DNA Immunization against Herpes Simplex Virus: Enhanced Efficacy Using a Sindbis Virus-Based Vector

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Previously we reported the development of a plasmid DNA expression vector system derived from Sindbis virus (T. W. Dubensky, Jr., et al., J. Virol. 70:508–519, 1996). In vitro, such vectors exhibit high-level heterologous gene expression via self-amplifying cytoplasmic RNA replication. In the present study, we demonstrated the in vivo efficacy of the Sindbis virus-based pSIN vectors as DNA vaccines. A single intramuscular immunization of BALB/c mice with pSIN vectors expressing the glycoprotein B of herpes simplex virus type 1 induced a broad spectrum of immune responses, including virus-specific antibodies, cytotoxic T cells, and protection from lethal virus challenge in two different murine models. In addition, dosing studies demonstrated that the pSIN vectors were superior to a conventional plasmid DNA vector in the induction of all immune parameters tested. In general, 100- to 1,000-fold-lower doses of pSIN were needed to induce the same level of responsiveness as that achieved with the conventional plasmid DNA vector. In some instances, significant immune responses were induced with a single dose of pSIN as low as 10 ng/mouse. These results indicate the potential usefulness of alphavirus-based vectors for DNA immunization in general and more specifically as a herpes simplex virus vaccine.

One promising new approach in vaccine development is the use of plasmid DNA for immunization. Immunization with antigen-encoding DNA plasmids has been used to induce both humoral and cell-mediated immune responses against a growing number of infectious disease agents, including viruses, bacteria, and parasites (reviewed in references 9, 12, 37, and 45). While numerous investigations have demonstrated the ability of DNA vaccine to induce protective immune responses in certain animal models, other studies have shown that in some systems, the level of immunity induced is not complete (16, 29, 30). In particular, the use of DNA vaccines to elicit mucosal immune responses remains inconsistent (27). Moreover, the efficacy of DNA vaccines in nonhuman primates, as well as in humans, has not been established, with few such reports appearing in the literature. However, one recent report of a study using chimpanzees indicates that DNA vaccines may indeed provide protection against experimental infection with human immunodeficiency virus type 1 (5).

Currently, a variety of methods are being used to increase the effectiveness of DNA immunization. Some of these approaches include the use of facilitators such as the anesthetic bupivacaine (47, 49), the coinjection of DNA vectors encoding immunomodulatory cytokines (15, 24, 44, 51) or costimulatory molecules (8), and the injection of plasmid DNA-transfected dendritic cells (32). Improving DNA delivery represents another area of active investigation and includes such devices or agents as the gene gun (14, 19), cationic lipids (17, 41, 50), and synthetic polymers (35). The continued improvement of DNA-based vectors also remains an important way to enhance DNA immunization (20, 34).

Recently, we (10, 11) and others (21) described the development of layered plasmid DNA-based expression systems derived from Sindbis virus, the type species of the alphaviruses (reviewed in references 13, 22, 28, and 42). The mode of heterologous gene expression from these alphavirus-derived expression plasmids differs from that of conventional eukaryotic expression plasmids. Conventional expression plasmids incorporate an RNA polymerase II expression cassette to drive the transcription of mRNA encoding the heterologous gene product. The first layer of the alphavirus-derived expression system also utilizes an RNA polymerase II cassette, but instead of driving the expression of a heterologous gene, this layer controls the expression of a second layer which is comprised of a self-replicating alphavirus RNA vector expression vector (replicon). This replicon component is essentially an alphavirus genome consisting of the alphavirus nonsymmetrical replicase genes, the 5′- and 3′-end genome sequences required in cis for replication, and a heterologous gene which has been substituted in place of the viral structural genes. Expression of the heterologous gene is achieved by linking it to the highly active alphavirus subgenomic promoter (52). Thus, primary transcription in vivo produces an RNA vector which is capable of cytoplasmic amplification and expression via the natural alphavirus replication cycle. For this reason, self-replicating vectors of this type are expected to express at higher levels than conventional plasmid DNA vectors where the heterologous gene is linked directly to a polymerase II promoter.

