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Comparison of Monovalent Glycoprotein B with Bivalent gB/pp65 (GP83) Vaccine for Congenital Cytomegalovirus Infection in a Guinea Pig Model: Inclusion of GP83 Reduces Antibody Response but Provides Equivalent Protection Against Mortality

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Abstract

Cytomegalovirus (CMV) subunit vaccine candidates include glycoprotein B (gB), and phosphoprotein ppUL83 (pp65). Using a guinea pig cytomegalovirus (GPCMV) model, this study compared immunogenicity, pregnancy outcome, and congenital viral infection following pre-pregnancy immunization with a three-dose series of modified vaccinia virus Ankara (MVA)-vectored vaccines consisting either of gB administered alone, or simultaneously with a pp65 homolog (GP83)-expressing vaccine. Vaccinated and control dams were challenged at midgestation with salivary gland-adapted GPCMV. Comparisons included ELISA and neutralizing antibody responses, maternal viral load, pup mortality, and congenital infection rates. Strikingly, ELISA and neutralization titers were significantly lower in the gB/GP83 combined vaccine group than in the gB group. However, both vaccines protected against pup mortality (60.5% in controls vs. 11.4% and 8.3% in gB and gB/GP83 combination groups, respectively; $p < 0.0001$). Reductions in pup viral load were noted for both groups compared to control, but preconception vaccine resulted in a significant reduction in GPCMV transmission in the monovalent gB group only (26/44, 59 % v. 27/34, 79 % in controls; $p < 0.05$). We conclude that, in

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Contributors

Mark R. Schleiss and Don J. Diamond developed and designed the study concept. Beth Swanson, Peter Gillis, Nelmary Hernandez-Alvarado, Claudia Fernández-Alarcón, Megan Schmit, Jason Zabeli, and Felix Wussow acquired the data. All authors were involved in the interpretation of the data. Beth Swanson wrote the first draft of the manuscript and all authors were involved in the further drafting of the manuscript or revising it critically for important intellectual content. All authors approved the manuscript, before it was submitted by the corresponding author. All authors had full access to the data and had final responsibility to submit for publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

the MVA platform, adding GP83 to a gB subunit vaccine interferes with antibody responses and diminishes protection against congenital GPCMV infection, but does not decrease protection against pup mortality.

Keywords

Cytomegalovirus (CMV); congenital CMV infection; CMV vaccine; glycoprotein B; CMV pp65; guinea pig cytomegalovirus; CMV immune modulation; pentameric complex

1. Introduction

Infection with human cytomegalovirus (HCMV) causes considerable morbidity and occasional mortality in transplant recipients, HIV-infected individuals, and newborns infants that acquire infection *in utero*[1-3]. A preconception vaccine capable of preventing virus transmission to the fetus would represent a highly cost-effective public health advance[4-6]. Unfortunately, the lack of a clear immunological correlate of protective immunity has hampered development of an HCMV vaccine[7]. Antibody responses targeting viral envelope glycoproteins, as well as cellular immune responses (CD4+ and CD8+) targeting structural and regulatory proteins, play important roles in protection against acquisition and/or reactivation of infection[8]. Accordingly, recombinant HCMV subunit vaccines have focused on the immunodominant glycoprotein B (gB) as well as the major CD8+ target, pp65 (ppUL83), in clinical trials[9-12].

Small animal models for the study of CMV vaccines require use of the strain unique to that specific species[13, 14]. Guinea pigs provide a particularly valuable model for study of vaccines against congenital infection, since the guinea pig CMV (GPCMV) is able to cross the placenta and cause congenital infection[15-19]. The GPCMV homolog of HCMV gB has been identified as a major target of neutralizing antibody response and has been studied as a vaccine[20-22]. Similarly, the GPCMV homolog of HCMV pp65 (ppUL83)[23], referred to as GP83, is a target of CD4+ and CD8+ T cell responses, and has also been evaluated as a subunit vaccine[24,25].

