus (HBcAg) to residues 120–140 in mice bearing the H-2 and H-2 haplotypes. Herein, we demonstrate that B10.S (H-2) and B10 (H-2) H-2 congenic strains recognize distinct T-cell sites within the p120–140 (a synthetic peptide corresponding to residues 120–140 of HBcAg) sequence defined by p120–131 and p129–140, respectively. Peptide p120–131 stimulates B10.S HBcAg-primed T cells, and reciprocally p120–131-primed T cells recognize HBcAg. Similarly, the p129–140 sequence is a T-cell recognition site relevant to the native HBcAg in the B10 strain. It is also shown that these 12-residue peptides efficiently prime T-helper cells, which are capable of eliciting antibody production to HBcAg in vivo. These observations prompted us to examine the ability of the HBcAg-specific p120–140 sequence to function as a T-cell carrier moiety as a component of a totally synthetic hepatitis B vaccine. For this purpose a synthetic B-cell epitope from the pre-S(2) region (p133–140) of the viral envelope was chosen because this sequence represents a dominant antibody-binding site of the envelope. Immunization of B10.S and B10 strains with the synthetic composite peptide c120–140–(133–140) elicited anti-antibody production, which was crossreactive with the native viral envelope. Furthermore, c120–140–(133–140) immunization primed p120–131-specific T cells in the B10.S strain and p129–140-specific T cells in the B10 strain, which recognized HBcAg and provided T-helper cell function for anti-envelope antibody production in vivo. These results demonstrate the feasibility of constructing complex synthetic immunogens that represent multiple proteins of a pathogen and are capable of engaging both T and B cells relevant to the native antigens.

The hepatitis B virus (HBV) consists of an outer envelope, composed of three terminal polypeptides (P25, GP33, and P39), and an inner nucleocapsid, which encapsidates the viral genome. The nucleocapsid of the HBV is a 27-nm particle composed of multiple copies of a single polypeptide of M, 21,000 (P21), and the intact structure exhibits hepatitis B core antigenicity (HBcAg). A nonparticulate form of HBcAg, designated HBeAg, may be present in the serum during HBV infection. Although HBcAg and HBeAg are serologically distinct, the primary amino acid sequences show significant identity (serum HBeAg lacks the C-terminal 34 residues of HBcAg) (1). The hepatitis B surface envelope (HBsAg) is composed of a major polypeptide (P25) (2). The larger polypeptides [glycoprotein of M, 33,000 (GP33) and P39] share the 226 amino acids of P25 (S region) at the C-terminals and possess additional residues at the N terminus. The pre-S(2) region consists of 55 residues N-terminal to the S region (3), and the pre-S(1) region consists of 119 residues N-terminal to the pre-S(2) region (4).

Vaccination with envelope [S or pre-S(2)] or nucleocapsid antigens has been reported to protect against HBV infection (5–7). Because antibodies to HBcAg (anti-HBc antibodies) are not virus neutralizing, the mechanism of protection by HBcAg is unknown. However, an interrelationship between the immune responses to HBsAg and HBcAg has recently been reported. HBCAg-specific T cells are able to provide T-helper (Th) cell activities for anti-S, anti-pre-S(2), and anti-pre-S(1)-specific antibody production if HBcAg and the envelope proteins are present within the same particle (i.e., the virion) (8). This phenomenon may explain the mechanism of protection by HBcAg vaccination (8).

Recently, we demonstrated that HBcAg functioned as a T-cell-independent antigen in athymic mice (9) and stimulated efficient T-cell activation in euthymic mice (9, 10). Examination of the fine specificity of T-cell recognition of HBcAg revealed multiple T-cell sites within the HBcAg sequence, and the site recognized is dependent on the H-2 haplotype of the responding strain (10). It is noteworthy that the H-2 and H-2 strains both recognize p120–140, a synthetic peptide corresponding to residues 120–140 of the HBcAg sequence. Herein, it is shown that the H-2 and H-2 strains actually recognize distinct T-cell sites within the p120–140 sequence. Additionally, the ability of defined synthetic T-cell sites to prime functional Tc cell activity and induce anti-HBc production in vivo is also demonstrated. These observations and the ability of HBcAg-specific Tc cells to elicit anti-envelope antibody production (8) prompted us to examine the potential of p120–140 to act as a T-cell carrier moiety as component of a prototypic synthetic HBV vaccine. An eight-residue peptide previously shown to represent a dominant antibody-binding site within the pre-S(2) region of the envelope (11) was selected as the synthetic B-cell site. The results indicate that a totally synthetic vaccine, encompassing T- and B-cell recognition sites, can be constructed, which will elicit Tc-cell function and T-cell memory as well as antibody production relevant to multiple native proteins of the virus.

