Primed MHC-I-Restricted Cytotoxic T Lymphocyte Responses to Exogenous Hepatitis B Surface Antigen Is CD4+ T Cell Dependent

Jens Wild, Michael J. Grusby, Reinhold Schirmbeck and Jörg Reimann

*J Immunol* 1999; 163:1880-1887; 
http://www.jimmunol.org/content/163/4/1880

**References**

This article cites 79 articles, 50 of which you can access for free at: http://www.jimmunol.org/content/163/4/1880.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Priming MHC-I-Restricted Cytotoxic T Lymphocyte Responses to Exogenous Hepatitis B Surface Antigen Is CD4⁺ T Cell Dependent

Jens Wild,* Michael J. Grusby, † Reinhold Schirmbeck,* and Jörg Reimann²*

MHC-I (L²)-restricted, S28–39-specific CTL responses are efficiently primed in H-2² BALB/c mice injected with low doses of native hepatitis B surface Ag (HBsAg) lipoprotein particles without adjuvants. Priming of this CTL response by exogenous HBsAg required CD4⁺ T cell “help” and IL-12: this CTL response could be neither induced in mice depleted of CD4⁺ T cells by in vivo Ab treatment, nor in (CD4⁺ T cell-competent or CD4⁺ T cell-depleted) IL-12-unresponsive STAT4⁻/⁻ knockout BALB/c mice. Codelivery of oligonucleotides (ODN) with immunostimulating CpG sequences (ISS) with exogenous HBsAg reconstituted the CTL response to exogenous HBsAg in CD4⁺ T cell-depleted normal mice and in CD4⁺ T cell-competent and CD4⁺ T cell-depleted STAT4⁻/⁻ BALB/c mice. Injection (by different routes) of “naked” pCI/S plasmid DNA encoding HBsAg into IL-12-responsive or −unresponsive BALB/c mice efficiently primed the MHC-I-restricted, HBsAg-specific CTL response. CTL priming was not detectable when CD4⁺ T cell-depleted animals were subjected to genetic immunization. In vivo priming of the well-characterized CD8⁺ CTL response to HBsAg in “high responder” BALB/c mice either by exogenous surface lipoprotein particles or by DNA vaccination is thus CD4⁺ T cell dependent. CTL priming by exogenous HBsAg, but not by genetic immunization, is IL-12 dependent. The dependence of CTL priming by exogenous HBsAg on CD4⁺ T cells can be overcome by codelivering ODN with ISS motifs, and this “adjuvant effect” operates efficiently in IL-12-unresponsive mice. The data characterize a feature of the adjuvant effect of ISS-containing ODN on CTL priming that may be of major interest for the design of CTL-stimulating vaccines with efficacy in immunodeficiency conditions. The Journal of Immunology, 1999, 163: 1880–1887.

Interactions between T cells and APC regulate the induction, amplification, and differentiation of cellular immune responses. In different experimental systems, the priming of precursors of MHC-I-restricted CD8⁺ CTL to viral, tumor, or minor H Ags has been shown either to depend on, or not to depend on, CD4⁺ T cell “help.” CTL responses to some Ags could be induced in vivo in the absence of CD4⁺ T cells (1–10). In contrast, the majority of CTL responses requires “T cell help” for priming and/or differentiation (11–14). Most prominent and informative are factors operating at the APC level that have been demonstrated to decisively influence CTL priming (15–21). Understanding these T-T and T-APC interactions is of central interest for the elucidation of immunoregulation controlling the induction of immunity or tolerance, as well as of practical relevance for designing CTL-stimulating vaccines.

Some 10- to 100-nm multimeric protein particles effectively prime class I-restricted CTL of different species when injected as exogenous Ags in low doses without adjuvants. This has been shown, e.g., for heat-inactivated virus particles (22–24), yeast-de-
M. J. Grusby (Department of Immunology and Infectious Diseases, Harvard School of Public Health and Department of Medicine, Harvard Medical School, Boston, MA) (35). A breeding colony of these mice was established in Ulm. Female mice were used at 10–16 wk of age.

**Cell lines and vector constructs**

The H-2<sup>d</sup> mastocytoma cell line P815 (TIB-64) was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The BMGneo vector was a generous gift of Drs. Y. Karasuyama and F. Melchers, Basel, Switzerland) (36). The establishment of HBsAg (subtype ayw)-expressing P815/S transfectants has been described (26).