Preconception immunization of guinea pigs with gB expressed by a variety of modalities has demonstrated varying levels of protection against congenital GPCMV infection[15,18, 25-27]. GP83 elicits both an antibody and T cell response following vectored immunization with an alphavirus replicon vaccine, and this vaccine also conferred partial protection against congenital GPCMV infection and disease[25]. However, there is little information about potential synergy of combined gB/pp65 vaccination in any animal model of CMV vaccines. Combination gB/pp65 vaccines have been evaluated for immunogenicity in the rhesus macaque CMV (RhCMV) model, with these antigens co-administered with either the RhCMV-IE1 protein or the viral homolog of Il-10[28, 29], but there has been no head-to-head comparison of these combination vaccines with monovalent gB vaccine in study of congenital infection. In the murine CMV (MCMV) model, the pp65 homolog, M84, has been evaluated as a component in a multiple-subunit vaccine[30], but was not compared to single antigen vaccination. We therefore undertook these studies, using a modified vaccinia

virus Ankara (MVA)-vectored platform, to compare the protective efficacy of a combination gB/GP83 vaccine to monovalent gB vaccine in the GPCMV model.

2. Materials and methods

2.1 Guinea Pigs

GPCMV-seronegative outbred Hartley guinea pigs were purchased from Elm Hill Laboratories (Chelmsford, MA), and housed under conditions approved by the Institutional Animal Use Committee policy at the University of Minnesota, Minneapolis.

2.2 Virus and cells

Salivary gland-passaged GPCMV virus stocks were prepared in strain-2 guinea pigs as previously described[31]. Cell culture was carried out in guinea pig fibroblast lung cells (GPL; ATCC CCL158) in F-12 medium supplemented with 10% fetal calf serum (FCS, Fisher Scientific), 10,000 IU/l penicillin, 10 mg/l streptomycin (Gibco-BRL) and 0.75% NaHCO₃ (Gibco-BRL). For neutralization assays, *gpt* selection[32] was used to generate an eGFP-tagged virus, vJZ848, with an intact pentameric complex[33-35], using previously described protocols[36].

2.2 Generation of the MVA-gB and MVA-GP83 vaccines

Recombinant MVA vaccines were generated using MVA transfer vector pZWIIA[24, 37]. Details of generation of these vaccines have already been described elsewhere[24]. Briefly, for the gB vaccine, a truncated, secreted form of gB was cloned via PCR-mediated insertion into pZWIIA (truncated at Ile₆₈₇), and for GP83 vaccine, the full-length ORF was cloned. MVAs were generated on chicken embryo fibroblasts via homologous recombination[38, 39], and viruses subjected to plaque purification by limiting dilution. Correct insertions and orientations of gB and GP83 ORFs in recombinant MVA genome were confirmed by PCR and DNA sequencing.

2.4 Study design

A preliminary dose response study demonstrated 5×10^7 as an optimal gB vaccine dose in non-pregnant animals (data not shown). For analysis of vaccine protection against congenital GPCMV infection, young female Hartley guinea pigs were divided into 3 groups of 12. Group 1 was immunized subcutaneously on 3 occasions at 1-month intervals with MVA-gB (5×10^7 pfu/dose). Group 2 animals were immunized with both MVA-gB and with a MVA-GP83 vaccines (5×10^7 pfu/dose) administered subcutaneously at a separate sites with a separate syringe. The control group was unimmunized. Anti-GPCMV ELISA titers were measured 30 days following each dose of vaccine. Following completion of the immunization series, animals were mated and examined weekly for evidence of pregnancy. At midgestation (30-35 days gestation), dams were challenged with 1×10^5 PFU of salivary gland-passaged GPCMV (SG-GPCMV) and observed daily until delivery[40]. Animals that failed to become pregnant (one animal in group 2), or dams that gave birth <7 days following SG-GPCMV challenge (one animal in group 1; one animal in group 2, and two animals in the control group), were included in the vaccine immunogenicity analyses, but were not included in the final pregnancy outcome analyses. Following delivery, pup tissue

was immediately harvested from dead pups, or within 72 hours post-delivery for live-born pups. There were 11, 10, and 10 evaluable pregnancies in the MVA-gB, MVA-gB and MVA-GP83, and unvaccinated groups, respectively.