Although enhanced heterologous gene expression from layered DNA-based Sindbis virus vectors has been demonstrated in vitro compared to conventional DNA vectors (10, 11, 21), little is known about their relative activities in vivo. However, it has been shown that replicon-containing, recombinant alphavirus vector particles can induce both humoral and cell-mediated immune responses in animal models (6, 53, 54). In this report, we demonstrate the in vivo efficacy of plasmid
approximately 5 vectors expressing HSV-1 gB was performed by using Lipofectamine (Gibco/BRL, Gaithersburg, Md.), followed by a secondary goat anti-mouse IgG (1:20,000) peroxidase (HRP) antibody (Southern Biotechnology Associates, Birmingham, Ala.). The blot was developed by exposing autoradiography film to a chemiluminescence reaction, using an ECL kit (Amersham, Arlington Heights, Ill.).

**Plasmid DNA preparation.** Large-scale preparations of plasmid DNA were obtained by using Qiagen DNA purification columns as instructed by the manufacturer (Qiagen, Inc., Chatsworth, Calif.). Plasmids were resuspended in sterile TE buffer (10 mM Tris-HCl [pH 7.5], 0.1 mM EDTA) at a final concentration of 1 mg/ml. Only preparations that resolved as a single band on an agarose gel were used. The concentration of the plasmid DNA was determined by the *Lima*us amebocyte lysate assay (E-Toxate kit; Sigma, St. Louis, Mo.) were used for immunization.

**Immunization of mice.** The hind legs of anesthetized 5- to 8-week-old female BALB/c (H-2d) mice (Harlan Sprague-Dawley, Indianapolis, Ind.) were shaved with an electric razor and injected bilaterally in the tibialis anterior (TA) muscle with different doses of plasmid DNA diluted in sterile saline. In all cases, a total volume of 50 μl was injected per muscle, using an insulin syringe with 28.5-gauge needle (Becton Dickinson, Franklin Lakes, N.J.).

Viral challenge. Two different murine models of HSV infection were used to determine the in vivo efficacy of pCI and pSIN-gB vectors to induce virus-specific protective immune responses. In the McKrae challenge model, 5-week-old BALB/c mice received a single intramuscular (i.m.) immunization with various doses of the vectors. At 2 weeks postimmunization, all mice were bled and then challenged intraperitoneally (i.p.) with a lethal dose of virulent HSV-1 McKrae (5 × 10⁶ PFU/mouse). Ensuring morbidity and mortality were scored for 14 days postchallenge, and only those which remained alive at the end of the observation period were considered protected. The lethal challenge dose of HSV-1 McKrae represents the amount of virus which kills 100% of phosphate-buffered saline-injected negative controls and was predetermined for the mouse strain used and the age of the mice at the time of challenge (1).

In the zosteriform challenge model (40), 5-week-old BALB/c mice were immunized as described above. At 2 weeks postimmunization, the left flank of each mouse was shaved with an electric razor and then depilated by using the chemical Nair (Nair-Walker, Inc., New York, N.Y.). On the following day, the smooth flank was gently scarified with a 2.85-gauge needle, and 10⁵ PFU of HSV-1 McKrae was applied to the abraded area (approximately 4 mm²). During the 14-day postchallenge period, the mice were scored for the appearance and spread of lesions, as well as overall morbidity and mortality. Mice that did not develop lesions were considered protected, whereas mice that developed lesions and were subsequently moribund were scored as unprotected (2).

**Determination of HSV-1-specific antibodies.** Sera collected from immunized mice were analyzed for HSV-1-specific total IgG antibody by enzyme-linked immunosorbent assay (ELISA). EIA Costar plates (Corning Costar Corp., Cambridge, Mass.) were coated with HSV-1-infected cell extract (100 μl/well; AB) diluted 1:500 in carbonate buffer. The plates were blocked for 1 h at room temperature, washed once, and virally inactivated overnight with serially diluted mouse serum (100 μl/well). After multiple washes, each well received 100 μl of goat anti-mouse IgG (H + low light chain) conjugated to HRP (Southern Biotechnology Associates), and the plates were incubated at 37°C for 2 h. For detection, anti-IgG isotypes, goat anti-mouse IgG1-HRP, IgG2a-HRP, and IgG2b-HRP (Southern Biotechnology Associates) were used. After a final series of washes, 100 μl of peroxidase substrate solution (EIA substrate kit: Bio-Rad Laboratories, Hercules, Calif.) was added to each well, and the optical density at 450 nm (OD₄₅₀) was read. Serum OD₄₅₀ readings were scored positive if the optical density OD₄₅₀ reading exceeded the mean OD₄₅₀ reading of sera from sham-immunized controls by 3 standard deviations. The reciprocal of the last serum dilution which scored positive was taken as the endpoint titer.

