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By exchanging sequences from the middle-surface (MS) and small-surface (S) Ag of hepatitis B virus (HBV) with corresponding sequences of the MS Ag of woodchuck hepatitis virus, we constructed chimeric MS variants. Using these constructs as DNA vaccines in mice, we selectively primed highly specific (non-cross-reactive) Ab responses to pre-S2 of the HBV MS Ag and the “a” determinant of the HBV S Ag, as well as Ld- or Kb-restricted CTL responses to HBV S epitopes. In transgenic mice that constitutively express large amounts of HBV surface Ag in the liver we could successfully suppress serum antigenemia (but not Ag production in the liver) by adoptive transfer of anti-pre-S2 or anti-“a” immunity but not CTL immunity. DNA vaccines greatly facilitate construction of chimeric fusion Ags that efficiently prime specific, high-affinity Ab and CTL responses. Such vaccines, in which sequences of an Ag of interest are exchanged between different but related viruses, are interesting tools for focusing humoral or cellular immune responses on selected antigenic determinants and elucidating their biological role. The Journal of Immunology, 2001, 166: 1405–1413.

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3 Abbreviations used in this paper: VLP, virus-like particle; MHC-I, MHC class I; MHC-II, MHC class II; HBsAg, hepatitis B surface Ag; WhAg, woodchuck hepatitis surface Ag; S, small-surface HBsAg; MS, middle-surface (pre-S2/S) HBsAg; LS, large-surface (pre-S1/pre-S2/S) HBsAg; HBV, hepatitis B virus; WHV, woodchuck hepatitis virus; wt, wild type; B6, C57BL/6J; tg, transgenic; HBs-tg, tg line B6-TgN(Alb1HBV)44Bri expressing in the liver LS, MS, and S; CH0, Chinese hamster ovary.

In DNA vaccination, proteins are expressed after transient in vivo transfection and subjected to the same posttranslational modifications, changes in conformation, or oligomerizations as during virus infection. In contrast to recombinant Ags purified from eukaryotic or prokaryotic expression systems, genetic vaccination readily maintains the integrity of epitopes that stimulate neutralizing Ab (B cell) responses. DNA (or RNA) immunization is known to be exceptionally potent in stimulating T cell responses because antigenic peptides are efficiently generated from intracellular or extracellular protein Ags expressed from introduced genes in endogenous or exogenous processing pathways (without interference by viral proteins) (3, 4). In DNA vaccines, sequences can easily be exchanged between Ag-encoding genes to construct chimeric immunogens. We constructed chimeric VLP from the middle-surface (MS) Ag containing woodchuck hepatitis virus (WHV) and/or hepatitis B virus (HBV) determinants. Three surface protein species are present in the envelope of HBV virions, designated the large-surface (LS) (pre-S1/pre-S2/S), MS (pre-S2/S), and small-surface (S) proteins. In the LS protein (p39, gp42), the 108-residue pre-S1 sequence and the 55-residue pre-S2 sequence precede the S protein; in the MS protein (p31), the 55-residue pre-S2 sequence precedes the S protein (reviewed in Ref. 1). Using DNA-based vaccination, we characterized the humoral (serum Ab) and cellular (CTL) response of mice to selected determinants or epitopes of HBV expressed by these VLP. The MS proteins from HBV and WHV have sequence homology of about 70% (1). We show that Ab-defined pre-S2 determinants (of MS) and “a” determinants (of S), as well as the Ld- and Kb-restricted CTL epitopes of HBV, show no cross-reaction to the respective determinants of MS or S from WHV. This approach allowed us to assay the biological role of selected Ab- or CTL-defined determinants of the MS Ag in a transgenic (tg) model.
Materials and Methods

Mice

C57BL/6 (B6) mice (H-2b) and BALB/c (H-2d) mice were kept under standard pathogen-free conditions in the animal colonies of Ulm University (Ulm, Germany). B6-TgN(A112BHB8)44Bri-tg (HBs-tg) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were used at 10–16 wk of age.