2.5 ELISA, western blot, antibodies, and INF- γ ELISPOT

GPCMV-specific serum IgG titers were determined by ELISA (Figure 1) and neutralization (Figure 2) assays. Previously described protocols[36] were utilized to generate ELISA antigen, with some modifications. GPL cells were inoculated with GPCMV and antigen purified at 7 days post-inoculation by subjecting supernatants to gradient centrifugation as described elsewhere[33] to purify viral particles. Aliquots of 100 ng/well were utilized for ELISA assay using serial two-fold dilutions of serum. A peroxidase-conjugated rabbit anti-guinea pig antibody was used as a secondary antibody (Accurate Scientific, Westbury, NJ) following the manufacturer's specifications. ELISA titer was defined as that dilution of serum that produced an absorbance of >0.1 following addition of substrate and was twice the absorbance of that noted against an identical amount of control antigen purified from uninfected GPL cells. The eGFP-tagged recombinant vJZ848 virus was used for neutralization assays. Polyclonal anti-GPCMV serum used as a control for these assays was prepared as described previously[31].

ELISA responses were confirmed by western blot assay (Figure 3) using polyclonal anti-GPCMV antisera; a rabbit monoclonal antibody targeting GP83; and a mouse anti-GPCMV gB monoclonal, IE3-21[13]. GPCMV particles were subjected to SDS-PAGE and membranes processed as previously described[27]. Antibody binding was detected using enhanced chemiluminescence. As a control for the GP83-specificity of the antibody response, a GST fusion protein expressing GP83 I₄₈₇ through Q₅₆₅ was used as a target antigen.

Methods for study of interferon- γ ELISPOT studies of cell-mediated immune responses in immunized animals are described in Appendix A (Supplementary Data).

2.6 Real-time PCR analysis

Maternal blood was obtained on day 7 post-challenge with SG-GPCMV for real-time PCR as described previously[32]. Data were analyzed with the LightCycler Data Analysis Software (version 1.5; Roche) using standard curves generated by serial dilutions of a GP83 plasmid at known concentrations. Viral load (Figure 4) was expressed as genome copies per ml of blood or, for tissue (Figure 5), genome copies per mg of tissue. For blood, the limit of sensitivity for detection in this assay was 200 copies/ml. For tissue, the limit of detection of the PCR assay (based on extraction of 0.1 g homogenized tissue) was 4 genome copies/reaction (corresponding to ~ 2 genomes/mg tissue). For statistical comparison, tissue samples that were negative in the PCR assay were assigned a value of 1 copy/mg.

2.7 Statistical analyses

GraphPad Prism (version 6.0) was used for statistical analysis. Pup mortality and transmission were compared using Fisher's exact test with one-sided comparisons. Pup weights were compared with Dunn's multiple comparisons. Antibody titers were compared

using Mann-Whitney (for 2 group comparisons) and Kruskal-Wallis (for 3 group comparisons). Parametric data sets included INF- γ assays, and viral load measurements in blood and pup tissue, and these were compared using ANOVA followed by Tukey's multiple comparison test.

