Glycoprotein B (gB) vaccines adjuvanted with AS01 or AS02 protect female guinea pigs against cytomegalovirus (CMV) viremia and offspring mortality in a CMV-challenge model

Mark R. Schleiss[a,*], **K. Yeon Choi[a,1]**, **Jodi Anderson[a]**, **Janine Gessner Mash[a]**, **Martine Wettendorff[b]**, **Sally Mossman[b]**, and **Marc Van Damme[b]**

Mark R. Schleiss: schleiss@umn.edu; K. Yeon Choi: kchoi@tamhsc.edu; Jodi Anderson: kaise024@umn.edu; Janine Gessner Mash: jmash@natera.com; Martine Wettendorff: MARTINE.WETTENDORFF@GSK.COM; Sally Mossman: SALLY.P.MOSSMAN@GSK.COM; Marc Van Damme: MARC.VAN-DAMME@GSK.COM

[a]Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota, Minneapolis

[b]GlaxoSmithKline Vaccines, Rue de l'Institut, 89, B-1330 Rixensart, Belgium

Abstract

The transmission of cytomegalovirus (CMV) from mother to fetus can give rise to severe neurodevelopment defects in newborns. One strategy to prevent these congenital defects is prophylactic vaccination in young women. A candidate vaccine antigen is glycoprotein B (gB). This antigen is abundant on the virion surface and is a major target of neutralization responses in human infections. Here, we have evaluated in a challenge model of congenital guinea pig CMV (GPCMV) infection, GPCMV-gB vaccines formulated with the clinically-relevant Adjuvant Systems AS01$_B$ and AS02$_V$, or with Freund's adjuvant (FA). Fifty-two GPCMV-seronegative female guinea pigs were administered three vaccine doses before being mated. GPCMV-challenge was performed at Day 45 of pregnancy (of an estimated 65 day gestation). Pup mortality rates in the gB/AS01$_B$, gB/AS02$_V$, and gB/FA groups were 24% (8/34), 10% (4/39) and 36% (12/33),

*Corresponding Author: Dr. Mark Schleiss, Division of Infectious Diseases, University of Minnesota Medical School, Department of Pediatrics, Division of Infectious Diseases, Center for Infectious Diseases and Microbiology Translational Research, 420 Delaware Street SE, MMC 296, Minneapolis, Minnesota 55455. Phone: 612-624-1112, Fax 612-624-8927, schleiss@umn.edu.
[1]Present address: Texas A&M Health Science Center, Microbial & Molecular Pathogenesis, 407 Reynolds Medical Building, College Station, TX 77845

Conflict Of Interest

All authors completed the ICMJE Form for Disclosure of Potential Conflicts of Interest and declared that the following interests are relevant to the submitted work. Martine Wettendorff, Sally Mossman and Marc Van Damme are employees of the GlaxoSmithKline (GSK) group of companies; Martine Wettendorff and Sally Mossman report ownership of shares or options to shares in GSK.

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respectively, and in the unvaccinated control group was 65% (37/57). Hence, efficacies against pup mortality were estimated at 64%, 84% and 44% for gB/AS01$_B$ ($p<0.001$), gB/AS02$_V$ ($p<0.001$) and gB/FA ($p=0.014$), respectively. Efficacies against GPCMV viremia (i.e DNAemia, detected by PCR) were estimated at 88%, 68% and 25% for the same vaccines, respectively, but were only significant for gB/AS01$_B$ ($p<0.001$), and gB/AS02$_V$ ($p=0.002$). In dams with viremia, viral load was approximately 6-fold lower with vaccination than without. All vaccines were highly immunogenic after two and three doses. In light of these results and of other results of AS01-adjuvanted vaccines in clinical development, vaccine immunogenicity was further explored using human CMV-derived gB antigen adjuvanted with either AS01$_B$ or the related formulation AS01$_E$. Both adjuvanted vaccines were highly immunogenic after two doses, in contrast to the lower immunogenicity of the unadjuvanted vaccine. In conclusion, the protective efficacy and immunogenicity of adjuvanted vaccines in this guinea pig model are supportive of investigating gB/AS01 and gB/AS02 in the clinic.

Keywords

Cytomegalovirus; Guinea pig cytomegalovirus; Cytomegalovirus vaccine; Glycoprotein B; Adjuvant; Vaccine efficacy; Guinea pig challenge model

1. Introduction

Maternal infection with human cytomegalovirus (HCMV) during pregnancy occasionally causes severe disease in newborns, and can lead to neurodevelopmental sequelae and sensorineural deafness [1]. Congenital HCMV infection complicates an estimated 40 000 pregnancies annually in the United States [2]. A vaccine capable of protecting newborns from the sequelae of congenital HCMV infection is a major public health priority [3]. However, it is not clear what would constitute an optimal vaccine. Subunit vaccines that target the major envelope glycoprotein gB (gpUL55) have been evaluated for immunogenicity and safety in clinical trials [4–7]. Although there has been no efficacy evaluation of an HCMV vaccine for prevention of congenital infection to date, a clinical study has demonstrated the proof-of-concept of protection against primary CMV infection in women with an adjuvanted gB-based formulation [5].