Ag-encoding plasmid DNA used for nucleic acid vaccination

The coding regions of hepatitis B surface Ag (HBsAg) and woodchuck hepatitis surface Ag (WHsAg) were dissected by PCR. The following primers were used for constructing chimeric genes from HBsAg and WHsAg: PCR-defined coding regions included HBsAg-55/120 with primers HBs55s ATCTCCAGCAGCATCGTGG (sense, 3163a)/HBs120s CTGCAATTTGCGCCGTTGCTTGAAGTT (antisense, 521a), HBsAg-55/147 with primers HBs55s ATCTCCAGCAGCATCGTGG (sense, 3163a)/ HBs147a ATACAGTGGCAATTTCCTGCC (antisense, 733b), and WHs-55/120 with WHs55s CAGTCAATTGCAGACAATGC (sense, 636a)/WHs120s GTCTGCAATTGACTGTGTTGTTTC (antisense, 650b). The correctness of the sequences. These fragments were recloned into pcDNA3 (sense, 593a)/HBs147a ATACAGTGGCAATTTCCTGCC (antisense, 605a), HBs-121/147 with primers HBs121s CGGGCAATTGCAGAACCTGCATGACT (sense, 509a)/HBs147a ATACAGTGGCAATTTCCTGCC (antisense, 733a) and HBs-155/226, construct III with WHs-55/147 and HBs-148/226, and construct IV with HBs121s CAGTCAATTGCAGACAATGC (sense, 636a)/WHs120s GTCTGCAATTGACTGTGTTGTTTC (antisense, 650a). The numbering of the HBV genomea and WHV genomex is according to Refs. 5 and 6.

Sequences of eight primers were modified to create MunI and Bbrpl sites for the construction of chimeric HBsAg/WHsAg genes. PCR fragments were cloned into pcMR2.1 according to the manufacturer’s instructions. The cloned fragments were subjected to DNA sequencing analysis to verify the correctness of the sequences. These fragments were recloned into pcDNA3 at the restriction sites HindIII and XhoI. Four chimeric genes of HBsAg and WHsAg were constructed by ligation of fragments: construct I with HBs-55/120 and WHs-121/226, construct II with HBs-55/147 and WHs-148/226, construct III with WHs-55/147 and HBs-148/226, and construct IV with WHs-55/120, HBs-120/147, and WHs-148/226. The fragments were ligated either using the MunI site at junction 120 or by the Bbrp I site at junction 147/148. In the generated plasmid constructs, the Ags were expressed under control of the human CMV immediate early promoter.

Vaccination of mice

Adult mice were immunized i.m. into the tibialis anterior muscle with the indicated amounts of plasmid DNA as described previously (7). For the adoptive transfer experiments, spleens were obtained from B6 mice primed and boosted with the indicated vaccines. Single-cell suspensions were prepared from these spleens in PBS/BSA. A total of 3 × 104 spleen cells were injected i.p. or i.v. into HBs-tg hosts. Where indicated, CD4+ T cells were suppressed in mice by repeated i.p. injections of 200 μg anti-CD4 mAb YTS 191.1 (in 200 μl PBS) as described previously (8, 9). For Ab transfer (serotherapy), 200 μl sera containing the indicated levels of anti-“a” reactivity or anti-pre-S2 reactivity from DNA-immunized mice was injected i.p.

HBsAg expression in transiently transfected cells

LMH-chicken hepatoma cells (a generous gift of Dr. H.-J. Schlücht, Ulm, Germany) were transiently transfected with the indicated plasmid DNA using the CaP04 precipitation method or the commercial liposome-based FuGENE transfection reagent (cat. no. 1815091; Roche Diagnostics, Mannheim, Germany). Two days later, steady-state levels of secreted HBsAg particles purified from crude yeast extracts by adsorption to silica gel, column chromatography, and isopyknic ultracentrifugation were obtained from Dr. K. Melber (Rhein Biotech, Düsseldorf, Germany) (12). Mixed HBsAg particles were expressed in CHO cells (a generous gift from Drs. M. Goreki and N. Moav, Bio-Technology General, Rehovot, Israel) as described (13). In these cells, HBsAg genes are expressed under the control of the S gene promoter. The transfected CHO cells synthesize and secrete HBsAg particles, which are harvested from the growth medium using a combination of gel exclusion and ion exchange chromatography. The peptide composition, analyzed by SDS-PAGE with a reducing agent, revealed glycosylated and nonglycosylated LS (pre-S1/pre-S2/S), MS (pre-S2/S), and S proteins. WHsAg was extracted from serum stocks of chronically WHV-infected woodchucks as described previously (14). These recombinant Ags allowed us to detect HBV- or WHV-specific pre-S2 and/or S Ab responses.