3. Results

3.1 Immune response to immunizations

Both monovalent MVA-gB and MVA-gB/GP83 vaccine proved to be immunogenic, eliciting anti-GPCMV ELISA responses and virus-neutralizing responses. However, there was a significantly reduced ELISA response in the combination vaccine group compared to the monovalent MVA-gB group. ELISA responses (mean and SEM) at 30 days following each dose of vaccine were: $3.0 \pm 0.2 \log_{10}$ (dose 1), $4.2 \pm 0.1 \log_{10}$ (dose 2) and $4.3 \pm 0.1 \log_{10}$ (dose 3) for the MVA-gB monovalent vaccine, and $2.5 \pm 0.1 \log_{10}$ (dose 1), $3.6 \pm 0.1 \log_{10}$ (dose 2) and $3.7 \pm 0.1 \log_{10}$ (dose 3) for the MVA-gB/GP83 vaccine group. The differences after dose 2 and dose 3 were highly significant between the two vaccine groups ($p < 0.001$; Figure 1). ELISA antibody was not detected in any unvaccinated controls (data not shown).

Neutralization activity of post-vaccine guinea pig serum was measured against an eGFP-expressing recombinant GPCMV, vJZ848. Consistent with anti-GPCMV ELISA data, dams in the MVA-gB group engendered a higher neutralizing response, with a mean $\log_{10}$ neutralization titer of 2.9 ± 0.1 , compared to 2.3 ± 0.1 in the MVA-gB/GP83 group ($p < 0.001$; Fig. 2).

To confirm that vaccination engendered antibody responses that were immunoreactive with gB and/or GP83, western assays were performed using purified virion particles (and, for animals in the gB/GP83 group, a GST-GP83 fusion protein). These studies (Fig. 3) demonstrated that immunized guinea pigs generated antibodies reactive with gB and GP83 proteins.

GP-83 specific ELISPOT responses were observed in a subset of dams (3/group) from each group. There was a trend towards increased numbers of IFN- γ producing splenocytes following gB/GP83 vaccination, compared to gB vaccine or SG-GPCMV only (control dams; Appendix A).

3.2 Pregnancy outcomes after GPCMV challenge

A total of 24 female guinea pigs were immunized with either MVA-gB, or the combination MVA-gB/GP83 vaccine ($n = 12$ per group). Controls were unimmunized ($n = 12$). Pregnancy was established in all animals, with the exception of one guinea pig in the combination MVA-gB/GP83 vaccine group. All other dams were challenged with SG-GPCMV. Three dams gave birth < 7 days following challenge and were excluded from the mortality and transmission analyses. For the remaining 31 evaluable pregnancy outcomes, there were 84 live-born pups and 34 dead pups (29% mortality). Control dams had significantly higher pup mortality (24/38, 63%) than vaccinated dams (combined 10/80, 12.5%; $p < 0.0001$, Fisher's exact test). For individual vaccine group compared to controls,

differences in mortality were highly significant. Mortality for the gB group was 5/44 (11.4%) and for the gB/GP83 group, 5/36 (13.8%; both $p < 0.0001$ vs. control). Control pups also had significantly lower mean birth weights (Table 1). The mean birth weight of pups born to evaluable dams in the MVA-gB group was 87.3 g, and 93.4 g in the MVA-gB/GP83 vaccine group. In contrast, mean pup birth weight was 55.7 g in controls ($p < 0.0001$ compared to both vaccine groups, Kruskal-Wallis and Dunn multiple comparisons).

3.3 Maternal and pup viral load analyses and congenital GPCMV infection transmission

After SG-GPCMV challenge, blood viral load in dams was examined by real-time qPCR. Monovalent gB vaccination resulted in a five-fold reduction, and gB/GP83 vaccine a two-fold, the magnitude of day 7 DNAemia compared to controls. This difference was significant or the gB vaccine group ($5.5 \pm 0.1 \log_{10}$ genomes/ml), compared to unvaccinated controls (viral load $6.3 \pm 0.2 \log_{10}$ genomes/ml; $p = 0.02$; Fig. 4).

