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Prime-Boost Immunization with DNA Vaccine: Mucosal Route of Administration Changes the Rules[1]

Seong Kug Eo,* Malgorzata Gierynska,* Amal Abou Kamar,[†] and Barry T. Rouse[2]*

In this study we assessed prime-boost immunization strategies with a DNA vaccine (gB DNA) and attenuated recombinant vaccinia virus vector (rvacgB), both encoding the gB protein of HSV, for their effectiveness at inducing mucosal as well as systemic immunity to HSV. Confirming the reports of others, systemic priming with gB DNA and systemic boosting with rvacgB were the most effective means of inducing serum Ab and splenic T cell responses. Nevertheless, the systemic prime-boost approach failed to induce detectable humoral or T cell responses at mucosal sites. However, such responses, at both proximal and distal locations, were induced if immunizations, especially the priming dose, were administered mucosally. Curiously, whereas optimal immunity with systemic priming and boosting occurred when gB DNA was used to prime and rvacgB was used as a boost, mucosal responses were optimal when animals were mucosally primed with rvacgB and boosted with gB DNA given mucosally. Furthermore, notable mucosal responses also occurred in animals mucosally primed with rvacgB and subsequently boosted systemically with gB DNA. Because the mucosal prime-boost immunization protocol also induced excellent systemic immune responses, the approach should be useful to vaccinate against agents for which both mucosal and systemic immunity are important for protection. *The Journal of Immunology,* 2001, 166: 5473–5479.

D eoxyribonucleic acid immunization represents an anticipated approach for the control of numerous infectious diseases (1). Used alone, however, DNA vaccines appear to be inferior immunogens, especially for inducing protective Ab production (2). Recently Ramshaw and colleagues, and later several other groups, demonstrated that if DNA vaccines were used to prime, and recombinant viral vectors were used as a booster, superior levels of immunity were achieved (3). Such prime-boost regimens have produced encouraging results against HIV in a primate model (4–6) and may well represent the long-sought successful approach to malaria vaccination (7).

Currently, the prime-boost strategy has been investigated in situations where both priming and boosting immunizations were administered systemically (4–7). Moreover, measurements of efficacy focused on components of systemic immunity (4–7). Because for many pathogens the portal of entry is by way of mucosal surfaces, and immunity at such sites can limit or even preclude infection (8), it is also important to evaluate prime-boost strategies for their effects on mucosal defense. In the present report we have compared the efficacies of the prime-boost approach using a DNA vaccine encoding glycoprotein gB of herpes simplex virus (gB DNA)[3] and that using a recombinant vaccinia virus vaccine encoding gB (rvacgB) given mucosally as well as systemically for

their effects on systemic and mucosal immunity. Our results confirm that systemic priming with gB DNA followed by boosting with rvacgB resulted in superior systemic humoral and T cell-mediated immunity. However, this approach failed to induce detectable immunity at mucosal sites. In contrast, mucosal priming with gB DNA and mucosal boosting with rvacgB induced excellent mucosal immune responses and also caused enhanced systemic immunity. Of particular interest, maximal mucosal immunity was achieved if rvacgB was used to mucosally prime animals and gB DNA was used as a mucosal booster. Thus, our results demonstrate that the prime-boost strategy may result in excellent mucosal immunity, but the order of immunization should be to prime mucosally with recombinant viral vector and to use DNA vaccines given mucosally to boost responses.

Materials and Methods

Mice and viruses

Female 4- to 5-wk-old BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were purchased from Harlan Sprague Dawley (Indianapolis, IN) and housed in the animal facilities at the University of Tennessee. HSV-1 KOS and McKrae strains were grown in Vero cells obtained from American Type Culture Collection (Manassas, VA), whereas rvacgB and vaccinia virus tk$^-$ (vvtk$^-$) were grown in CV-1 cells (American Type Culture Collection). The viruses were concentrated, titrated, and stored in aliquots at $-80°C$ until use.

Preparation of plasmid DNA vaccine

Plasmid DNA encoding gB (gB DNA) under the CMV promoter has been described in detail previously (9). The plasmid DNA was purified as previously described (10).

Immunization and sample collection

Groups of mice (3- to 4-wk-old female mice) were immunized with either 100 μg of gB DNA or 10^7 PFU of rvacgB via the intranasal (i.n.) or the intramuscular (i.m.) route and then boosted 10 days later with alternative vaccine vehicle via the same or a different route. Serum samples from mice were collected by retro-orbital bleeding. Vaginal lavages were obtained by introduction of 100 μl of PBS (pH 7.2) into the vaginal canals, followed by recovery with micropipette.