MATERIALS AND METHODS

Mice. C57BL/10 (B10), B10.S, and B10.BR H-2 congenic murine strains were obtained from the breeding colony of the Research Institute of Scripps Clinic.

HBcAg Particles, HBeAg, and Synthetic Peptides from HBcAg. Recombinant HBcAg particles and recombinant HBeAg of the ayw subtype (12) were provided by Stephen Stahl (Biogen, Geneva). These reagents were positive in HBcAg-specific and HBeAg-specific ELISA using monoclonal anti-HBc and anti-HBe antibodies (antibodies to HBcAg

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; Tc, T helper; CFA, complete Freund’s adjuvant.

†To whom reprint requests should be addressed.
and HBeAg, respectively) (1) supplied by M. Mayumi (Jichi Medical School, Tochigi-Ken, Japan). The peptides were synthesized by the Merrifield solid-phase method and were subjected to HPLC on a C18 reverse-phase column. All peptides used eluted as a single major peak (>90%). The structural polypeptide (P21) of the HBCAg particle consists of 183 amino acids. The following synthetic peptides were utilized for this study and are designated by amino acid position from the N terminus of HBCAg: p120–140, Val-Ser-Arg-Pro-Pro-Val-Val-Val-Val-Arg-Thr-Pro-Pro-Ala-Tyr-Arg-Pro-Pro-Asn-Ala-Pro-Ile-Leu; p120–131, Val-Ser-Phe-Gly-Val-Tyr-Ile-Arg-Thr-Pro-Pro-Ala-Tyr-Arg-Pro-Pro-Asn-Ala-Pro-Ile-Leu; and p129–140, Pro-Pro-Ala-Tyr-Arg-Pro-Pro-Asn-Ala-Pro-Ile-Leu. Additional peptides used were p133–140 [a pre-S(2) region peptide; N terminus designated as residue 120], Asp-Pro-Arg-Val-Arg-Gly-Leu-Thr and the composite peptide c120–140 (133–140) consisting of residues 120–140 of the HBCAg sequence and residues 133–140 of the pre-S(2) region sequence, which was produced in a single synthesis.

**T-Cell Proliferative Assay.** Groups of four mice were primed with either 4 μg of HBCAg or 100 μg of synthetic peptide in complete Freund’s adjuvant (CFA) by hind footpad injection. Eight days after immunization, draining popliteal lymph node cells were harvested, and 5 × 10⁶ cells in 0.1 ml of Click’s medium (13) were cultured with 0.1 ml of medium containing either HBCAg, HBSAg/GP33 (see below), various synthetic peptides, or medium alone. Cells were cultured for 96 hr at 37°C in a humidified 5% CO₂ atmosphere, and during the final 16 hr, 1 μCi of [³H]thymidine (6.7 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) was added. The cells were then harvested onto filter strips for determination of [³H]thymidine incorporation. The data are expressed as cpm corrected for background proliferation in the absence of antigen (Δcpm). All T-cell activation experiments were performed at least twice, and representative experiments are depicted.

**In Vivo T-cell Assay.** T-cell activity was determined by the ability of peptide-primed mice to produce anti-HBc antibody in vivo after challenge with a suboptimal dose of HBCAg. Groups of five mice were primed i.p. with 100 μg of peptide in CFA. Control mice were primed with CFA alone. Three weeks after priming, the mice were challenged with 0.1 μg of HBCAg in incomplete Freund’s adjuvant or adjuvant alone, and sera were collected for measurement of anti-HBc IgG 7 days after challenge.

**Measurement of in Vivo Antibody Production.** Pooled murine sera were evaluated for antibody in an indirect, solid-phase RIA using solid-phase HBCAg (0.1 μg per well), HBSAg/GP33 (0.1 μg per well), or synthetic peptides (1 μg per well) and goat anti-mouse IgG as second antibody. Antibodies were quantitated with an ¹²⁵I-labeled, affinity-purified swine anti-goat immunoglobulin as described (14). Recombinant HBSAg particles containing approximately 35% GP33 (15) were provided by P. Tiollais (Pasteur Institute, Paris) and are designated HBSAg/GP33. The data are expressed as antibody titer representing the highest dilution to yield 4 times the cpm of preimmunization sera.