Results

Construction of chimeric MS Ags of HBV and WHV

The MS Ag of HBV comprises the 55-aa pre-S2 and the 226-aa S sequence. It contains different Ab-binding determinants and (H-2d) CTL-defined epitopes (Fig. 1A). Most human and murine anti-S Abs bind to the conformational “a” determinant located at the S120–147 region (1, 15–17). In H-2d and H-2b mice, Ab responses but not CTL responses are readily primed against determinants in the pre-S2 domain of HBsAg (11). The S Ag contains the L1-restricted CTL epitope S28–39 (18) and the K-restricted CTL epitopes S208–215 (19) and S172–191 (this study).

We designed four plasmids encoding chimeric MS that contained surface Ag sequences from either HBV or WHV (Fig. 1B). Construct I encodes the pre-S2 domain and the N-terminal 1–20 aa of the S Ag of HBV and the C terminus of the S Ag of WHV. It contains the L1-restricted CTL epitope S28–39, but neither the
Kb-restricted CTL epitopes nor the Ab-defined “a” determinant of HBV S Ag. Construct II encodes the pre-S2 domain and the N-terminal 1–147 aa of the S Ag of HBV and the C terminus of the S Ag of WHV. It contains the “a” determinant, as well as the Ld-restricted S28–39 CTL epitope, but does not include the Kb-restricted CTL epitopes of HBV because the C-terminal sequence is from WHV. Construct III encodes the C-terminal 148–226 aa of S Ag of HBV and the corresponding N terminus (including the pre-S2 domain) of S Ag of WHV. It contains only the Kb-restricted CTL epitopes of S Ag from HBV. Construct IV encodes the C-terminal 148–226 aa of S Ag of HBV and the corresponding N terminus (including the pre-S2 domain) of S Ag of WHV. It contains only the “a” determinant of the S Ag of HBV. These four constructs allowed us to prime humoral and CTL responses to selected determinants of the MS Ag of HBV by DNA vaccination in mice.

Priming CTL responses to HBsAg by injecting recombinant plasmid DNA encoding chimeric HBV/WHV MS Ag

BALB/c and B6 mice were injected i.m. with 100 μg plasmid DNA encoding wild-type (wt) MS (pre-S2/S) from either HBV or WHV. Immune spleen cells were restimulated in vitro with irradiated syngenic transfectants expressing either the LS (pre-S1/pre-S2/S) or the S of HBV (19–21) and assayed for specific CTL reactivity. HBV S-specific CTL reactivity was readily detected in mice vaccinated with the wild-type HBV S Ag. However, vaccination with the WHV-derived S Ag did not prime the detection of HBV S-specific CTL. Vaccination of BALB/c mice with DNA encoding the chimeric expression constructs I-IV. Vaccination of BALB/c mice with DNA from construct I or II but not construct III or IV elicited S-specific CTL (Fig. 2A, lanes c-f).
These CTL were Ld-restricted and specific for the well-known S28–39 epitope (Fig. 2B, lanes c and d). Vaccination of B6 mice with DNA from construct III but not construct I, II, or IV primed CTL that were specific for the Kβ-restricted HBV S172–191 peptide generated during endogenous processing of HBsAg (Figs. 2C, lanes c–f; and 2D, lane c) and the Kβ-restricted HBV S208–215 peptide generated during exogenous processing of HBsAg (Figs. 2E, lanes c and d; 2F, lane c; and data not shown). All CTL expressed the CD3+CD8+CD4- surface phenotype (data not shown). These results confirm the known MHC-I-restricted epitopes of S Ag from HBV that are recognized by CTL from vaccinated H-2d or H-2b mice. They demonstrate that these epitopes are equally well processed from wt or chimeric MS Ag.