**Recombinant HBsAg**

HBsAg, subtype ayw, was produced in the *Hansenula polymorpha* host strain RB10 (37). HBsAg particles were purified from crude yeast extracts by adsorption to silica gel, column chromatography, and isopyknic ultracentrifugation (37). HBsAg particles were obtained from Dr. K. Melber (Rhein Biotech, Düsseldorf, Germany).

**Peptides**

The synthetic 12-mer S28–39 peptide IPQSLSDWWTSL of HBsAg that binds to L<sub>1</sub> was synthesized in an Applied Biosystems (Foster City, CA) peptide synthesizer model 431A and purified by reverse-phase HPLC. The peptide were dissolved in a DMSO solution at a concentration of 10 mg/ml and diluted with culture medium for use. A<sup>1</sup>C<sup>1</sup>-labeled peptides (10<sup>10</sup>) suspended in 250 μl serum-free UltraCulture medium (cat. no. 12-725F; Bio-Whittaker, Walkersville, MD) were incubated with 10<sup>7</sup>-10<sup>9</sup> M of this peptide for 1 h. Subsequently, cells were washed and used as targets in cytotoxic assays.

**HBsAg-encoding plasmid DNA used for nucleic acid vaccination**

The HBsAg-encoding XhoI/Bg/II fragment of HBV (subtype ayw) was obtained from plasmid pTkTKitB2 (a generous gift of Dr. M. Meyer, Munich, Germany) and cloned into the XhoI/BamH-cut pCI vector (cat. no. E1731; Promega). In the generated plasmid pCI/S, the HBsAg is expressed under control of the human CMV immediate early promoter.

**In vivo suppression of CD4<sup>+</sup> T cells in mice**

CD<sup>4</sup><sup>+</sup> T cells were suppressed in mice by three injections of the anti-CD4 mAb YTS 191.1. Two days before, at the time of, and two days after the vaccination, mice were i.p. injected with 200 μl PBS containing 100 μg Ab. Flow cytometric analyses of PBMC populations demonstrated that >99% of the CD4<sup>+</sup> T cells expressing the respective phenotype were depleted for 4–6 days, about 4% of CD4<sup>+</sup> T cells reappeared at day 8 posttreatment, and 5–6% CD4<sup>+</sup> T cells reappeared 2 wk posttreatment.

**Protein immunization of mice**

Mice were injected once i.m. or s.c. (into the base of the tail) with the indicated dose of recombinant HBsAg in 200 μl PBS. In some experiments, 5 μg HBsAg were mixed with either 50 μg of the oligonucleotide (ODN) TCATTGGAAAAGGTCTTGAGGGGG containing one CpG-immunostimulating sequence (ISS), or with 50 μg of the ODN TCATTGGAAAAGGTCTTGAGGGGG containing no ISS, or with 50 μg of the ODN TCATTGGAAAAGGTCTTGAGGGGG containing no ISS, containing methylated CpG motifs (38). The phosphorothioate-modified ODNs were produced by MWG-Biotech (Ebersberg, Germany). HBsAg mixed with the ODN was injected into mice without adding further adjuvants. In some experimental groups, HBsAg was coadministrated with 1000 μg recombinant murine IFN-γ (cat. no. 1276905; Boehringer, Mannheim, Germany), or 100 ng recombinant murine IL-12 (cat. no. 19361V; Phar-Mingen, Hamburg, Germany).

**Nucleic acid immunization of mice**

We injected 50 μl of 1 μg/μl plasmid DNA in PBS into each tibialis anterior muscle (39–41). All mice received bilateral i.m. injections once.

**In vitro restimulation of primed, HBsAg-specific CTL**

Spleens were removed from immunized mice 8 days postvaccination. Single cell suspensions were prepared in αMEM tissue culture medium supplemented with 10 mM HEPES buffer, 5 × 10<sup>−3</sup> M 2-ME, antibiotics, and 10% v/v FCS (Life Technologies, Eggenstein, Germany). A selected batch of Con A-stimulated rat spleen cell supernatant (2% v/v) was added to the culture medium. Responder cells (3 × 10<sup>5</sup>) were cocultured with 1 × 10<sup>6</sup> irradiated, syngeneic P815/S transfectants. Cultures were performed in 10 ml medium in upright 25-cm<sup>2</sup> tissue culture flasks in a humidified atmosphere/7% CO<sub>2</sub> at 37°C. After 5 days of culture, CTL were harvested, washed, and assayed for HBsAg-specific cytolytic activity. All CTL lines generated displayed the CD<sup>3</sup> CD<sup>4</sup> CD<sup>8</sup> TCRαβ<sup>+</sup> phenotype.