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[3] Abbreviations used in this paper: gB DNA, DNA vaccine encoding glycoprotein gB of herpes simplex virus; rvacgB, recombinant vaccinia virus vaccine encoding gB; i.n., intranasal(ly); ELISPOT, enzyme-linked immunospot; LN, lymph node; DLN, draining LN; ILN, iliac LN; CTB, cholera toxin B; i.m., intramuscular(ly).

ELISA for gB-specific Abs

The gB-specific Abs in the samples were determined by standard ELISA as described previously (11). Briefly, ELISA plates were coated with gB protein (provided by Chiron, Emeryville, CA) and goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL) or rabbit anti-mouse IgA (Zymed, San Francisco, CA), then incubated overnight at 4°C. The plates were washed with PBST (PBS containing Tween 20; three times) and blocked with a 3% solution of dehydrated milk. Samples were 2-fold serially diluted, incubated for 2 h at 37°C, and then incubated with goat anti-mouse IgG-conjugated HRP (IgG-HRP) for 1 h. For measurement of IgA levels in vaginal lavage, biotinylated goat anti-mouse IgA was first added for 2 h at 37°C, followed by peroxidase-conjugated streptavidin (Jackson ImmunoResearch, West Grove. PA). After color development, Ab concentrations were calculated with an automated ELISA reader (Spectra MAX340; Molecular Devices, Sunnyvale, CA).

Quantification of IFN-γ-producing CD4^{+} Th cells

The enzyme-linked immunospot (ELISPOT) assay was used for quantification of cytokine-producing cells as described previously (10). Briefly, ELISPOT plates (Millipore, Molseheim, France) were previously coated with IFN-γ anti-mouse Ab. The immune T cells (responder cells) were mixed with syngeneic splenocytes (stimulator cells) pulsed with UV-inactivated HSV (multiplicity of infection, 5.0 before UV inactivation). Coincubation of the responder and stimulator cells was continued for 72 h at 37°C. The ELISPOT plates were washed three times with PBS and three times with PBST, then biotinylated IFN-γ Ab was added to the plates for 1 h at 37°C. The spots were developed using nitro blue tetrazolium (Sigma, St. Louis, MO) and 5-bromo-4-chloro-3-indolylphosphate (Sigma) as a substrate following incubation with alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch) for 1 h and counted 24 h later under a stereomicroscope.

CTL assay

CTL activity was accessed by a standard 5-h ^{51}Cr release assay against labeled target cells as previously described (12). Splenocytes and corresponding draining lymph node (DLN) lymphocytes (effector cells) were restimulated in vitro with syngeneic splenocytes pulsed with gB$_{498-505}$ (SSIEFARL) peptide specific for MHC class I (H-2^{b})-restricted CD8^{+} T lymphocytes at a concentration of 10 μg/ml for 5 days. The effector cells were then mixed at various ratios with ^{51}Cr-labeled target cells for 5 h. The target cells included MHC-matched EL-4 (H-2^{b}) and MHC-mismatched EMT-6 (H-2^{d}) pulsed with SSIEFARL peptide. Spontaneous release of ^{51}Cr was determined by incubating the target cells with medium alone, and maximum release was determined by adding Triton X-100 to a final concentration of 5%. To calculate the specific lysis of targets, the percent lysis of irrelevant targets was subtracted from the percent lysis of specific targets. The percent specific lysis was calculated as follows: 100 × ([experimental release − spontaneous release]/[maximum release − spontaneous release]). Each experiment was performed twice, using triplicate samples.

Preparation of vaginal and iliac lymph node (LN) cells

Vaginal T lymphocytes were prepared as previously described (13, 14) with some modification. Briefly, vaginas were excised, cut longitudinally, and minced with a sterile scalpel in HBSS without calcium and magnesium (Life Technologies, Gaithersburg, MD). After washes with HBSS containing 1 mM EDTA (four time), minced tissues were digested in RPMI medium containing 1 mg of collagenase type VIII (Sigma)/ml and 1 mg of dispase II (Roche, Indianapolis, IN)/ml. Digestion was performed under stirring (1 h, 37°C). Cells were filtered through a sterile gauze mesh and washed with RPMI medium. Additional tissue debris was excluded by low speed centrifugation (200 × g, 10 min). Cells were collected by an additional centrifugation (400 × g, 10 min), resuspended in RPMI medium, and enriched on a nylon-wool column. Vaginal cells for Ab-producing cells were used before application to the nylon-wool column. Approximately 2–3×10^{6} cells were collected from seven mice. After eluting vaginal cells through the nylon-wool column, the vaginal cells usually showed 40–60% CD4^{+} T cells by flow cytometric analysis. Iliac LN cells were isolated from excised iliac LN, and then contaminating erythrocytes were lysed by hypotonic shock with a 0.83% ammonium chloride solution.