The studies did not reveal cryptic or subdominant CTL-defined epitopes of the MS Ag of HBV that could prime CTL when immunodominant epitopes are deleted.

Vaccination with plasmid DNA encoding chimeric HBV/WHV MS Ag primes species-specific Ab responses to pre-S2 and “a” determinants

Mice were primed and boosted with plasmid DNA encoding either the wt MS Ag from HBV or WHV, or one of the chimeric HBV/WHV MS Ags. Sera were collected at different time points post-vaccination. Serum Abs binding to the conformational “a” determinant of the HBsAg were detected in the commercial

FIGURE 2. Induction of HBV S-specific CTL.

**Balb/c (H-2b)**

A. 

B. 

C. 

D. 

E. 

F. 

**C57BL/6 (H-2b)**

endogenous stimulation

exogenous stimulation

**Induction of HBV-specific CD8+ CTL**

Balb/c (H-2b)

A. 

B. 

C. 

D. 

E. 

F. 

The studies did not reveal cryptic or subdominant CTL-defined epitopes of the MS Ag of HBV that could prime CTL when immunodominant epitopes are deleted.
IMxAUSAB test (Abbott) and were measured in mIU/ml. This read-out revealed “a”-specific Abs in mice vaccinated with plasmid DNA encoding either the wt MS Ag of HBV or the chimeric MS Ags II and IV (Fig. 3A, lanes a, d, and f). No “a”-specific Abs were measurable in mice vaccinated with wt MS Ag from WHV or with the chimeric MS Ags I and III (Fig. 3A, lanes b, c, and e). Thus, the murine Ab response to the “a” determinant of the HBsAg shows no cross-reactivity to the “a” determinant of the WHsAg.

Pre-S2-specific IgG Abs binding to the N-terminal domain of the MS Ag of HBV were measured in an end-point dilution ELISA using different recombinant HBsAg for detection (Fig. 3, C and D). A similar pattern of Ab binding to HBV S Ag was seen, both when yeast-derived S particles were used as the test Ag in the ELISA and when the commercial ELISA test was used (compare Fig. 3, A and C). An apparently similar Ab-binding pattern was seen when CHO-derived HBV large surface Ag (composed of LS, MS, and S Ag) (13) was used for Ab detection (Fig. 3D, lanes a, d, and f). With the latter test Ag we detected Abs binding to the native pre-S2 domain of the MS Ag of HBV that were elicited by vaccination with DNA encoding construct I (Fig. 3D, lane c).

**FIGURE 3.** Induction of surface Ag-specific Ab responses. B6 mice were injected i.m. with 100 μg DNA encoding HBV MS, WHV MS, or the hybrid constructs I-IV. Eight weeks postvaccination, mice were boosted with the same constructs, and serum samples were collected 2–8 wk after the booster. We tested serum probes for the presence of specific Abs using the commercial anti-HBV S ELISA (A). Expression of secreted MS particles carrying the HBV “a” determinant was detected in transiently transfected cells (B). LMH cells were transfected with the indicated plasmid constructs. At 36 h, the culture medium was changed. After 12 h, supernatants were analyzed for secreted HBsAg levels (ng/ml) by a commercial ELISA. HBV- and WHV-specific IgG serum Abs were determined by end-point dilution ELISA using yeast-derived HBV S Ag particles (C), CHO-derived HBV LS particles (containing LS, MS, and S Ag of HBV) (D), or WHV LS particles (containing LS, MS, and S Ag of WHV) (E) for detection. Furthermore, serum Abs were tested in Western blot analyses using the cT-HBV/pre-S1/pre-S2 fusion Ag for detection (F). Mean Ab titers ± SEM of two to five mice/group are shown.
that these mice had no Abs in the serum that bound to the “a” determinant of the S Ag of HBV (Fig. 3, A and C, lane c). Abs binding the pre-S2 domain of the MS Ag of HBV were also detected in Western blot analyses. We have described a system that allows stable expression of mutated, truncated, or chimeric protein domains fused to a hsp73-binding cytosolic T-Ag fragment (11, 22, 23). We expressed the N-terminal 163-aa pre-S (i.e., pre-SIpre-S2) domain of the LS Ag of HBV in this system (11). These analyses showed that Abs binding to linear epitopes of the HBV pre-S2 domain were present in the serum of mice vaccinated with DNA encoding construct I or II (or wt MS Ag of HBV) but not with DNA encoding wt MS Ag of WHV or construct III or IV (which all contained a pre-S2 domain of the MS Ag of WHV) (Fig. 3f, and data not shown).