**Cytotoxic assay**

Serial dilutions of effector cells were cultured with 2 × 10<sup>5</sup><sup>3</sup>Cr-labeled targets in 200 μl round-bottom wells. Specific cytolytic activity of cells was tested in short-term <sup>3</sup>Cr-release assays against P815/S transfectant or peptide-pulsed P815 targets. After a 3.5-h incubation at 37°C, 50 μl of supernatant was collected for gamma radiation counting. The percentage specific release was calculated as [(experimental release – spontaneous release)/total release – spontaneous release] × 100. Total counts were measured by resuspending target cells. Spontaneously released counts were always less than 15% of the total counts. Data shown are the mean of triplicate cultures. The SD of triplicate data was always less than 20% of the mean.

**Results**

**Priming CD8<sup>+</sup> CTL responses to exogenous HBsAg is CD4<sup>+</sup> T cell dependent**

The HBsAg system is a well-characterized model to study cross-priming of CTL to exogenous Ag. The injection of low doses of native HBsAg lipoprotein particles (without adjuvants) by different routes efficiently primes murine MHC-I-restricted CTL (26, 27). This is confirmed by the data shown in Fig. 1. A single s.c. injection of HBsAg (without adjuvants) into H-2<sup>b</sup>BALB/c mice primed a CTL response (Fig. 1A). Similar responses were primed by single i.m. or s.c. injections of 2–10 μg HBsAg into BALB/c mice (data not shown). The S28–39-specific, L<sub>1</sub>-restricted CTL reactivity was readily detected as early as 5 days postvaccination in lymph node and spleen cells from immunized mice (data not shown).

Repeated injections of the rat anti-mouse mAb YTS 191.1 specific for murine CD4 into mice (as described in Materials and Methods) completely depleted CD4<sup>+</sup> T cells from peripheral blood and spleens of treated animals for almost 10 days. CD4<sup>+</sup> T cell-depleted BALB/c mice were immunized by a single s.c. injection of 5 μg HBsAg. Cells from spleen of vaccinated mice were obtained after 8 days postvaccination when only very low numbers of CD4<sup>+</sup> T cells (<4%) had reappeared. The cell populations were specifically restimulated in vitro in cultures supplemented with CD4<sup>+</sup> T cell-conditioned medium providing “T cell help.” No HBsAg-specific CTL reactivity was detectable in any of the mice vaccinated with 5 μg HBsAg (Fig. 1E). Neither repeated in vitro restimulations nor increasing the dose of HBsAg used for vaccination revealed evidence for CTL priming in CD4<sup>+</sup> T cell-depleted mice (data not shown). The number of independently performed experiments for each group is listed in table I. These data showed that priming MHC-I-restricted CTL responses to exogenous HBsAg in “high responder” BALB/c mice is CD4<sup>+</sup> T cell dependent. The findings confirm data from another Ag system that “cross-presentation” of peptides in the context of MHC-I molecules to CD8<sup>+</sup> CTL is CD4<sup>+</sup> T cell dependent (34).

Injection of exogenous HBsAg into CD4<sup>+</sup> T cell-suppressed mice did not induce specific tolerance to this Ag. Vaccination of CD4<sup>+</sup> T cell-competent) mice 6 wk after HBsAg injection and CD4<sup>+</sup> T cell depletion with an immunogenic dose of exogenous HBsAg without adjuvant efficiently primed a CTL response (data not shown).