**Splenocyte restimulation in vitro.** For cytotoxicity assays, splenocytes were harvested from mice that had received a single i.m. immunization 3 weeks previously. In vitro restimulation of primed splenocytes was achieved by using retrovirus vector-transduced syngeneic cells expressing HSV-1 gB. The BC-gB1 cell line was made essentially as described previously (48) by transducing the parent BC10ME cell line (H⁻²) with a retrovirus vector expressing HSV-1 gB. For in vitro restimulation, single-cell suspensions of splenocytes were prepared from individual mouse spleens in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 1 mM sodium pyruvate, and 50 μM 2-mercaptoethanol (RPMMI complete medium). A total of 3 × 10⁵ splenocytes were then restimulated with 6 × 10⁴ γ-irradiated (10,000 rads) BC-gB1 cells for 7 days at 37°C in 5% CO₂.

**Cr³⁺ release assay.** Target cells expressing HSV-1 gB (BC-gB1) or a control β-galactosidase antigen (BC-βgal) were labeled for approximately 1 h with Na²⁴CrO₄ (200 μCi per 2 × 10⁶ cells; 21.4 mCi/ml; Amersham). Following labeling, the target cells were washed three times and added in triplicate at the ratio of 100,000 cells/well to 96-well-bottom plates containing 10⁴ effector cells. The resulting effector-target cell ratios ranged from 100:1 to 1:1. The plates were incubated for 4 h at 37°C in 5% CO₂, after which the radioactivity released into the supernatants was determined with a beta-emitter counter. The cytolytic index was calculated from the corrected per hour minute according to the following formula: (experimental cpm – spontaneous cpm) / (control cpm – spontaneous cpm) × 100.

**Western blot analysis.** Transfection of BHK-21 cells with pCI and pSIN vectors expressing HSV-1 gB was performed by using Lipofectamine (Gibco/BRL, Gaithersburg, Md.) according to the manufacturer’s instructions. Approximately 5 μg of plasmid DNA was used to transfect 5 × 10⁵ cells. After 48 h, protein extracts were obtained by lysis in buffer containing 1% Nonidet P-40, 150 mM NaCl, and 10 mM Tris (pH 7.4). Approximately 2 × 10⁶ cell equivalents were then denatured, separated in 8 to 16% gradient sodium dodecyl sulfate (SDS)–polyacrylamide gels, and transferred onto Problott membranes (Applied Biosystems, Foster City, Calif.). Western blotting was performed by reacting the membrane with a primary mouse monochonal anti-HSV-1 gB antibody (ABI, Columbus, Mo.), followed by a secondary goat anti-mouse IgG (1:50,000) peroxidase (HRP) antibody (Southern Biotechnology Associates, Birmingham, Ala.). The blot was developed by exposing autoradiography film to a chemiluminescence reaction, using an ECL kit (Amersham, Arlington Heights, Ill.).

**MATERIALS AND METHODS**

**Viruses.** The virulent McKrae and attenuated KOS strains of HSV-1 were grown in Vero cell monolayers as described previously (2) and stored in aliquots at −80°C. Titers were measured by standard plaque assay using Vero cells and expressed as PFU/milliliter.