In addition, we analyzed by ELISA the serum Ab response of vaccinated mice against the pre-S2 or “a” determinant of the surface Ag of WHV, using surface particles purified from the serum of chronically WHV-infected woodchucks (which contain LS, MS, and S Ag from WHV) (Fig. 3e). Abs binding WHV-derived surface particles were present in sera of mice vaccinated with DNA encoding construct I or II but not from HBV (Fig. 3e, lanes a and b). These Abs do not cross-react with the S and LS Ag of HBV (Fig. 3, C and D, lane b). Similar to the exquisite species specificity of the murine CTL response to epitopes on the S Ag of HBV, the murine Ab response to the pre-S2 and to the “a” determinant of the MS Ag of HBV is specific and does not cross-react with homologous determinants of the MS Ag from WHV.

CD4+ T cell-dependent Ab responses against the pre-S2 or the “a” domain of the MS Ag suppress HBsAg antigenemia of HBs-tg mice

We used HBs-tg mice (24) to test the biological effect of Ab responses primed to selected domains of the MS Ag. HBs-tg mice produce large amounts of surface particles from HBV (containing LS, MS, and S Ag) in the liver, and readily detectable levels of antigenemia build up in their blood and peripheral tissues. Using an adoptive transfer system described previously (9), we engrafted immune cells with different well-characterized immune reactivities to MS Ag into congenic HBs-tg hosts. We have shown that this adoptive transfer of congenic immune spleen cells into HBs-tg hosts establishes a stable and rising CD4+ T cell-dependent Ab response to HBsAg that suppresses antigenemia (9, 25).

B6 mice were primed and boosted by injections of plasmid DNA encoding either the wt MS Ag of HBV or the hybrid MS Ag constructs I-IV. The immune mice were shown to have developed the expected Ab and CTL reactivity against MS described above (Figs. 2 and 3). Injection of serum from immune B6 mice into HBs-tg hosts (serotherapy) transiently suppressed HBsAg antigenemia in some (Fig. 4, F–H and K) but not all (Fig. 4I) groups of treated tg mice. We injected i.p. either 200 μl serum containing either 260 mIU (Fig. 4F), 190 mIU (Fig. 4I), or 40 mIU (Fig. 4K) anti-“a” seroreactivity, or antisera with a 1:3000 anti-pre-S2 titer (Fig. 4G). Suppression of HBsAg antigenemia was transient, and serum HBsAg always reappeared 2–10 days after the serotherapy. HBsAg antigenemia was transiently suppressed in mice injected with sera from immune donor B6 mice vaccinated with plasmid DNA encoding wt MS Ag from HBV or chimeric MS Ag expressed from constructs I, II, and IV (Fig. 4, F–H and K). HBsAg antigenemia was not suppressed in HBs-tg hosts injected with immune sera from B6 donor mice vaccinated with plasmid DNA encoding chimeric MS Ag expressed by construct III or injected with 100 μl nonimmune sera (from donor B6 mice injected with noncoding plasmid DNA) (Fig. 4I, and data not shown). The transfer of Abs specific for either the “a” determinant (Fig. 4K) or the pre-S2 determinant (Fig. 4G) of MS from HBV could therefore transiently suppress HBsAg antigenemia in HBs-tg mice.