ODNs containing ISS override the CD4<sup>+</sup> T cell dependence of CD8<sup>+</sup> CTL priming by exogenous HBsAg

ODNs with ISS enhance the immunogenicity of Ags in mice and tend to bias immune responses toward the Th1 phenotype (42, 43). A single s.c. injection of 5 μg recombinant HBsAg mixed with 50
FIGURE 1. ODN containing ISS support priming of CD8\(^+\) CTL precursors to exogenous HBsAg in the absence of CD4\(^+\) T “helper” cells. CD4\(^+\) T cell competent (A-D) or CD4\(^-\) T cell-depleted (E-H) BALB/c mice were vaccinated by a single s.c. injection of 5 \(\mu\)g HBsAg particles. The HBsAg particles were either not mixed with adjuvant (A and E), or mixed with 50 \(\mu\)g immune-stimulating ODN (ISS\(^+\)) (B and F), 50 \(\mu\)g nonstimulating, mutated ODN (ISS\(^-\)) (C and G), or 50 \(\mu\)g nonstimulating, methylated ODN (ISSM) (D and H). Their spleens were removed 8 days postvaccination, specifically restimulated in vitro with inactivated, HBsAg-expressing transfectants, and tested in a 3 h \(^{51}\)Cr-release assay against HBsAg-expressing P815/S targets or nontransfected P815 control targets. Mean specific lysis values (of triplicates) at the indicated E:T ratios are shown.

\(\mu\)g ISS-containing ODN into CD4\(^+\) T cell-competent H-2\(d\) BALB/c mice enhanced CTL priming in response to injection of this exogenous Ag (Fig. 1, A and B). Injection of the same dose of HBsAg mixed with 50 \(\mu\)g ODN containing mutant, nonstimulating sequences (ISS\(^-\)) or methylated ISS (ISSM) had no detectable influence on CTL priming by exogenous HBsAg (Fig. 1, C and D).

In the next series of experiments we immunized CD4\(^+\) T cell-depleted BALB/c mice with exogenous HBsAg. A CD8\(^+\) CTL response to exogenous HBsAg was not primed by an injection of 5 \(\mu\)g exogenous HBsAg in the absence of CD4\(^+\) “helper” T cells (Fig. 1E). This CTL response was completely restored when HBsAg mixed with ISS-containing ODN (ISS or ODN) was injected into CD4\(^+\) T cell-depleted mice (Fig. 1F). Exogenous HBsAg mixed with ODN containing mutated, nonstimulating sequences (ISS\(^-\)) or methylated CpG sequences (ISSM) could not reconstitute the CTL response to exogenous Ag in mice lacking CD4\(^+\) T cells (Fig. 1, G and H). These data indicate that ODN containing ISS can override the CD4\(^+\) T cell dependence of the CD8\(^+\) CTL response to exogenous HBsAg.

ISS-containing ODN efficiently induce the Th1 cytokines IL-12 and IFN-\(\gamma\) (43–47). When we injected HBsAg particles mixed with either 100 ng recombinant murine IL-12, or 10^3 units recombinant murine IFN-\(\gamma\), we could not prime CTL responses to this exogenous viral Ag in CD4\(^+\) T cell-depleted BALB/c mice (data not shown). We therefore vaccinated IL-12-unresponsive STAT4\(^{−/−}\) KO BALB/c mice (35) with exogenous HBsAg to find evidence for a role of IL-12 in CTL priming in this system.

Vaccination of IL-12-nonresponsive STAT4\(^{−/−}\) BALB/c mice with exogenous HBsAg primes CD8\(^+\) CTL responses only in the presence of immune-stimulating ODN

A single s.c. injection of 5 \(\mu\)g or 10 \(\mu\)g HBsAg particles without adjuvants into CD4\(^+\) T cell-competent BALB/c mice specifically and efficiently primed CTL (Fig. 1A). In contrast, no evidence for priming of a HBsAg-specific CTL response was detectable after injections of 5 \(\mu\)g HBsAg into congenic, IL-12-unresponsive STAT4\(^{−/−}\) BALB/c mice (Fig. 2A). The injection of 10 \(\mu\)g or 20 \(\mu\)g HBsAg into these genetically engineered “knockout” mice also failed to prime CTL although this vaccination stimulated high and specific serum Ab responses against HBsAg (data not shown). This suggested that IL-12 plays a role in CTL priming to exogenous Ag. ODN-containing ISS facilitated priming of CTL to exogenous HBsAg in an IL-12-deficient environment. We could prime CTL from STAT4\(^{−/−}\) KO mice to exogenous HBsAg by adjuvanting it with ISS-containing ODN (Fig. 2B). Using this vaccine formulation, HBsAg-specific, L\(^d\)-restricted CD8\(^+\) CTL reactivity was efficiently induced in STAT4\(^{−/−}\) KO mice by a single injection of 5 \(\mu\)g adjuvanted, exogenous HBsAg.