To compare rates of congenital GPCMV transmission and viral load in infected pups between vaccine and control groups, real-time PCR was performed on extracted liver, lung, and spleen from live-born and stillborn pups. Examination of tissue homogenates following completion of pregnancy demonstrated that 27/34 (79.4%) of pups from the control group had evidence of congenital GPCMV transmission. By comparison, there was a significant reduction in congenital transmission in pups born to dams in the gB vaccine group (26/44, 59.1%; $p < 0.05$, Fisher exact test), but not in the gB/GP83 vaccine group (25/36, 69.4%, $p = \text{NS}$ compared to controls; Table 1). Viral load was reduced by at least 10-fold in both vaccination groups compared to controls (Fig. 5). Mean viral load $\pm$ SEM in pup tissue from the gB group was 0.8 ± 0.1 , 1.1 ± 0.2 , and $0.5 \pm 0.1 \log_{10}$ genomes/mg, for spleen, lung, and liver tissue, respectively. For the gB/GP83 vaccine group, viral load was 1.0 ± 0.2 , 1.5 ± 0.2 , and $0.9 \pm 0.2 \log_{10}$ genomes/mg for these tissues. In controls, viral load was 1.6 ± 0.3 , 1.9 ± 0.3 , and $2.0 \pm 0.3 \log_{10}$ genomes/mg in spleen, lung, and liver, respectively. Control pups had significantly higher viral load compared to pups in the gB group for spleen ($p < 0.01$) and liver ($p < 0.0001$), and when compared to pups from the gB/GP83 vaccine group for liver tissue ($p < 0.001$).

Discussion

In this report, we describe the use of an MVA-vectored approach to develop and test vaccines against congenital CMV infection, using a well-established guinea pig model. Previous work in this model demonstrated a range of 56% - 83% reduction in congenital GPCMV transmission using a variety of subunit gB vaccines[13]. A GP83 vaccine was also evaluated in the GPCMV model using an alphavirus replicon as a delivery system. This study demonstrated a 45% reduction in congenital infection, and a reduction in mortality from 57% in controls to 13% in the GP83 vaccine group, similar to the findings in the current study[25]. However, no strategy evaluated to date in the GPCMV model has evaluated a subunit approach in which gB and GP83 subunit vaccines were administered simultaneously. It was therefore of considerable interest to compare a monovalent vaccine strategy, targeting gB, with a combined gB/GP83 vaccine, to examine whether the

combination of the two immunogens could confer synergistic protection against mortality and vertical transmission.

The protection studies summarized in this report examined MVA as the vaccine vector. Although MVA can infect and replicate DNA efficiently, it is essentially avirulent and unable to produce infectious progeny[41], making it a desirable viral vector for vaccine development. Several studies have examined MVA as a vector for subunit CMV vaccination. A recombinant MVA expressing both full-length pp65 and exon4 of the HCMV IE1 induced a robust primary cell-mediated response to both antigens in HLA A2.1 transgenic mice[39]. MVA-vectored vaccines in the RhCMV model suggested that the combination of immune targets is a useful strategy, since a trivalent vaccine consisting of gB, pp65 and IE1 was capable of reducing viral loads in saliva (compared to control monkeys) by 1 to 3 orders of magnitude [42], and demonstrated statistically greater reductions than gB vaccine administered alone.

Surprisingly, our study demonstrated significantly reduced immunogenicity for the MVA gB vaccine when it was co-administered with GP83 vaccine. The molecular basis for the apparent interference between gB and GP83 is unclear. HCMV UL83 protein is capable of several immune modulation functions, include the blocking of the induction of interferon-response genes[43], primarily through modulation of the interferon response factor 3, IRF-3[44]. Conceivably, expression of the GP83 homolog via vectored vaccination may have lead to down-regulation of interferon-response genes, in the process reducing the immune response to gB vaccine. There was possible evidence of interference between HCMV gB and pp65 plasmid immunization in a preclinical study in rabbits which revealed, upon high-dose inoculation, that the combination of both plasmids results in a 20% reduction in gB antibody titer compared to monovalent gB-only[45]. Although anti-vector immunity has been proposed as a concern that could limit the effectiveness of MVA-vectored vaccines, studies in both the murine model and rhesus model do not demonstrate any significant impact on immune response following serial immunization[42]. Since there was evidence of reduced immunogenicity after a single dose of combination gB/GP83 vaccine in our study, vaccine interference, and not anti-vector immunity, may be responsible for reduced ELISA titers in the combination group. In future studies, the inclusion of an empty MVA will serve as a useful control group for this possibility. Although the fact that the target antigen used for the ELISA assay (containing both gB and GP83) complicates these analyses, the reduction in the neutralizing response in the combination group compared to the gB-only group further suggests interference between GP83 and gB immunogens. This reduction in neutralizing response did not impact the magnitude of protection, possibly because GP83 contributes to protection[25], compensating for reductions in the anti-gB response. Future studies using more precise anti-specific ELISA will be required to sort out the relative contributions of anti-gB and anti-GP83 to protection in this model.