Efficacy evaluations of vaccines against homologous, species-specific CMVs can be performed using the guinea pig cytomegalovirus (GPCMV) model [8;9]. In contrast to the CMVs of most small animals, CMV in guinea pigs causes maternal viremia and disease, can cross the placenta, and can infect fetuses resulting in still-born offspring [10;11]. Furthermore the anatomy of the guinea pig placenta more closely resembles the human placenta than in other small laboratory animals [12–15]. Preconception immunization of guinea pigs with recombinant GPCMV gB, administered either as a DNA vaccine or purified baculovirus-expressed protein vaccine, has been shown to protect against GPCMV challenge made during pregnancy, as measured by the reduction of pup mortality and infection, and by the reduction of maternal and fetal viral load [16;17]. Moreover, protection conferred by gB subunit vaccination has been found to be highly dependent upon the adjuvant employed. Lower rates of maternal GPCMV-viral load and maternal-fetal transmission have been observed in dams immunized with gB formulated with Freund's

adjuvant (gB/FA) compared with dams immunized with gB formulated with alum (gB/alum) [17]. Although effective in non-clinical vaccines, FA is not a clinically-relevant adjuvant, and observations regarding the efficacy of this adjuvant are of limited applicability to human clinical trials. In contrast, GlaxoSmithKline's (GSK's) proprietary Adjuvant Systems $AS01_B$, $AS01_E$ and $AS02_V$ are clinically relevant and have been included in a variety of candidate vaccines evaluated up to Phase III [18–20]. These three Adjuvant Systems contain the immunostimulants 3-O-desacyl-4′-monophosphoryl lipid A (MPL) and QS-21 and can promote both antibody and cell-mediated immune responses to subunit antigens [18]. Therefore we have performed two preclinical studies to evaluate gB antigens formulated with these Adjuvant Systems.

In the first study, GPCMV gB vaccines formulated with $AS01_B$ or $AS02_V$, were evaluated in comparison with gB/FA, in the guinea pig challenge model of congenital CMV infection. Vaccine efficacies were estimated from maternal protection against GPCMV viremia and offspring mortality, in addition to measuring vaccine immunogenicity. In the second study, the immunogenicity of the human (H)CMV-derived gB antigen (hgB) formulated with $AS01_B$ or its related formulation $AS01_E$ was evaluated in guinea pigs.

2. Materials and methods

2.1. Animal husbandry

Study 1 was performed at the University of Minnesota (Minneapolis, MN, USA). Inbred adult strain-2 guinea pigs for preparation of salivary gland passaged-GPCMV stocks were obtained from the University of Minnesota. Age-matched young female and breeder male Hartley guinea pigs were obtained from Harlan Laboratories (Indianapolis, IN, USA). All animals were confirmed to be GPCMV-seronegative by ELISA [17]. Animals were housed under conditions approved by the American Association of Accreditation of Laboratory Animal Care, in accordance with institutional animal use committee policies at the University of Minnesota. Study 2 was performed using age-matched female Hartley guinea pigs (obtained from Charles River, Lyon, France) at GSK Vaccines (Rixensart, Belgium) in accordance with the Belgian national guidelines for animal experimentation.

2.2. CMV stocks

GPCMV (strain no. 22122, ATCC VR682) used in neutralization assays was propagated in guinea pig fibroblast lung cell cultures (GPL; ATCC CCL 158) maintained 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 7.5% $NaHCO_3$ (Gibco-BRL). Salivary gland-passaged GPCMV stocks (SG virus) used for animal challenge studies were prepared by sequential passage in strain-2 guinea pigs. HCMV (AD169 strain) from Novasep was propagated in MRC5 fibroblast cell line culture (obtained from Novasep).

2.3. Vaccines

Viral stocks of recombinant baculovirus expressing GPCMV gB were maintained in *Spondoptera frugiperda* (Sf9) cell cultures. GPCMV gB was produced in *Trichoplusia ni* cells infected at high multiplicity with recombinant baculovirus. Infected-cell lysates were