ELISPOT for Ab-producing cells

The numbers of gB-specific IgG and IgA-producing cells in iliac LN and vaginal tract were determined by ELISPOT assay as described previously (10). Briefly, ELISPOT plates (Millipore) were coated with 200–400 ng of gB protein/well and incubated overnight at 4°C. For determination of the total number of IgG and IgA spot-forming cells, the plates were coated

with 200 ng of rabbit anti-mouse IgG or IgA (Zymed)/well. After blocking with RPMI medium with 10% FBS, the cells were loaded in 100-μl volumes and incubated for 72 h at 37°C. The plates were then washed three times with PBS and subsequently with PBST, then incubated with biotinylated goat anti-mouse IgG or IgA (Zymed) for 1 h at 37°C. The spots were developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate substrates after incubation with alkaline phosphatase-streptavidin conjugate for 1 h and counted 24 h later under a stereomicroscope.

Statistical analysis

Significant differences between groups were evaluated using Student's *t* test.

Results

Influence of systemic or mucosal priming with DNA vaccine and recombinant vaccinia virus vaccine on humoral responses

To compare the effectiveness of systemic and mucosal immune induction following systemic or mucosal immunization using a prime-boost regimen, groups of BALB/c mice were primed mucosally (i.n.) or systemically (i.m.) with gB DNA or rvacgB and boosted 10 days later via the same route used for priming with the alternative vector. Some mice were immunized with vector DNA or vvtk^{-} to act as negative controls. Ten days following boosting, levels of systemic and mucosal immunity were evaluated by determining serum gB-specific IgG and vaginal gB-specific IgA responses. Firstly, confirming results in other systems (3, 6, 7), priming systemically with gB DNA and boosting systemically with
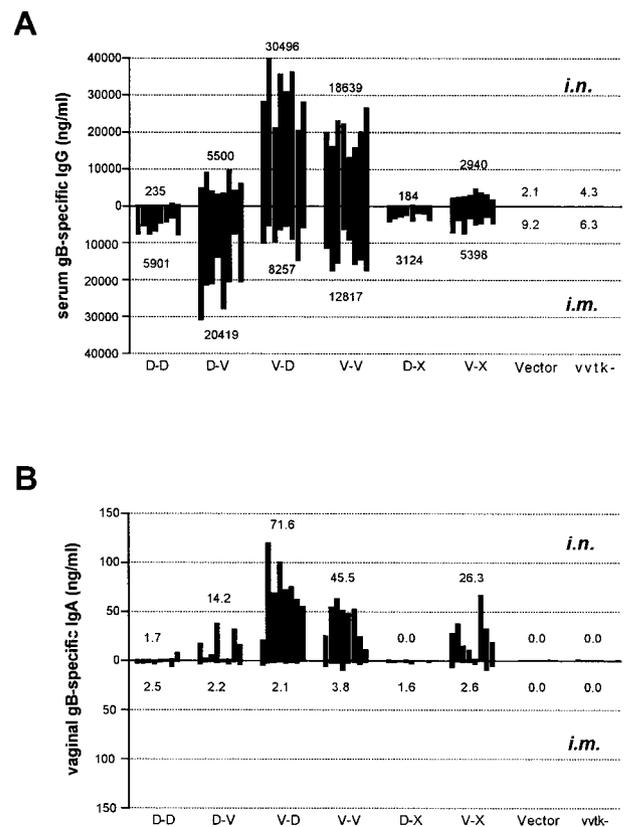
FIGURE 1. Mucosal administration of recombinant vaccinia virus as the priming vaccine followed by mucosal boosting with DNA vaccine elicits a maximal level of humoral responses at mucosal as well as systemic sites. Groups of BALB/c mice were immunized mucosally (i.n.) or systemically (i.m.) with gB DNA (D) or rvacgB (V) and boosted via the same route used for priming with the alternative vaccine type. Ten days later, gB-specific serum IgG (*A*) and vaginal IgA (*B*) levels were measured by the ELISA as described in *Materials and Methods*. The figures in the graph represent the average of each group (*n* = 8).

Table I. *Frequencies of gB-specific Ab and IFN-γ producing cells in ILN and vaginal tract of immunized mice following HSV vaginal infection[a]*