In summary, although the basis for interference between gB and GP83 is unclear, the known role of HCMV UL83 in immune modulation may deserve further scrutiny in future subunit vaccine studies. In spite of robust ELISA and neutralization responses, vertical transmission of GPCMV still occurred in 59% of pups, and, surprisingly, no augmentation of protection

was observed by inclusion of GP83 – a known T cell target capable of inducing modest protection in a previous monovalent vaccine study[25] – in a bivalent MVA-vectored vaccine. Other T-cell targets may be required in a multivalent subunit CMV vaccine. In a recent report in RhCMV, it was shown that the pp65 homolog elicited a rapid immune response that controls viremia, but that pp65-specific T cell responses were not sufficient to recapitulate the level of protective immunity generated by actual viral infection, leading these authors to suggest that sole use of pp65 as a subunit vaccine candidate may be suboptimal[46]. Further study of MVA vectored vaccines, which have recently been shown to be safe and immunogenic for other viral pathogens[47], is warranted for CMV. The CMV PC proteins merit particular focus, given the ability of antibodies targeting the PC to block infection of epithelial and endothelial cells[48-50], desirable traits in a CMV vaccine. Future studies of an MVA-vectored GPCMV PC vaccine could provide insight into the optimal effectors of protection against transplacental transmission in this model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- ☐ Guinea pig CMV gB and pGP83 were expressed in vaccinia virus Ankara (MVA).
- ☐ Animals were vaccinated with gB (monovalent group), or gB/GP83 (bivalent group).
- ☐ Inclusion of GP83 in a bivalent vaccine interfered with the immune response to gB.
- ☐ Both monovalent and bivalent vaccine protected against pup mortality.
- ☐ Bivalent vaccine did not improve pup outcome compared to gB monovalent vaccine.

Figure 1. Post-vaccine anti-GPCMV IgG ELISA titers

Serum samples were collected from young female Hartley guinea pigs at 30 day intervals following each of a 3-dose vaccine series with MVA-gB alone (n=12) or combination MVA-gB/GP83 vaccines (n=12). Log₁₀ mean ELISA titers with SEM are shown in vaccinated animals, and data corresponding to each bleed is summarized in the text. Mean IgG titers were significantly higher in the MVA-gB group compared to combination MVA-gB/GP83 vaccines group after dose 2 and dose 3 (*p < 0.001, Mann-Whitney). ELISA antibodies to GPCMV viral particles were undetectable for all bleeds in unvaccinated control animals (n=12, data not shown).

Serum was collected 30 days following the final dose of each 3-dose vaccine series, from which neutralization titers were measured in GPL cells against the recombinant GPCMV, vJZ848. The highest neutralizing response was elicited in serum from the MVA-gB group, with a log₁₀ mean ELISA $\pm$ SEM of 2.9 ± 0.1 , compared to 2.3 ± 0.1 in the combination MVA-gB/GP83 group (*p<0.001 gB v. gB/GP83, Mann-Whitney). Neutralizing activity was undetectable in the unvaccinated control group (data not shown).