A stable suppression of HBsAg antigenemia and the establishment of stable MS-specific Ab titers were obtained in HBs-tg mice by adoptive transfer of immune spleen cells from B6 donors vaccinated with plasmid DNA encoding wt or some of the chimeric variants of MS (Fig. 4, A–E). The transfer of immune spleen cells (3 × 107 cells/mouse) from B6 donor mice vaccinated with plasmid DNA encoding wt MS of HBV (Fig. 4A) or with the chimeric variant MS constructs I, II, or IV (Fig. 4, B, C, and E) but not III (Fig. 4D) led to stable suppression of HBsAg antigenemia and the appearance of a HBV S-specific serum Ab titer in the HBs-tg host (Fig. 4, A, C, and E). The HBsAg-specific serum Ab titers increased for months posttransplantation in the HBs-tg hosts, suggesting a restimulation of the Ab response in the adoptive host by transgene-encoded HBsAg (Refs. 9, 25, and 26, and data not shown). A stable suppression of HBsAg antigenemia in the absence of anti-S serum Ab titers was observed in tg mice transplanted with cells from immune donors vaccinated with plasmid DNA encoding construct I (Fig. 4B). However, sera of these transplanted mice contained increasing serum Ab levels binding the HBV pre-S2 domain of MS that efficiently clear up serum HBsAg antigenemia (Fig. 4B). Hence, all serum HBsAg particles in HBs-tg mice carry Ab-binding pre-S2 determinants (present on the LS or MS Ags) and can be eliminated by an Ab-dependent effector mechanism specific for this epitope.

The establishment of stable humoral immunity to HBsAg was CD4+ T cell dependent because the depletion of CD4+ T cells from the immune donor cell inoculum prevented its establishment in the tg host (data not shown), confirming previously published data (9, 26). Thus, primed donor-derived CD4+ T cells are critical for the establishment of HBsAg-specific Ab responses in the HBs-tg host. The immunity to HBsAg we adoptively established in HBs-tg mice did not suppress HBsAg expression in the liver from the transgene. Despite the rising titers of anti-HBsAg Abs and the stable suppression of HBsAg antigenemia in transplanted HBs-tg mice, we detected no decrease in the HBsAg content of the liver (data not shown), confirming previous data (9, 26). Furthermore, we detected no increase in serum transaminase levels after immune cell transfer and/or Ab transfer, indicating that the adoptive transfer of this type of immunity does not damage HBsAg-expressing liver cells.

A MHC-I (H-2d)-restricted CTL reactivity against epitopes in the C terminus of HBsAg was present in immune cell populations primed by plasmid DNA encoding either the complete wt MS Ag of HBV or the chimeric MS Ag III (Figs. 1 and 2). Using immune spleen cells from B6 mice vaccinated with DNA of construct III, we selectively transferred the Kb-restricted CTL (immune cells did not contain anti-HBV “a” or pre-S2-reactivity) to HBs-tg hosts (Fig. 4D). However, this transfer established neither humoral (Fig. 4D) nor cellular immunity to HBsAg (data not shown) in the tg host, nor did it induce histopathological changes in the liver or a rise in serum transaminase levels (data not shown) when tested at different time points postransfer. In addition, the tg-specific HBsAg expression in the liver was not reduced. Following transfer of immune spleen cells from B6 donors vaccinated with these two vector DNA constructs, we could not recover this HBsAg-specific CTL reactivity from the adoptive tg host postransfer (data not shown). This further confirms our previously published data (9). It seems that the H-2d-restricted, HBsAg-specific CTL that were transferred in these experiments were rapidly silenced in the tg recipients. This is in contrast to the observation that transplantation of large numbers of H-2d-restricted, HBsAg-specific CTL were
detectable for 2–4 wk posttransfer in adoptive tg hosts (reviewed in Ref. 27).