	ILN			Vaginal Tract		
	gB-specific Ab SFC/10^6 cells		IFN-γ SFC/ 10^6 cells	gB-specific Ab SFC/10^6 cells		IFN-γ SFC/ 10^6 cells
Regimens	IgA	IgG		IgA	IgG	
Mucosal immunization						
D-D	3 ± 3	18 ± 9	46 ± 23	1 ± 2	1 ± 2	6 ± 4
D-V	85 ± 37	114 ± 16	56 ± 27	26 ± 3	15 ± 6	37 ± 12
V-D	141 ± 27[b,c]	151 ± 14[b,c]	102 ± 19[b,c]	35 ± 11[b,c]	22 ± 5	62 ± 12[b,c]
V-V	93 ± 30	115 ± 26	60 ± 29	25 ± 6	17 ± 3	43 ± 14
D-X	5 ± 6	11 ± 6	28 ± 19			9 ± 3
V-X	84 ± 16	120 ± 6	46 ± 17	30 ± 6	20 ± 8	35 ± 18
Vector	1 ± 2	ND[g]	18 ± 25		ND	4 ± 4
Vvtk-	7 ± 5	ND	29 ± 16	5 ± 4	ND	12 ± 3
Systemic immunization						
D-D	36 ± 14	54 ± 14	49 ± 17	18 ± 3	ND	14 ± 3
D-V	53 ± 14[e]	77 ± 17[e]	80 ± 15[f]	8 ± 4[e]	ND	22 ± 7[e]
V-D	46 ± 13	62 ± 8	49 ± 13	16 ± 4	ND	18 ± 3
V-V	31 ± 8	36 ± 5	55 ± 15	10 ± 3	ND	9 ± 3
D-X	56 ± 6	48 ± 11	42 ± 10	10 ± 3	ND	12 ± 10
V-X	40 ± 10	76 ± 25	61 ± 13	18 ± 3	ND	11 ± 5
Vector	ND	ND	ND	ND	ND	ND
Vvtk-	ND	ND	ND	ND	ND	ND

[a] BALB/c mice (six per group) were immunized mucosally (i.n.) or systemically (i.m.) with gB DNA (D) or rvacgB (V) and boosted 10 days later via the same route used for priming with the alternative vaccine type. Control mice were given vector plasmid DNA or 10^7 PFU of vvtk-. Eight weeks following boosting, the mice were challenged vaginally with 10^6 PFU of HSV-1 McKrae. Four days later, the ILN and the vaginal tract were collected from each group. The frequencies of gB-specific Ab and IFN-γ-producing cells were determined by ELISPOT assay. SD is based on the number of SFC from quadruplicates per group.

[b] $p < 0.05$ compared with gB DNA mucosal priming-rvacgB mucosal boosting or rvacgB mucosal priming-rvacgB mucosal boosting.

[c] $p < 0.05$ compared with gB DNA systemic priming-rvacGb systemic boosting.

[d] $p = 0.08$ compared with gB DNA systemic priming-rvacgB systemic boosting.

[e] $p < 0.05$ compared with rvacgB systemic priming-gB DNA systemic boosting.

[f] $p = 0.0002$ compared with rvacgB systemic priming-gB DNA systemic boosting.

[g] ND, Not done.

rvacgB led to excellent serum IgG levels (Fig. 1A). These exceeded those achieved by priming and boosting with the same vaccine and were also superior to responses induced by systemic priming with rvacgB and systemic boosting with gB DNA. However, none of the systemic prime-boost approaches induced detectable gB-specific vaginal IgA responses (Fig. 1B).

In contrast, mucosal IgA responses were induced if mice were mucosally primed with gB DNA and boosted mucosally with rvacgB (Fig. 1B). Of particular interest, the maximal gB-specific IgA responses were induced if animals received rvacgB mucosally as the priming vaccine, followed by mucosal boosting with gB DNA (Fig. 1B). Moreover, such a protocol provided significantly higher serum IgG levels than did gB DNA systemic priming-rvacgB systemic boosting ($p < 0.05$; Fig. 1A). It is worth noting that vaginal IgA as well as serum IgG responses were higher in mice that received rvacgB priming-gB DNA boosting than in mice primed and boosted mucosally with the same vaccine type.

To further evaluate the efficacy of the different prime-boost approaches, mice immunized as described above were challenged vaginally with a virulent strain of HSV-1 8 wk after boosting and were killed 4 days later to measure mucosal immunity by a more quantitative assay. Accordingly, the ELISPOT approach was used to measure the frequency of gB-specific IgA- and IgG-producing cells in the vaginal tract and iliac LN (ILN). Once again, it was evident that the rvacgB mucosally prime-gB DNA mucosally boost protocol induced the highest frequency of gB-specific IgA and IgG producers in both ILN and the vaginal tract (Table I). With regard to systemic immunization, gB DNA systemic priming-rvacgB systemic boosting provided detectable, but markedly fewer, numbers of gB-specific Ab-producing cells than was attained by mucosal immunization (Table I). These results further demonstrate that maximal mucosal humoral immunity resulted from administration of the priming-boost regimen via the mucosal