Figure 3. Western blot analysis of antibody response following vaccination

Guinea pig sera collected prior to vaccination and 30 days following the 3rd vaccine dose from animals in each vaccine group was used to confirm immunoreactivity by western blotting. Results from one representative preimmune animal and from one animal from each vaccine group are shown. *A*, The antigen target in this series of western blots was GPCMV viral particles. Preimmune serum (“Pre”) did not demonstrate immunoreactivity. MVA-gB post-vaccine serum resulted in a band at ~58 kDa, consistent with the M_r of gB protein (carboxy-terminal subunit). An anti-gB monoclonal antibody (gB moab) recognizing the 58 kDa moiety of GPCMV gB produced a similar band when probed against viral particles [arrowhead; 13]. MVA-gB/GP83-vaccinate serum produced bands at both ~58 and ~65 kDa, corresponding with gB and GP83 proteins, respectively. The ~65 kDa band is noted with a rabbit GP83 moab [arrow]. *B*, The antigen target in this series of western blots was a GST-GP83 fusion protein with a predicted M_r ~36 kDa. As expected, preimmune (pre) and MVA-gB immunized sera failed to detect the fusion protein. Both serum from MVA-gB/GP83 vaccinated animals, and GP83 moab, detect a band at ~36 kDa corresponding to GST-GP83 fusion protein (arrowhead).

Figure 4. Mean day 7 post-challenge dam blood GPCMV loads by qPCR

Dams were challenged at midgestation with SG-GPCMV, bled at day 7 post-infection, and DNA isolated for qPCR. All dams with evaluable pregnancies in both vaccine groups and the control group (n=31) had DNAemia at day 7 following challenge. MVA-gB monovalent-vaccinated dams had log transformed mean whole blood viral load of $5.5 (\pm 0.1, \text{SEM})$ genomes/ml. Dams from the combined MVA-gB/GP83 vaccine group had a $\log_{10}$ mean viral load of $5.6 (\pm 0.3, \text{SEM})$ genomes/ml. By comparison, the control dams had a $\log_{10}$ mean viral load of $6.3 (\pm 0.2, \text{SEM})$ genomes/ml. Only the MVA-gB monovalent vaccine group demonstrated a statistically significant reduction in whole blood viral load compared to controls ($p=0.02$, one-way ANOVA with Tukey's multiple comparisons test).

Figure 5. GPCMV viral load in pups

Pup organs were removed from both dead and live pups and DNA extracted for real-time qPCR. Values for log transformed mean tissue viral loads in copies/mg are noted in text. Viral loads were significantly reduced in organs harvested from pups in the gB monovalent vaccine group (solid black bars) compared to controls (solid grey bars) for both spleen and liver (* $p < 0.01$, *** $p < 0.0001$, respectively). Viral load in tissues harvested from the gB/GP83 vaccine group (striped bars) was significantly lower than that in corresponding control pup tissues for liver tissue only (** $p < 0.001$; ANOVA with Tukey's multiple comparisons test).

Table 1

Pup Mortality and Congenital Transmission in MVA-Vector Vaccinated Dams Following Pregnancy Challenge.

Vaccine Group	Pup mortality		Transmission rate		Mean pup weight, g
	Litters, no.	Deaths/n (%)	Litters tested, no.	PCR positive/n (%)	
MVA-gB	11	5/44 (11.4)	11	26/44 (59.1)	87.3
MVA-gB/GP83	10	5/36 (13.8)	10	25/36 (69.4)	93.4
Control	10	24/38 (60.5)	9	27/34 [§] (79.4)	55.7

Mortality was reduced in each vaccine group v. control, $p < 0.0001$, Fisher's exact test. Transmission was reduced in the gB group compared to controls, ($p = 0.047$ gB v. control, Fisher's exact test). The mean pup weights were significantly reduced in controls compared to each vaccine group ($p < 0.0001$ Kruskal-Wallis, Dunn's multiple comparison).

[§]Tissues from four pups in this group were unavailable for PCR testing.