**RESULTS**

**Fine Specificity of T-Cell Recognition of the HBCAg-Specific Peptide p120–140 Is Dependent on the H-2 Haplotypic of the Responding Strain.** Previously we demonstrated that HBCAg-primed T cells from mice of the H-2a and H-2b strains predominantly recognized the p120–140 sequence of HBCAg (10). It was of interest to determine if each haplotype recognized the same or distinct sites within the p120–140 sequence. For this purpose, an "N-terminal" (p120–131), a "C-terminal" (p129–140), and an overlapping (p125–136) peptide were synthesized. As illustrated in Fig. 1a, B10.S (H-2b) HBCAg-primed T cells proliferated in response to HBCAg, the complete peptide (p120–140), and p120–131 but not p129–140. p125–136 was also active, but it was 500-fold less efficient as compared to p120–131 (data not shown). Note that the dose–response curves for HBCAg-primed T cells stimulated with HBCAg or the active peptides were similar. The reciprocal experiments involved priming with peptides and challenging in vitro with HBCAg or peptides. B10.S p120–140-primed T cells recognized HBCAg, the immunizing peptide (p120–140), and p120–131, but not p129–140 (Fig. 1b). Therefore, similar to T cells primed with native HBCAg, p120–140-primed B10.S T cells recognized p120–131. B10.S T cells primed with p120–131 were activated by p120–140, p120–131, and HBCAg (Fig. 1c). Therefore, the 12-residue peptide p120–131 was sufficiently immunogenic to prime HBCAg-specific T cells in vivo. p120–140 was only marginally immunogenic in the B10.S strain (Fig. 1d).

Similar experiments were performed in H-2 congenic B10 (H-2d) mice (Fig. 2). B10 HBCAg-primed T cells proliferated in response to HBCAg, p120–140, and p129–140 but not to

---

**Fig. 1.** Specificity of T-cell recognition of HBCAg in the B10.S (H-2b) strain. Groups of four B10.S mice were immunized in the hind footpads with 4.0 μg of HBCAg (a) or 100 μg of either p120–140 (b), p120–131 (c), or p129–140 (d) in CFA. Eight days after the priming, draining popliteal lymph node cells were harvested, pooled, and cultured with the indicated concentrations (0.00015–16 μg/ml) of HBCAg, p120–140, p120–131, p129–140, or media alone, and T-cell proliferation was measured by [³H]thymidine incorporation. The data are expressed as cpm corrected for background proliferation in the absence of antigen (Δcpm). Background proliferation ranged from 800 to 2000 cpm.
p120–131 or p125–136 (Fig. 2a), in contrast to B10.S HBcAg-primeed T cells. Because the p120–140 sequence is common to both HBcAg and HBeAg, these results predict that HBcAg and HBeAg are crossreactive at the level of T-cell proliferation in the B10.S and B10 strains. The availability of recombinant HBeAg allowed us to confirm this prediction because B10 HBcAg-primeed T cells recognized HBeAg (Fig. 2a). B10 p120–140- or p129–140-primeed T cells were specific for p129–140 and recognized HBeAg approximately as well as the immunizing peptide (Fig. 2b and d). p129–140-primeed T cells also proliferated in response to HBeAg (Fig. 2d). p120–131 was nonimmunogenic in the B10 strain (Fig. 2c). Identical experiments performed in B10.BR (H-2b) mice revealed that, although B10.BR HBcAg-primeed T cells recognized HBcAg and HBeAg, the p120–140 sequence and the truncated peptides were neither stimulatory nor immunogenic (data not shown).

Synthetic Peptides p120–140, p120–131, and p129–140 Can Prime Functional T helper-Cell Activity in Vivo. As an alternative method of examining the fine specificity of T-cell recognition of HBcAg and as a means of determining the functional ability of synthetic T-cell sites to prime antibody production in vivo, T helper-cell assays were performed. Priming with synthetic T-cell sites, p120–140, p120–131, and p129–140, did not elicit anti-HBc antibody production in B10.S mice, and p129–140 induced only minimal anti-HBc in B10 mice (Fig. 3). Therefore, although these peptides contain T-cell recognition sites, the B-cell epitopes present on the peptides are not relevant to or not exposed on the native HBcAg (i.e., peptide-specific). Therefore, it was possible to examine the ability of these peptides to prime Th cells in vivo directly as opposed to performing T-cell transfer experiments. This approach requires that the memory Th cells primed by immunization with peptide be recalled by challenge with HBcAg, indicating the relevance of the synthetic T-cell recognition site to the native molecule.