**Construction of vectors expressing HSV-1 gB.** The HSV-1 KOS gB gene (3,555 bp) was obtained from Martin Muggeridge (LSU Medical Center, Shreveport, La.) and subcloned into pCI (Promega, Madison, Wis.) and Sindbis virus-based expression vectors (pSIN1.5 and pSIN2.5) as depicted in Fig. 1. Essential features of the pCI vector include a human CMV major intermediate-early promoter/enhancer, a β-globin-immunoglobulin G (IgG) chimeric intron, and a simian virus 40 late-region polycladenylation signal. The vector pCI-gB was constructed by insertion of gB into pCI, using XhoI and XbaI to create a construct 7,563 bp in size in which the expression of gB is linked directly to the CMV promoter. The Sindbis virus vectors pSIN1.5-gB and pSIN2.5-gB were constructed by insertion of gB into pCI, using XhoI and XbaI to create recombinant plasmids totaling 15,963 and 16,549 bp in size, respectively.

Transcription initiation from the CMV promoter in pSIN vectors occurs at the authentic Sindbis virus 5′ end (11) to ensure maintenance of the necessary cis replication sequences. Other essential features include the nonstructural protein (replicase) genes, the subgenomic junction region promoter for heterologous gene expression, the Sindbis virus 3′-end cis replication sequences, a synthetic polyadenylation tract, and the bovine growth hormone transcription termination signal. The pSIN2.5 vector also contains the hepatitis B virus posttranscriptional polyadenylation tract, and the bovine growth hormone transcription termination signal (TT) in the pCI vector, bovine growth hormone transcription termination signal (TT) in the pSIN vectors, hepatitis B virus posttranscriptional regulatory element (PRE), and HSV-1 gB.

DNA-based Sindbis virus vectors encoding the glycoprotein B (gB) (pSIN-gB) of herpes simplex type 1 (HSV-1) as a DNA vaccine and compared it to a conventional cytomegalovirus (CMV) promoter-driven DNA vector also expressing HSV-1 gB (pCI-gB). While both types of vector systems were able to induce virus-specific and protective immune responses in two different murine models of HSV infection, dosing studies demonstrated that the pSIN-gB vectors were consistently superior to pCI-gB in the induction of all immune parameters tested, including virus-specific antibody, cytotoxic T lymphocytes (CTL), and protection from lethal viral challenge. These findings illustrate the potential utility of aliphavirus-based vectors as DNA vaccines. Moreover, the continued development and refinement of such systems may extend their usefulness to other areas of gene transfer as well.
RESULTS

Expression of HSV-1 gB in vitro. Recombinant vectors expressing HSV-1 gB were constructed by using the conventional CMV promoter-based pCI vector and the Sindbis virus-based DNA vectors pSIN1.5 and pSIN2.5 (Fig. 1). To verify that all of the vectors expressed authentic gB protein, BHK-21 cell monolayers were transfected with the individual plasmids. Lysates were collected at 48 h posttransfection, separated by SDS-polyacrylamide gel electrophoresis, transferred onto membranes, and probed by Western blot analysis. A murine monoclonal antiserum specific for gB was used to assay for the expected 110-kDa protein species. The protein was detected in each of the pCI-gB-, pSIN1.5-gB-, and pSIN2.5-gB-transfected cell lysates (Fig. 2, lanes 3 to 5, respectively) but not in the mock-transfected BHK cell lysate (lane 2). The gB-specific protein species detected in plasmid DNA-transfected cells was similar to that observed in transduced cells expressing HSV-1 gB (Bc-gB1; lane 1). Moreover, the amount of gB protein expressed from the pSIN vectors appeared comparable to that produced by the pCI vector. While the reason for these results is not entirely clear, we believe that the ability of the pSIN vectors to transfect BHK-21 cells transfected with the pSIN or pCI vectors, we have observed expression differences between these vectors when other antigens and doses are used (11).