As expected, the injection of even high doses of exogenous HBsAg did not prime a CD8\(^+\) CTL response in CD4\(^+\) T cell-depleted STAT4\(^{−/−}\) mice (Fig. 2C). Unexpectedly, the coadministration of ODN with exogenous HBsAg successfully induced specific and MHC-I-restricted CTL responses also in CD4\(^+\) T cell-depleted STAT4\(^{−/−}\) KO mice (Fig. 2D). This indicated that ISS-containing ODN facilitate CTL priming to exogenous HBsAg in the absence of CD4\(^+\) T cells and in the absence of a functional IL-12 response.

Primin L\(^d\)-restricted, HBsAg-specific CTL by DNA vaccination is CD4\(^+\) T cell dependent

DNA vaccination is the most efficient way available to prime MHC-I-restricted CTL to HBsAg in different mouse strains (39–41, 48–55). We have shown that the i.m. and the s.c. injection of 50–100 \(\mu\)g “naked” plasmid DNA into mice elicits potent CTL responses of defined epitope and restriction specificity (56). This was confirmed in experiments in which we injected s.c. or i.m. a single dose of 100 \(\mu\)g DNA of the plasmid pCI/S (encoding the small surface Ag of HBV) into BALB/c mice. Both vaccination
Protocols induced readily detectable HBsAg-specific CTL responses (Fig. 3, A and B). When mice were depleted of CD4\(^+\) T cells, none of the DNA vaccination protocols tested primed this anti-viral CTL response (Fig. 3, C and D). Neither the pretreatment of the muscle with cardiotoxin before the plasmid DNA injection (39) nor extending the in vitro restimulation period of in vivo primed spleen cells revealed evidence of CTL priming in CD4\(^+\) T cell-depleted, vaccinated mice (data not shown). These data demonstrate that CD8\(^+\) CTL priming by DNA vaccination is dependent on CD4\(^+\) T cell “help” and therefore resembles the help-dependence of cross-primed CTL responses. Furthermore, the data indicate that the efficient priming of murine CTL responses by DNA vaccination cannot be explained by the potent adjuvanticity of codelivered bacterial CpG-containing DNA.

**Priming HBsAg-specific CD8\(^+\) CTL responses in IL-12-nonresponsive STAT4\(^{-/-}\) BALB/c mice by DNA vaccination is CD4\(^+\) T cell dependent**

HBsAg-specific CTL responses could not be primed in IL-12-nonresponsive STAT4\(^{-/-}\) KO BALB/c mice by injecting exogenous HBsAg lipoprotein particles (Fig. 2A). In contrast, the i.m. injection of HBsAg-encoding pCI/S plasmid DNA into STAT4\(^{-/-}\) KO mice readily primed CTL specific for this viral surface protein (Fig. 2E). The elicited cytolytic effector cells expressed the CD8\(^+\) phenotype and were specific for the S28–39 epitope of HBsAg recognized in the context of L\(^d\) (data not shown). The i.m. and the s.c. routes of plasmid DNA injection were equally effective (data not shown). IL-12 is therefore not a critical cytokine required for CTL priming by DNA vaccination.

As in normal, IL-12-responsive BALB/c mice (Fig. 3, A and D), efficient priming of HBsAg-specific CTL by DNA vaccination was also CD4\(^+\) T cell dependent in IL-12-nonresponsive STAT4\(^{-/-}\) KO BALB/c mice (Fig. 3F). Also, under these conditions, the bacterial plasmid DNA could not provide an adjuvant stimulus that was as efficient as ISS-containing ODN in facilitating CTL priming to HBsAg.

**Discussion**

“Cross-priming,” i.e., the stimulation of an MHC-I-restricted specific CTL response by exogenous, immunogenic material has been known for many years (57–59). It has regained interest because it seems to be involved in CTL priming by tumor cells (60–62), by DNA vaccination (63–65), and by autoantigens (66). Recently, several virus-like particles (VLP) have been shown to prime CTL responses when delivered to animals as exogenous Ags. We are interested in the MHC-I-restricted T cells of mice to the HBsAg lipoprotein particle of HBV. Injection of low doses of native HBsAg lipoprotein particles (by different routes) without adjuvants failed to generate specific CTL reactivity against HBsAg.