In the B10.S strain, unprimeed mice challenged with HBcAg produced minimal anti-HBc antibodies (1:40) in vivo, whereas p120–140-primeed mice challenged with HBcAg produced anti-HBc IgG efficiently (1:5120) 7 days after the challenge (Fig. 3 Upper). To examine the fine specificity of the Th-cell activity, B10.S mice were primeed with p120–131 or p129–140 and then challenged with HBcAg. Priming with p120–131 elicited significant anti-HBc production (256 times the unprimeed value), and p129–140 was only marginally reactive (4 times the unprimeed value) (Fig. 3). Similarly, immunization with p120–140 primeed anti-HBc production in the B10 strain (1:10,240). However, in contrast to the B10.S strain, p129–140 primeed anti-HBc production in the B10 strain, and p120–131 was inactive (Fig. 3 Lower). The p120–140 sequence of HBcAg did not prime anti-HBc production in B10.BR mice (data not shown). These results are consistent with the T-cell proliferation results and indicate a concordance between proliferative T-cell and Th-cell fine specificities.

The HBcAg-Specific Peptide p120–140 Can Function As a T-Cell Carrier Møiety for a Synthetic B-Cell Epitope from the...
Envelope of HBV. Because the p120-140 sequence was shown to encompass distinct T$_{\text{h}}$-cell recognition sites for B10.S and B10 mice capable of inducing anti-HBc production in vitro, it was tested whether p120-140 coupled directly to a synthetic B-cell epitope could act as a T-cell carrier for that epitope. The B-cell epitope chosen was the pre-S(2) region peptide p133-140, which was previously shown to represent a dominant antibody-binding site within the pre-S(2) region of HBsAg/GP33 particles (11). The unconjugated p133-140 sequence of the pre-S(2) region is nonimmunogenic in the B10.S, B10, and B10.BR strains (data not shown). After primary immunization with c120-140-(133-140), the B10.S strain produced antibody to the HBcAg-specific peptide (p120-140), which did not crossreact with native HBcAg (i.e., was peptide-specific), and antibody to the pre-S(2) region peptide (p133-140), which did crossreact with native HBsAg/GP33 particles (Table 1). After the secondary immunization, antibody titers increased 4-fold (anti-p120-140) and 16-fold (anti-p133-140). Note that the secondary anti-p120-140 antibody was only minimally reactive with the native HBcAg protein, whereas the anti-p133-140 antibody was highly crossreactive with the native HBsAg/GP33 protein. The B10 strain was less responsive to c120-140-(133-140) immunization than the B10.S strain, as evidenced by the necessity for a booster immunization to elicit anti-p133-140 antibody production, which was 4 to 8 times less as compared to the secondary response of the B10.S strain (Table 1). Similar to the B10.S strain, the B10 anti-p133-140 antibody was highly crossreactive with native HBsAg/GP33, whereas anti-p120-140 was only minimally crossreactive with native HBcAg. Both the B10.S and B10 strains produced high-titered, predominantly peptidespecific antibody to the p120-140 sequence. In both strains, the antibody was specific for p129-140 (data not shown). Predictably from the T-cell proliferation and T$_{\text{h}}$-cell experiments, the B10.BR strain was a nonresponder to immunization with c120-140-(133-140). These results indicate that the HBcAg-specific synthetic peptide p120-140 can function as a T-cell carrier for a synthetic B-cell epitope represented on the envelope of the HBV in strains that recognize the p120-140 sequence of HBcAg at the T-cell level.

Fine Specificity of T-Cell Recognition of the Synthetic Immunogen c120-140-(133-140). In order to confirm that the predicted sites within the composite immunogen were functioning as T-cell recognition sites, c120-140-(133-140) was coupled to HBcAg to form a novel synthetic immunogen.