Enhanced protection from viral challenge. To test the in vivo expression of HSV-1 gB and the induction of specific immune responses, animals were injected i.m. with the DNA vectors encoding gB. As described in Materials and Methods, the immunized mice were then challenged in two different murine models of HSV infection (Table 1). In initial experiments, mice were immunized on a single occasion with relatively large doses (100 or 30 \( \mu \)g) of the pCI-gB, pSIN1.5-gB, or pSIN2.5-gB vectors. Immunization at these doses resulted in complete protection, with 100% of the mice surviving the lethal i.p. McKrae challenge (data not shown). As expected, the positive control group, consisting of mice immunized with HSV-1 KOS, also demonstrated complete protection from lethal i.p. McKrae challenge, whereas none of the mice immunized with 100-\( \mu \)g doses of either negative control plasmid (pCI-HBV or pSIN1.5-HBV) survived (Table 1). These vectors, which encode the precore protein from hepatitis B virus, were included to rule out the induction of nonspecific protection by an irrelevant viral protein.

In further experiments, we evaluated the in vivo efficacy of the pCI and pSIN-gB vectors at progressively lower doses. Strikingly, as the doses decreased, both pSIN1.5-gB and pSIN2.5-gB were still able to protect a majority of the challenged mice at doses where pCI-gB could no longer induce similar levels of protection (Table 1). This dosage difference becomes even more apparent when one takes into consideration the relative number of input DNA molecules. Because the pSIN-gB vectors are more than twice the size of pCI-gB, the same immunization dose of vector based on DNA mass (e.g., 100 \( \mu \)g/mouse) correspondingly reduces the effective number of DNA molecules or molar equivalents of pSIN-gB twofold compared to pCI-gB. As shown in Table 1, mice immunized with 3.0 \( \mu \)g of pSIN1.5-gB or pSIN2.5-gB were protected from lethal i.p. McKrae challenge at a rate of 93 or 97%, respectively. By comparison, only 49% of mice immunized with 3 \( \mu \)g of pCI-gB were protected following lethal challenge. Moreover, this trend continued as even lower doses were used for immunization. At the lowest dose tested (10 ng), approx-

![FIG. 2. Expression of HSV-1 gB in vitro from pCI and pSIN vectors. Cell lysates were prepared from BHK-21 cells transfected with the different plasmid vectors. Cell lysates were harvested 48 h posttransfection, separated on SDS–8 to 16% polyacrylamide gels, transferred, and probed with a mouse monodonal anti-HSV-1 gB antibody followed by a goat anti-mouse IgG2a-HRP antibody. Sizes are indicated in kilodaltons.](http://jvi.asm.org)
and mortality were monitored for 14 days following challenge. Since only 5 of 20 mice immunized with the same dose of pCI-gB was unable to induce antibody in any of the mice tested, overall, the antibody data appeared to correlate well with the ability to confer protection against lethal virus challenge. In addition, IgG isotype determination of positive sera collected from both pCI- and pSIN-gB-immunized mice revealed a clear preponderance of IgG2a over IgG1 (data not shown). These data indicate that both vector systems were able to induce an isotype pattern similar to that achieved by infection with HSV (36).

Another set of experiments was performed to determine if the pSIN vectors would demonstrate enhanced efficacy at extended periods of time following immunization. As shown in Fig. 4, 100% of mice immunized with various doses (1.0, 0.3, or 0.05 μg/mouse) of pSIN2.5-gB still demonstrated HSV-1-specific antibody even at 15 weeks postimmunization. In contrast, only a few of the mice immunized with the same doses of pCI-gB were antibody positive at this point in time. A similar trend was observed in sera from mice immunized with the same doses of the different vectors and analyzed at both 4 and 8 weeks postimmunization (data not shown). Overall, these results illustrate that the differences in efficacy first observed between these vectors at 2 weeks postimmunization (Fig. 3) still remain at 4, 8, and 15 weeks postimmunization.

**Induction of HSV-1 gB-specific CTL.** To examine bulk CTL levels in DNA-immunized mice, splenocytes were collected from individual mice 3 weeks following a single immunization with the pCI or pSIN2.5-gB vectors. Splenocyte cultures were restimulated in vitro for 7 days with BC-gB1 cells, a retrovirus vector-transduced syngeneic cell line expressing HSV-1 gB, and the cytolitic activity of the cultures was measured in a 51Cr-release assay with BC-gB1 and BC-gB1 target cells (Fig. 5). When BALB/c mice were immunized with a higher dose (100 or 30 μg) of pCI-gB or pSIN2.5-gB, positive HSV-1 gB-specific CTL responses were observed in all mice tested. To determine any potential differences in efficacy, lower doses of these vectors were then tested. As shown in Fig. 5, the pSIN2.5-gB induction of CTL correlated well with previous antibody and challenge data compared to pCI-gB. At 10 μg/mouse, pCI-gB was unable to induce consistently positive bulk CTL, whereas doses of 10 or 1 μg of pSIN2.5-gB per mouse induced CTL in all animals tested. Even at the lowest dose tested (10 ng/mouse), pSIN2.5-gB was able to induce CTL in two of three mice.