The data in Fig. 1, A and E, show that priming of the CTL response to exogenous HBsAg particles requires CD4\(^+\) T cell “help.” Mice injected with various doses of exogenous HBsAg without adjuvants failed to generate specific CTL reactivity against HBsAg in the absence of CD4\(^+\) T cells. This was observed irrespective of the route (s.c. vs i.m.) of immunization. We tested this because the CD4\(^+\) T cell dependence of CTL responses may differ...
with different routes of immunization (68). We could not test the intradermal route because this type of vaccination stimulates exclusively Th2 responses without priming detectable CD8 T cell reactivity (data not shown). Toxicity of the anti-CD4 Ab treatment in vivo is unlikely because CD8 T cell precursors could be primed in treated mice in the presence of ODNs, and we did not see an effect of this Ab treatment on serum Ab levels (data not shown).

Our data reproduce in a viral Ag system the only previously published report on the CD4 T cell dependence of “cross-priming” in a transgenic autoantigen system. These reports showed that the induction of a CD8 cytotoxic T lymphocyte response by cross-priming requires cognate CD4 T cell help and that class I-restricted cross-presentation of exogenous self Ags in the absence of CD4 T cell “help” leads to deletion of autoreactive CD8 T cells (34, 69).

CpG-containing ODNs have been reported to be potent enhancers of specific immunity in mice immunized with recombinant HBsAg (70, 71). ODN with ISS efficiently reconstituted the CD8 T cell response to exogenous HBsAg in CD4 T cell-depleted BALB/c mice. ODN showed this effect when it was mixed with HBsAg (without further adjuvants) and delivered i.m. or s.c. (R. Schirmbeck, unpublished observation). Control experiments demonstrated that nonmethylated ODN with well-established ISS motifs were required to observe this “adjuvant effect.” ODN may facilitate priming of CTL responses by activating dendritic cells in a way similar to signals physiologically delivered by Ag-stimulated CD4 T cells (72). The activation of dendritic cells seems to involve CD40/CD40 ligand (CD154) interactions, (73, 74) as well as cytokines. “Activation” results in “presentation-competent” dendritic cells that can activate naive CD8 CTL precursors.

CD4 T cell-competent, STAT4 KO BALB/c mice generated a CTL response to exogenous HBsAg, but CD4 T cell-competent, STAT4 KO BALB/c mice did not (Table I). This suggested a critical role for IL-12 in this type of CTL priming. Activation of APC may operate through the release of IL-12 by dendritic cells and/or IFN-γ by NK cells or macrophages (42, 43, 75–78). We could not reproduce the “adjuvant effect” of ODN by mixing HBsAg with recombinant IL-12 or IFN-γ (data not shown). This indicates that either ODN operate by a mechanism independent of these two Th1 cytokines, or ODN-induced release of these cytokines in situ is more stimulatory (e.g., relative quantities of bioactive factor or the kinetic of release) than exogenously substituted cytokines. The efficacy of ODN as an adjuvant in priming CTL to exogenous HBsAg in STAT4 KO mice shows that at least part of its mechanism of action is IL-12 independent and may operate either by other IFN-γ-inducing cytokines (e.g., IL-18) or by directly inducing an IFN-γ response.

CTL priming to HBsAg by DNA vaccination was CD4 T cell dependent but IL-12 independent. Evidence has been presented that i.m. DNA vaccination operates through a “cross-priming” mechanism (63–65). This may explain the CD4 T cell-dependent nature of this type of CTL priming. If this assumption is valid, s.c. DNA vaccination would also stimulate immune responses through “cross-priming.” The data point to a fundamental (and unexplained) difference between delivering exogenous HBsAg with