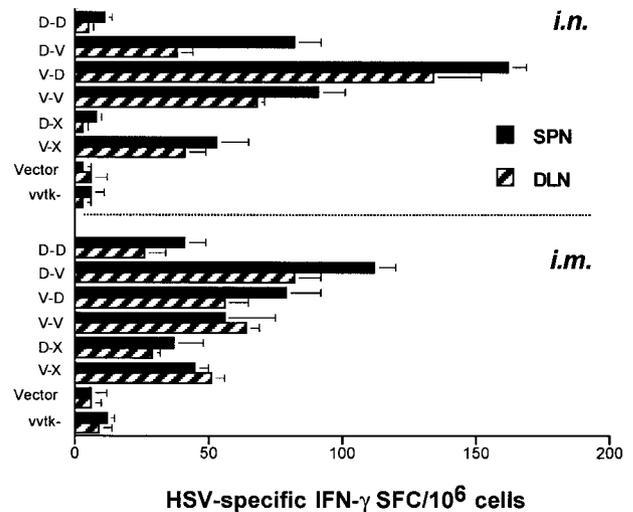route, and maximal immunity occurred if rvacgB was used for the priming step.

FIGURE 2. Influence of systemic or mucosal priming with DNA vaccine and recombinant vaccinia virus vaccine on the frequency of IFN-γ-producing CD4^{+} T cells in restimulated spleen and DLN. Groups of BALB/c mice immunized as described in *Materials and Methods* were killed on the 15th day postboosting. Spleen and DLN were collected to enumerate IFN-γ-producing CD4^{+} T cells by the ELISPOT approach. Cervical LN was used for mucosal immunization, and popliteal LN pooled with inguinal LN was used for systemic immunization. The collected lymphocytes were restimulated with UV-inactivated HSV-1 for 3 days. Results are the mean ± SD of six mice per group. D, gB DNA; V, rvacgB.

Influence of systemic or mucosal priming with DNA vaccine and recombinant vaccinia virus vaccine on T cell-mediated immunity

BALB/c mice immunized in various ways to measure systemic and mucosal T cell-mediated immunity were killed on the 15th day postboosting. Spleen and DLN were collected to enumerate numbers of IFN-γ-producing cells following stimulation with UV-inactivated HSV. This assay is considered primarily as a measurement of CD4^{+} T cell function (9). In other experiments C57BL/6 mice were immunized by the various protocols, because only in this mouse strain does gB have a strong MHC class I (H-2b)-restricted epitope (SSIEFARL), making measurement of CD8^{+} T cell responses more precise (15, 16).

As evident in Fig. 2, when both priming and boosting vaccines were given systemically, optimal IFN-γ-producing CD4^{+} T cells responses occurred in spleens of gB DNA-primed/rvacgB-boosted mice. However, in those mice immunized mucosally, maximal CD4^{+} T cell-mediated responses were evident in mice primed with rvacgB and boosted with gB DNA (Fig. 2). This pattern of IFN-γ-producing CD4^{+} T cell responses was also seen when DLN cells were quantified for IFN-γ spot-forming cells (Fig. 2). We also analyzed the frequency of IFN-γ-producing cells in ILN and vaginal tract of mice infected vaginally 4 days before killing with a virulent strain of HSV-1. Once again, mice primed mucosally with rvacgB and gB DNA boosted generated higher frequencies of IFN-γ-producing cells than occurred with other protocols (Table I).

An analysis of the pattern of CD8^{+} T cell responses in C57BL/6 mice immunized by the various prime-boost protocols revealed similar results as those observed with CD4^{+} T cell and Ab responses (Fig. 3). Most notably, immunization via the mucosal route generated optimal responses when rvacgB was used to prime and gB DNA was used as the boosting vaccine. Mucosal CD8^{+} T cell responses were also detectable in the LN draining the immunization site as well as distally in the mesenteric LN (Fig. 3, *B* and *D*). Whereas mucosal immunization generated responses in the mesenteric LN, all systemic immunization failed to cause such responses.
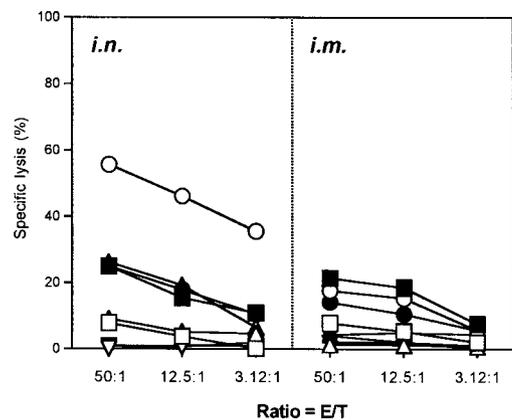
The priming step is critical for optimal mucosal immunity