Table 1. The HBcAg-specific p120-140 sequence can function as a T-cell carrier for a synthetic pre-S(2) region B-cell epitope, p133-140

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time</th>
<th>p120-140</th>
<th>HBcAg</th>
<th>p133-140</th>
<th>HBsAg/GP33</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10.S</td>
<td>Pre</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1°</td>
<td>10,240</td>
<td>0</td>
<td>640</td>
<td>1,280</td>
</tr>
<tr>
<td></td>
<td>2°</td>
<td>40,960</td>
<td>1,280</td>
<td>10,240</td>
<td>10,240</td>
</tr>
<tr>
<td>B10</td>
<td>Pre</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1°</td>
<td>1,280</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2°</td>
<td>81,920</td>
<td>5,120</td>
<td>2,560</td>
<td>1,280</td>
</tr>
<tr>
<td>B10.BR</td>
<td>Pre</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1°</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2°</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Groups of five mice of the indicated strains were immunized i.p. with 100 µg of the composite peptide, c120-140-(133-140), in CFA and boosted 4 weeks later with 50 µg i.p. in incomplete Freund's adjuvant. Sera were collected before immunization (Pre), 3 weeks after the primary immunization (1°), and 2 weeks after the secondary immunization (2°).

Antibody (IgG specific for the indicated antigens) was measured by solid-phase RIA and expressed as the reciprocal of the dilution to yield 4 times the cpm of the preimmunization sera.


immunized mice were evaluated at the T-cell level. B10.S c120-140-(133-140)-primed T cells responded to p120-140, p129-131, and to native HBcAg. The B-cell epitope (p133-140) and native HBsAg/GP33 particles were nonstimulatory at all concentrations (Fig. 4 Top). B10 c120-140-(133-140)-primed T cells were activated by p120-140, p129-140, and native HBcAg. The B-cell epitope (p133-140) and native HBsAg/GP33 were nonstimulatory at all concentrations (Fig. 4 Middle). The significantly higher peptide-specific T-cell proliferative responses of the B10.S strain as compared to the B10 strain may explain the greater anti-pre-S(2) antibody production observed in the B10.S strain after c120-140-(133-140) immunization (Table 1). B10.BR c120-140-(133-140)-primed T cells were nonresponsive to the entire antigen panel (Fig. 4 Bottom). These results and the antibody results depicted in Table 1 indicate that T-cell recognition of the composite peptide is H-2-dependent and correlates with the specificity patterns observed for free p120-140 in terms of T-cell proliferation and T$_{\text{h}}$-cell activities.

DISCUSSION

An ideal synthetic vaccine will be composed of T-cell as well as B-cell recognition sites identical to those recognized in the native molecule. Such a synthetic vaccine would not require a heterologous protein carrier and, more importantly, would induce neutralizing antibody and elicit T- and B-cell memory relevant to the pathogen should neutralizing antibody wane. A previous study demonstrated that T$_{\text{h}}$-cell recognition of HBcAg by H-2<sup>b</sup> and H-2<sup>d</sup> haplotype-bearing strains is focused on residues 120-140 of the HBcAg sequence (10). Herein, it was shown that B10.S (H-2<sup>b</sup>) mice recognized p120-131, whereas H-2 congenic B10 (H-2<sup>d</sup>) mice recog-
nized p129–140. It was reported that T cells of the H-2d and H-2e strains recognize residues 100–120 within the HBcAg sequence (10). Preliminary data indicate that these two haplotypes also recognize unique T-cell sites within the p100–120 sequence. A dominant T-cell site recognized by H-2d strains (B10.D2 and BALB/c) resides within residues 85–100 of HBcAg (10). Cumulatively, these results indicate that multiple T-cell recognition sites exist within HBcAg and that the specificity of T<sub>d</sub>-cell recognition is limited by H-2-linked genetic constraints and not by the absolute number of T-cell epitopes available. This realization presents a substantial problem to the design of completely synthetic vaccines. It appears that a multiplicity of synthetic T-cell sites will need to be incorporated into a synthetic vaccine to guarantee sufficient responsiveness in an outbred population. This will require a detailed mapping of T-cell recognition sites within the native protein. The enhanced T-cell immunogenicity of HBcAg (9, 10) makes this antigen an ideal candidate for study. In the HBV system there are also biological reasons to consider the use of HBcAg or synthetic fragments of HBcAg as a T-cell carrier moiety in a vaccine. An HBcAg-specific cytotoxic T-cell response has been suggested to mediate viral clearance (16), HBcAg vaccination was reported to protect against HBV infection (7), and HBcAg-specific T<sub>d</sub> cells can function to induce envelope-specific as well as nucleocapsid-specific antibody production (6). Antibodies to the envelope S region (5) and pre-S(2) region (6, 17) have been shown to be virus neutralizing.