**FIGURE 3.** Priming HBsAg-specific CD8 CTL responses by DNA-based vaccination (using different routes) in BALB/c mice and BALB/c STAT4−/− KO BALB/c mice is CD4 T cell dependent. CD4 T cell-competent (A and B) or CD4 T cell-depleted (C and D) BALB/c mice were immunized i.m. (A and C) or s.c. (B and D) by a single injection of 100 μg HBsAg-encoding pCI/S plasmid DNA. CD4 T cell competent (E) or CD4 T cell-depleted (F) STAT4−/− KO BALB/c mice were immunized i.m. by a single injection of 100 μg HBsAg-encoding pCI/S plasmid DNA. Splenic CTL reactivity was measured in primed mice 8 days postvaccination after restimulating cells in vitro for 5 days with syngeneic, HBsAg-expressing transfectants. Specific cytolytic reactivity was tested against HBsAg-expressing P815/S targets and nontransfected control targets. Mean specific lysis values (of triplicates) at the indicated E:T ratios are shown.
ISS-containing ODN as adjuvants, and delivering an HBsAg-encoding plasmid vaccine as “naked” DNA. Bacterial plasmid DNA is immunostimulatory, and insect DNA has been shown to support priming of naïve CD8\(^+\) CTL precursors (79). We found that bacterial pCI plasmid DNA mixed with HBsAg particles facilitated priming of HBsAg-specific CTL in “low responder” H-2\(^b\) mice (R. Schirmbeck, unpublished data). Injecting a mixture of (titrated amounts of) ISS-containing ODN and 100 \(\mu\)g pCI/S plasmid DNA i.m. completely suppressed the immunogenicity of HBsAg (R. Schirmbeck, unpublished observation) confirming a previously published report (80). The pCI/S plasmid DNA contains 20 immunostimulating CpG motifs (16 within the pCI vector and 4 within the HBsAg-encoding XholBglII fragment). Three of the motifs in the pCI vector DNA contain the 5’ AACGTT 3’ sequence identical to the one we used in the ODN. It is difficult to compare the relative efficacy of the adjuvant effects of synthetic nuclease-protected (PTO-modified), single-stranded ODN vs plasmid DNA. The described data in the HBsAg system indicate that the “adjuvant effect” of 100 \(\mu\)g pCI/S plasmid DNA is not comparable to that of 10–50 \(\mu\)g synthetic ODN. CTL priming by exogenous HBsAg delivered with ISS-containing ODN was CD4\(^+\) T cell independent, but CTL priming to HBsAg by genetic vaccination was CD4\(^+\) T cell dependent.

The described data have practical implications for the design of CTL-stimulating vaccine formulations in immunodeficiency conditions such as AIDS. In the absence of CD4\(^+\) T cell “help,” CD8\(^+\) CTL can be efficiently primed in a milieu deficient in the Th1 cytokine IL-12 by exogenous Ag formulated with ISS-containing ODN. Its surprising potency may carry the risk of activating autoreactive immune phenomena by bypassing CD4\(^+\) T cell help. This risk may be low in situations of “relative Th1 CD4\(^+\) T cell immunodeficiency.” Under such conditions, formulations using ODN adjuvants are expected to prove more efficient than DNA-based vaccination.

Acknowledgments

The expert technical assistance of Tom Krieg, Tanja Güntert, and Steffi Renninger is gratefully acknowledged. We thank Dr. K. Melber (Rhein-Biotech, Düsseldorf, Germany) for the HBsAg.

References


Table I. Requirements for CD4\(^+\) T cell “help” and IL-12 in priming CD8\(^+\) CTL responses to HBsAg by different vaccination protocols

<table>
<thead>
<tr>
<th>Vaccinated BALB/c Mouse</th>
<th>Exogenous HBsAg Particles</th>
<th>IL-12 Response</th>
<th>DNA Vaccination (pCI/S plasmid DNA)</th>
<th>CD8(^+) CTL Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>CD4(^+) T cells(^a)</td>
<td></td>
<td>Without adjuvants</td>
<td>With ISS(^b) ODN</td>
</tr>
<tr>
<td>1 Competent</td>
<td>Intact</td>
<td>3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2 Competent</td>
<td>Intact</td>
<td>3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3 Competent</td>
<td>Intact</td>
<td>3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4 Depleted</td>
<td>Intact</td>
<td>3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5 Depleted</td>
<td>Intact</td>
<td>3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>6 Depleted</td>
<td>Defective</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>7 Competent</td>
<td>Defective</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>8 Competent</td>
<td>Defective</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9 Competent</td>
<td>Defective</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>10 Depleted</td>
<td>Defective</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>11 Depleted</td>
<td>Defective</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>12 Depleted</td>
<td>Defective</td>
<td>2</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

\(^a\) BALB/c mice treated repeatedly with anti-CD4 Ab in vivo.

\(^b\) STAT4\(^−\) KO BALB/c mice.

\(^c\) Number of independent experiments.