The above results with prime-boost immunization indicate that optimal mucosal humoral and T cell-mediated responses required
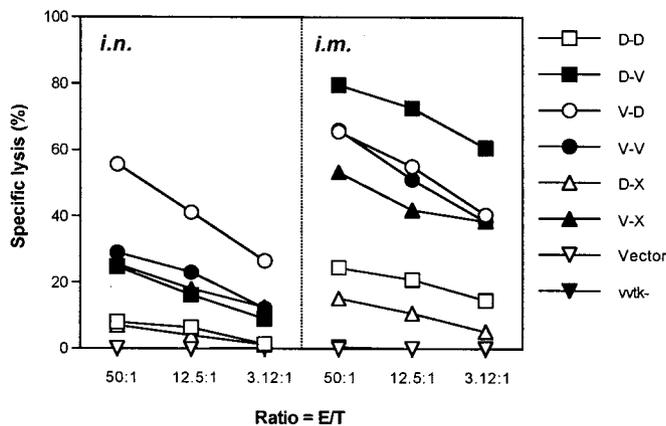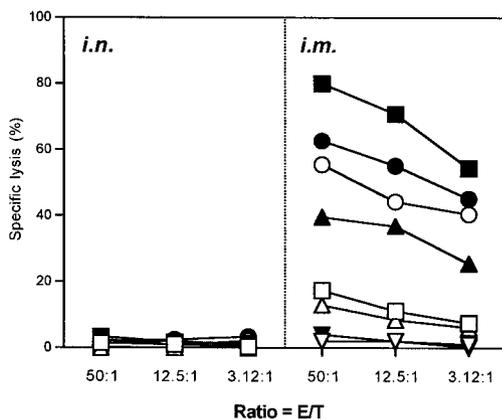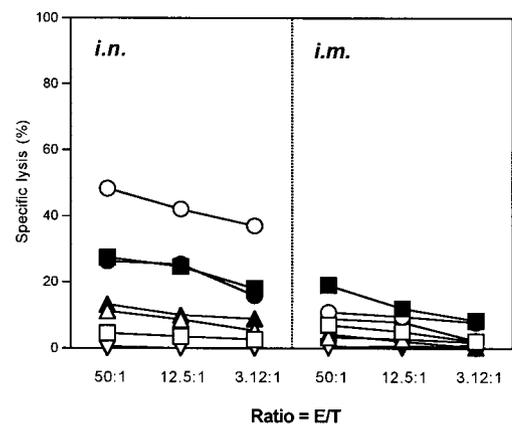

FIGURE 3. Effect of mucosal or systemic immunization of prime-boost protocols on CD8^{+} T cell-mediated CTL activity. C57BL/6 mice were immunized mucosally (i.n.) or systemically (i.m.) with gB DNA (D) or rvacgB (V) and boosted via the same route used for priming with the alternative vaccine type. Two weeks later, spleen (*A*), cervical LN (*B*), popliteal plus inguinal LN (*C*), and mesenteric LN (*D*) were collected to measure CD8^{+} T cell-mediated CTL activity. Collected lymphocytes of each LN were restimulated with the MHC class I-restricted (H-2b) immunodominant peptide (SSIEFARL) for 5 days. CTL activity was accessed by 5-h ^{51}Cr release assay as described in *Materials and Methods*. The results are plotted as the mean net percentage of specific lysis of EL-4 target cells (H-2b) pulsed with SSIEFARL peptide.

that the vaccines should be administered mucosally. To determine whether mucosal administration at both the priming and boosting steps was critically important, immune responses were compared in mice primed and boosted by the same route with those primed by one route and boosted by the other. The results summarized in Fig. 4 show systemic and mucosal humoral responses as well as IFN-γ-producing CD4$^+$ T cell responses in the various groups. It is evident that the maximal responses occurred if both priming and boosting were administered mucosally. However, mucosal priming appeared to be more important than mucosal boosting, because mice that received mucosal priming and systemic boosting had higher levels of mucosal immunity than those systemically primed and mucosally boosted (Fig. 4, A and B). These effects were most evident with gB-specific vaginal IgA responses (Fig. 4B), but were also noted with both IFN-γ-producing CD4$^+$ T cells (Fig. 4C) and CD8$^+$ T cell-mediated CTL responses (data not shown). Thus, these results indicate that mucosal administration at the priming step is critical for inducing maximal mucosal immunity in prime-boost approaches.

Discussion

In this study we assessed prime-boost immunization strategies with a DNA vaccine and attenuated recombinant vaccinia virus vector, both encoding the gB protein of HSV, for their effectiveness at inducing mucosal as well as systemic immunity to HSV.