Discussion

Serum Ab responses of mice to the pre-S2 and “a” determinant of the HBV MS Ag were elicited by DNA vaccines encoding natural or chimeric MS Ag and showed no cross-reactivity to the homologous determinants of the WHV MS Ag. Neither HBV, nor WHV, nor any other known species of the Hepadnavirus group are natural pathogens of the mouse. The exceptional serological specificity observed may result either from the two MS Ags chosen or from the use of DNA vaccination (which preferentially primes
high-affinity Abs). The HBV “a” determinant is the immunodominant, Ab-defined epitope of the S Ag. It has some variability, is a conformational epitope on the surface of HBsAg particles, and is formed exclusively by protein (although there is a glycosylation site in the “a” region). The large majority of variants of the HBsAg “a” determinants stimulate cross-reactive Ab responses; this underlies the efficacy of the currently used commercial HBV vaccine. Our data suggest that the “a” determinants on the WHsAg are quite different. Our data confirm the importance of anti-pre-S2 Abs, which has been suggested before (15, 16, 28). This supports the notion that a new generation of anti-HBV vaccine should contain this epitope. Similar to the data described by the group previously (9), the Ab responses to the pre-S2 and the “a” determinant described in this study were IgG, and their induction was CD4+ T cell dependent. This was shown in CD4+ T cell depletion experiments (data not shown). The adoptive-transferred immune CD4+ T cell populations were restimulated in HBs-tg mice by host HBsAg (9). No restimulation by WHV-derived determinants took place in the adoptive host (data not shown). The MHC class II (MHC-II)-binding epitopes of HBsAg that stimulate CD4+ T cell responses in B6 H-2d mice are not known. This suggests either that there is extensive cross-reactivity between CD4+ T cell-defined epitopes from WHsAg and HBsAg (in contrast to B cell- and CTL-defined epitopes) or that many CD4+ T cell-defined epitopes are present in HBsAg. The i.m. injection of a high dose of a DNA vaccine is known to prime Th1-biased responses. The comple- ment-fixing IgG2a isotype was largely over-represented in the Ab responses we measured (data not shown). Rising titers of these Abs in the serum of transplanted HBs-tg mice did not induce damage in the liver, the site of HBsAg secretion in the tg mice, confirming our previously published data (9).

We primed CTL with non-cross-reactive specificity to epitopes of the HBV S Ag by DNA vaccines encoding wt or chimeric Ag. The approach allowed us to confirm the presence of an N-terminal L5- and two C-terminal Kb-restricted CTL epitopes on the S Ag of HBV. Different (DNA- or protein-based) vaccination techniques have not revealed additional (subdominant or cryptic) CTL epitopes of HBsAg in B6 mice. These data generated in H-2d and H-2b mice make it unlikely that additional immunodominant or subdominant epitopes of the HBV S Ag will be discovered in these strains and confirm the absence of H-2d- and H-2b-CTL-defined epitopes in the HBV pre-S2 domain (11). This was independently confirmed in vaccination experiments with chimeric DNA vaccines expressing B0- to 100-aa fragments of HBsAg fused to heterologous viral Ags (data not shown). This was unexpected because different computer screening programs indicated a multitude of K5- and D8-binding 8-mer and 9-mer motifs in the HBsAg molecule. The two Kb-restricted epitopes of HBsAg we defined in B6 mice are unusual. The Kb/S172-191 epitope is only generated by cells processing exogenous HBsAg (19). Processing and presentation of this epitope is TAP and brefeldin A independent but blocked by NH4Cl or selected acid-protease inhibitors. In contrast, the K7/S172-191 epitope is generated by endogenous processing of HBsAg. Fine mapping of this epitope is difficult because the sequence is extremely hydrophobic. Within 12 h posttransfer, the adoptively transferred, Kb-restricted CTL immunity was lost in HBs-tg hosts. The mechanism that silences these CD8+ T cells in HBs-tg mice is unknown. Therefore, in the HBs-tg host, the maintenance of the Th1 CD4+ T cell-dependent humoral immunity to HBsAg in vivo seems independent of the immunoregulatory control of CD8+ T cells (29). The experimental approach of priming humoral or cellular immune responses to selected determinants of a complex viral Ag is thus an efficient technique for defining the biological role of individual components of anti-viral immune responses.

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