**CTLp frequency analysis.** To quantitatively assess the ability of the different vectors to induce CTL, HSV-1 gB-specific CTLp LDA was performed (Table 3). Consistent with all previous data, the pSIN2.5-gB vector exhibited greater efficacy than pCI-gB. At doses of 100 μg/mouse, pSIN2.5-gB induced a greater number of gB-specific CTLp (1 in 45,384) than did a similar dose of pCI-gB (1 in 102,239). These results indicate that even though bulk CTL induction at this dose by the pSIN2.5-gB and pCI-gB vectors were similar, induction of CTLp by pCI-gB did not appear to be as effective as that by pSIN-gB. Interestingly, at lower doses where pSIN2.5-gB was still able to induce bulk CTL, this plasmid induced essentially the same number of CTLp as obtained with the 100-μg dose. Only at a dose of 50 ng/mouse did the CTLp frequency drop to a frequency of 1 in 88,710. In comparison, the CTLp at this dose was still superior to that of pCI-gB at 100 μg (1 in 102,239). Lastly, because pCI-gB was not able to induce bulk CTL at the lower doses shown in Table 3, CTLp were not induced, as reflected by frequency values in excess of 2,000,000.

**DISCUSSION**

The goal of this study was to test the efficacy of Sindbis virus-based vectors for DNA immunization against HSV. Two

<table>
<thead>
<tr>
<th>Immunogena</th>
<th>Dose/mouse</th>
<th>No. of mice protected from lesion development/no. testedb</th>
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<tbody>
<tr>
<td>HSV-1 KOS</td>
<td>10⁷ PFU</td>
<td>9/10</td>
</tr>
<tr>
<td>pSIN1.5-gB</td>
<td>100 μg</td>
<td>0/10</td>
</tr>
<tr>
<td>pCI-gB</td>
<td>100 μg</td>
<td>1/10</td>
</tr>
<tr>
<td>pSIN2.5-gB</td>
<td>30 μg</td>
<td>10/10</td>
</tr>
<tr>
<td>pCI-gB</td>
<td>30 μg</td>
<td>10/10</td>
</tr>
<tr>
<td>pSIN2.5-gB</td>
<td>0.3 μg</td>
<td>17/20</td>
</tr>
<tr>
<td>pCI-gB</td>
<td>0.3 μg</td>
<td>5/20</td>
</tr>
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</table>

a BALB/c mice (5 to 6 weeks old) were immunized i.m. on a single occasion with different doses of pCI or pSIN vectors expressing HSV-1 gB. HSV-1 KOS (10⁷ PFU/mouse)-immunized mice served as positive controls, and pCI or pSIN vectors expressing the precore protein (e) from hepatitis B virus (HBVe) served as negative controls.

b Mice were challenged 14 days postimmunization by applying HSV-1 McKrae (10⁵ PFU/mouse) to the shaved and scarified flank. The development of lesions and mortality were monitored for 14 days following challenge.
Sindbis virus-based DNA vectors (pSIN1.5-gB and pSIN2.5-gB) and one conventional plasmid DNA vector control (pCI-gB) were constructed to express HSV-1 gB. gB was chosen because it is a well-characterized viral protein containing epitopes for both virus-specific antibody and CTL (7, 18). In addition, there are several murine models of HSV infection which can be used to assess protection from virus challenge (2, 40). Importantly, HSV infection continues to be a common