Confirming the reports of others, systemic priming with DNA vaccine and systemic boosting with recombinant vaccinia virus vaccine was the most effective means of inducing serum Ab and splenic T cell responses (3, 6, 7). Unfortunately, the systemic prime-boost approach failed to induce detectable humoral or T cell responses at mucosal sites. However, such responses, at both proximal and distal locations, were induced if immunizations, especially the priming dose, were administered mucosally. Curiously, whereas optimal immunity with systemic priming and boosting occurred when DNA vaccine was used to prime and recombinant viral vectors were used as a boost, mucosal responses were optimal when animals were mucosally primed with recombinant viral vector and boosted with DNA vaccine given mucosally. Furthermore, notable mucosal responses occurred in animals mucosally primed with recombinant viral vector and subsequently boosted systemically with DNA vaccine. Because the mucosal prime-boost immunization protocol also induced excellent systemic immune responses, the approach should be useful to vaccinate against agents for which both mucosal and systemic immunity are important for protection.

The discovery that plasmid DNA encoding viral proteins could be immunogenic initiated the idea of using DNA as a practical means of vaccination (1). Although this objective has yet to be met, recent observations that DNA vaccines given as the first step of a prime-boost protocol induced effective immunity have moved
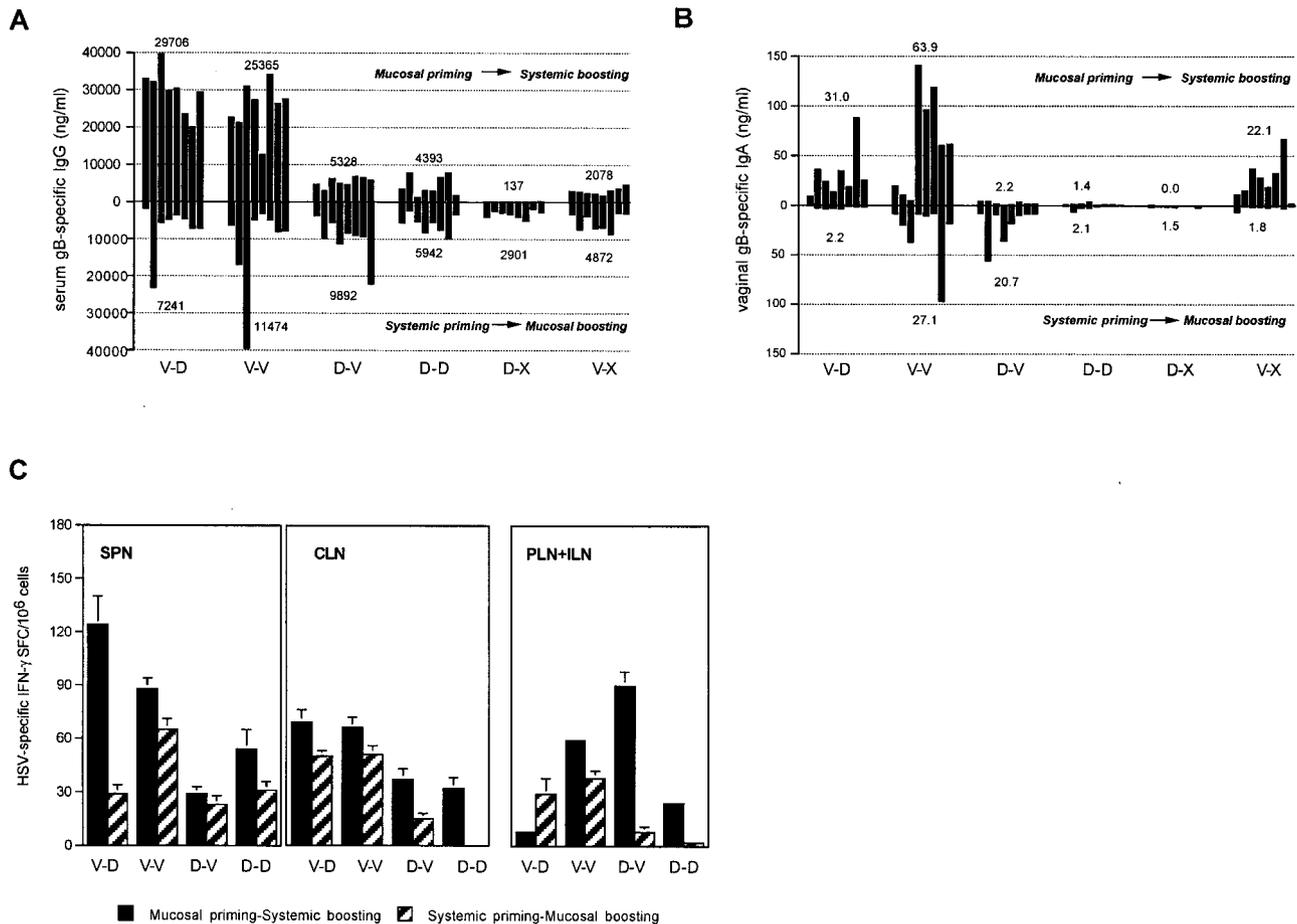