For these reasons we have initiated studies to determine the feasibility of constructing a totally synthetic HBV vaccine based on nucleocapsid-specific T-cell sites and an envelope-specific B-cell site. This requires that the peptide-primed T cells must recognize the natural HBcAg and provide functional helper activity for the B-cell clone specific for the synthetic B-cell site and that the anti-peptide antibody produced must react with the native envelope protein(s) of the virus. The HBcAg-specific synthetic peptide p120–140 was chosen as a candidate T-cell carrier because it represents a “complex” T<sub>d</sub>-cell carrier peptide consisting of at least two T-cell recognition sites (11), p120–131 being neutralizing to three H-2 haplotypes [the H-2<sup>d</sup> haplotype also recognizes the p120–140 sequence (10)]. These 12-residue peptides were shown to stimulate HBcAg-predimmed T cells and reciprocally to prime peptide-specific T cells that crossreacted with HBcAg. Evaluation of p120–131 and p129–140 at the T<sub>d</sub>-cell level paralleled the T-cell proliferation results in terms of the genetic restriction and the crossreactivity with HBcAg. Furthermore, the T<sub>d</sub>-cell analysis illustrated that the 12-residue peptides were sufficient to prime T<sub>d</sub> cells in vivo capable of eliciting anti-HBc production. Because the p120–140 sequence is common to both HBcAg and HBBeAg, these results indicate that T<sub>d</sub> cells that recognize HBcAg can function to elicit anti-HBc antibody production in vivo.

The pre-S(2) region peptide p133–140 was chosen as the B-cell epitope because it represents a dominant antibody-binding site on native HBsAg/GP33 after immunization with native particles (11). It is also conserved between subtypes of the HBV. Although neutralization studies have not been performed with p133–140, immunization with synthetic peptide p133–151 was shown to be protective against HBV infection in chimpanzees (6).

Immunization of the B10.S and B10 strains with the synthetic composite peptide, c120–140-(133–140), elicited anti-pre-S(2)-specific antibodies that were crossreactive with native HBsAg/GP33 particles. Examination of the fine specificity of T-cell recognition of c120–140-(133–140) revealed that the p120–131 sequence served as a T<sub>d</sub>-cell determinant for the B10.S strain and the p129–140 sequence served as a T<sub>d</sub>-cell determinant for the B10 strain. The B-cell epitope (p133–140) was not recognized by either strain at the T-cell level. It is noteworthy that p120–131 primed T cells (B10.S) and p129–140-primed T cells (B10) elicited by c120–140-(133–140) immunization were crossreactive with HBcAg and therefore may be expected to serve as memory T<sub>d</sub> cells relevant to this viral protein. Similarly, antibody elicited to the B-cell epitope p133–140 was crossreactive on native envelope particles. The anti-p120–140 antibodies produced in response to the T-cell carrier peptide were only minimally crossreactive on HBcAg. Because anti-HBc antibody is not protective against HBV infection, the lack of this antibody specificity is of no apparent consequence.

Although these initial results are promising in terms of the feasibility of an eventual synthetic HBV vaccine, numerous methods of optimization must be explored. For example, the effects on immunogenicity of altering the ratio of T- and B-cell sites per molecule, the use of spacer residues between T- and B-cell sites, and the spatial orientation of T- and B-cell sites require investigation. Most importantly, the absence of responsiveness to c120–140-(133–140) in the B10.BR strain emphasizes the necessity for continued effort to characterize and incorporate additional T-cell sites in order to broaden the T<sub>d</sub>-cell recognition potential in an outbred population. Additional synthetic T<sub>d</sub>-cell sites have been defined within the pre-S(1) region of the envelope of HBV (18). Additional neutralizing antibody sites are also likely to be required.

We thank S. Stahl for the recombinant HBcAg/HBeAg and P. Tiollais for the HBsAg/GP33. This work was supported by Grants AI05209, AI21583, and AI00855 from the National Institutes of Health and a grant from the Johnson and Johnson Company. This is publication number 4976MB from the Research Institute of Scripps Clinic.