FIG. 3. Induction of HSV-1-specific total IgG antibody in mice immunized with pCI or pSIN vectors expressing gB. BALB/c mice given a single i.m. injection of the different vectors at either 3.0 or 0.3 μg/mouse were bled at day 14 postimmunization, immediately prior to lethal challenge with HSV-1 McKrae. ELISA was used to measure total IgG antibody titers in individual mice. The normal serum control represents a pool from several nonimmune mice.
Several studies using gB and/or gD (3, 4, 26, 30, 32, 33, 38), or the nonstructural protein ICP27 (31, 38), as immunogens have demonstrated that DNA immunization can induce specific immune responses against HSV-1 or HSV-2 in murine and/or guinea pig models. However, some reports indicate that the level of immunity may be incomplete compared to HSV.
infection itself or immunization with recombinant vaccinia viruses expressing the same HSV proteins (16, 27, 30). In particular, the induction of CTL and mucosal immune responses appears to be inconsistent in spite of multiple immunizations (27, 30). As an alternative vaccine approach, we were interested in examining the induction of HSV-specific immune responses by our gB-expressing pSIN vectors. The data show that a single immunization with the pSIN-gB vectors efficiently in-

FIG. 5. Induction of HSV-1 gB-specific CTL in mice immunized with pCI or pSIN vectors expressing gB. Splenocytes from individual mice collected after a single i.m. injection with the pCI-gB or pSIN-gB vectors were restimulated in vitro with BC-gB1 and tested for cytolytic activity in a 4-h 51Cr release assay using BC-gB1 target cells (closed symbols) and BC-βgal target cells (open symbols). Cytolytic activity is shown as the percent specific lysis detected at each effector/target cell ratio tested and represents the mean of triplicates. This experiment was repeated on two additional occasions with similar results.
The pSIN vectors also contain additional features such as the nonstructural proteins, not present in conventional vectors, which will require further study. During the normal Sindbis virus life cycle, these nonstructural proteins are expressed at lower levels than the structural proteins. By analogy, the same should hold true for the pSIN vectors in which the structural proteins have been replaced by a heterologous protein. It is not clear what role, if any, the expression of the Sindbis virus nonstructural proteins may play in inducing antivector responses. However, for some applications, a single low-dose immunization with the pSIN vectors may be sufficient to induce the desired immune responses, obviating the need for readministration. In any case, preliminary experiments indicate that the pSIN-gB vectors can be successfully readministered. The superior performance of pSIN vectors in vivo compared to the conventional pCI vector is encouraging and suggests broadening the scope of these studies to larger animals. In addition, the efficacy of the pSIN vectors remains to be tested by using other antigens and other animal models, in particular nonhuman primates where repeat immunizations will likely be needed. The results of such studies should provide further information regarding the potential usefulness of pSIN vectors as DNA vaccines.

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REFERENCES


14. Fynan, E. F., R. G. Webster, D. H. Fuller, J. R. Haynes, J. C. Santoro, and

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TABLE 3. LDA of HSV-1 gB-specific CTLp frequencies induced by pCI-gB and pSIN-gB

<table>
<thead>
<tr>
<th>Dose (μg)</th>
<th>pSIN2.5-gB (5 SD)</th>
<th>pCI-gB (5 SD)</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>45,384 (38,309–55,664)</td>
<td>102,239 (82,096–135,478)</td>
</tr>
<tr>
<td>1.0</td>
<td>43,186 (38,025–49,966)</td>
<td>&gt;2,000,000</td>
</tr>
<tr>
<td>0.3</td>
<td>49,551 (41,960–60,496)</td>
<td>&gt;2,000,000</td>
</tr>
<tr>
<td>0.05</td>
<td>87,710 (72,534–114,172)</td>
<td>&gt;2,000,000</td>
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</table>

* Splenocytes obtained from BALB/c mice 3 weeks following a single immunization with the pCI or pSIN vectors expressing HSV-1 gB were cultured for 10 days by limiting dilution as described in Materials and Methods. Cytolytic activity was determined in a 4-h 51Cr release assay with BC-gB1 and BC-gB1 target cells. Calculated by using the minimum chi-square method (44). Data shown are means, with 95% confidence limits in parentheses. The LDA was performed on three separate occasions, and the results obtained in each experiment were similar.