FIGURE 4. Administration via the mucosal route at the priming step elicits more effective humoral and cell-mediated immunity than that via the systemic route. BALB/c mice (*n* = 8) were primed via the i.n. route with gB DNA (D) or rvacgB (V), then boosted via the i.m. route with the alternative vaccine type (mucosal priming-systemic boosting). Otherwise, mice primed via i.m. administration were boosted via the i.n. route (systemic priming-mucosal boosting). *A*, gB-specific serum IgG levels determined 10 days later after boosting. *B*, gB-specific vaginal IgA levels in mice immunized as described above. The figures in the graphs represent the average of each group. *C*, The frequency of IFN-γ-producing cells in spleen (SPN), cervical LN (CLN), and popliteal plus inguinal LN (PLN+ILN) restimulated with UV-inactivated HSV. Results are the mean ± SD of six mice per group.

DNA vaccines closer to practical reality (3–7, 17). Such prime-boost protocols induce excellent levels of humoral as well as T cell immunity, emphasizing $CD8^+$ and type 1 $CD4^+$ T cell responses important for defense against intracellular pathogens (6, 7, 18, 19). It is still not clear why the DNA prime-viral vector boost approach induces superior immunity or if the system can be even further improved by adding other components, such as cytokines and adjuvants.

Previous reports using prime-boost strategies administered vaccines parenterally and looked only at systemic immunity (3–7, 17–19). However, for many pathogens immunity at mucosal surfaces represents a critical determinant of immune defense (8). Moreover, many vaccines, while inducing excellent systemic immunity, may be inferior inducers of mucosal protection (1, 20, 21). As shown in this report, with i.m. administered gB DNA and rvacgB, excellent systemic humoral and T cell responses were induced, but mucosal responses were undetectable. However, mucosal Ab and T cell-mediated responses occurred if the vaccines were administered mucosally. As is often the case with mucosal immunization, immunity was evident at mucosal sites distal from the site of immunization, indicating the operation of an interlinked mucosal immune system (22–25). In addition, prime-boost vaccination by the mucosal route induced notable systemic immune responses. This was not surprising, because after mucosal administration of DNA vaccine, plasmid DNA is known to gain access to multiple organs, including central lymphoid tissues (26). In addition, as we have shown previously, i.n. administration of rvacgB not only induces excellent vaginal and intestinal immunity, but also elicits systemic humoral and T cell-mediated immunity (24). Accordingly, the prime-boost formulation given mucosally represents a valuable means of inducing mucosal as well as systemic immunity and should be valuable in situations when both types of immune induction represent the objective.

Unexpectedly, when using the prime-boost approach mucosally, the vaccine sequence that provided optimal results was the reverse of that which works best following systemic administration. Accordingly, rvacgB priming followed by DNA gB boosting achieved maximal responses both mucosally and systemically. Furthermore, when protocols were used in which mucosal and systemic routes were heterogeneous, priming mucosally was shown to be the critical event. Reasons why rvacgB priming via the mucosal route proved superior to gB DNA priming remains to be defined. One explanation could lie with the potency of the primary immunogen combined with possible interference by pre-existing immunity with boost immunizations. Thus, as we have shown previously (24), i.n. rvacgB immunization represents a more potent means of inducing a vaginal IgA response than does gB DNA immunization. The potency gap could be narrowed by incorporating an adjuvant such as cholera toxin B (CTB) along with DNA vaccine for i.n. administration (10). We have also found that gB DNA priming along with CTB followed by rvacgB boosting induces better mucosal immune responses than does gB DNA priming without CTB (data not shown). A second aspect of the explanation could lie with interference by pre-existing immunity. Thus, for example, DNA vaccines appear unaffected by pre-existing immunity (27, 28). This makes then potentially valuable vaccines in animals, such as newborns that possess high levels of passive immunity (28, 29). In contrast, immune responses to attenuated vaccines can be limited by pre-existing immunity (27–29), although in the case of recombinant vaccinia virus vectors this effect may be less when vaccines are administered mucosally (25). We are currently assessing the role of interfering humoral immunity resulting from priming using B cell-deficient (μK/O) animals unable to generate Ab responses.

In conclusion, our observations demonstrate that immunization via the mucosal route with a recombinant viral vector vaccine followed by boosting with a DNA vaccine represents an effective means of inducing both mucosal and systemic humoral and T cell-mediated immunities. In contrast to systemic protocols when used mucosally, the prime-boost immunization approach works most effectively if DNA vaccine is used to boost, rather than prime, immunity.

Acknowledgments

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