subjected to lectin column chromatography and elution as previously described [17;21]. Although one-step purification by lectin column chromatography did not purify GPCMV gB to complete homogeneity, the preparation was highly enriched for gB, as gauged by SDS-PAGE and western blot analysis (data not shown) [17;21]. The HCMV gB (hgB) antigen, a recombinant fusion protein consisting of the extracellular domain of the native gB (AD169 strain) and peptide sequences from glycoprotein gD of Herpes Simplex virus 2 (HSV2), was manufactured through a vaccine development and production agreement between GSK Biologicals SA and Henogen SA, Belgium. The hgB antigen had been purified to >95% homogeneity from transfected Chinese Hamster Ovary cells. One 1 mL vaccine dose contained 50 μg GPCMV gB antigen, or 15 μg of hgB antigen. The rationale for selecting the antigen quantities was based upon previously published and unpublished experience in the (independent) evaluations of immunogenicities and/or protective efficacies following vaccinations with these antigens [17;21]. $AS01_B$ and $AS02_V$ in one vaccine dose each contained 50 μg of MPL and 50 μg of QS-21, whereas $AS01_E$ in one vaccine dose contained 25 μg of MPL and 25 μg of QS-21. AS01 variants also contain liposome whereas $AS02_V$ is an oil-in-water emulsion. $AS01_B$, $AS01_E$ and $AS02_V$ were obtained from GSK Vaccines (Rixensart, Belgium). QS-21 was from Antigenics Inc, a wholly owned subsidiary of Agenus Inc., Lexington, MA, USA. For the gB/FA vaccination regimen, complete FA was used in the first vaccine dose and incomplete FA in the second and third doses. Standard complete and incomplete FA were obtained from Sigma/Aldrich. Adjuvants were mixed with antigen immediately prior to injection.

2.4. Experimental design

For Study 1, female Hartley guinea pigs were immunized with 3 subcutaneous doses, at monthly intervals, as previously described [17]. Blood samples were taken immediately prior to the second and third immunizations, 30 days after the third immunization, and 10 days after viral challenge. Mating was performed immediately after the third vaccine dose by co-caging one female and one male guinea pig for 2 weeks and the day that mating occurred was designated as pregnancy Day 0. GPCMV challenge was performed on ~45-day pregnant animals by subcutaneous injection of 1.5×10^5 pfu of GPCMV. Animals were observed daily up to parturition to determine pregnancy outcome, as previously described [16;17]. For Study 2, guinea pigs were intramuscularly injected twice, 28 days apart. Blood was taken 28 and 14 days after the first and the second immunization, respectively. In both studies, serum samples were prepared from blood using routine methodology.

2.5. ELISA, neutralization, and antibody avidity analyses

For Study 1, the ELISA was performed using plates coated with GPCMV antigens and a control antigen, as previously described [17]. ELISA titers were defined as the reciprocal of the highest dilution that produced an absorbance (OD_{450}) of at least 0.10, and twice the absorbance against the control antigen. GPCMV neutralizing titers were determined as previously described [17]. The avidity index (AI) of antibodies in a serum sample was determined from the ratio of the OD_{450} of GPCMV-bound antibodies (on plates coated with polypeptides extracted from GPCMV-infected fibroblasts) in the presence and absence of 4 M urea [22]. Antibodies were detected using the Radim diagnostic kit according to the manufacturer's specifications, with the exception that an HRP-conjugated rabbit anti-guinea

pig serum (Accurate Pharmaceuticals) was used as the secondary antibody. The AI was calculated as an average of values taken from those dilutions (1:160, 1:320, 1:640, and 1:1280) which gave measurements within the linear range of the ELISA assay (0.5–1.0 OD units) [22].

For Study 2, the ELISA was performed using HCMV gB as a coating antigen (0.4 μg/well). Anti-gB antibody titers were calculated in comparison with a reference pool of positive sera to which an arbitrary titer of 175000 ELISA units (EU)/mL had been attributed. The microneutralization assay was adapted from a previously published assay [23] and performed using MRC-5 cells as an infection substrate. After overnight incubation with a mixture of HCMV virus (30 $TCID_{50}$/50μl; strain AD169, Henogen SA, Belgium) and diluted serum samples, cells were fixed and virus infection was indicated by the detection of Immediate Early 1 protein (IE1) using a monoclonal antibody (MAB810R, Chemicon) and routine immunohistochemistry. The neutralizing titers were expressed as the reciprocal of the serum dilution inducing a reduction of 50% of infected cells relative to cells incubated with virus alone (calculation by point to point regression analysis).

2.6. Real-time PCR

Viremia was defined as the detection of GPCMV genomic DNA (DNAemia) and viral load was defined as the concentration of GPCMV genomic DNA in maternal blood, measured 10 days post-challenge. Maternal-fetal GPCMV transmission was confirmed by detection of GPCMV genomic DNA in the spleen and liver of necropsied pups. GPCMV genomic DNA was detected using quantitative real-time PCR. The following primers, based on the GPCMV gB gene sequence [24], were used: Forward primer, 5′-CTTCGTGGTTGAACGGG-3′; Reverse primer, 5′-GTAGTCGAAAGGACGTTGC-3′; Probe 1, 5′-TGGTGACCTTCGTTACCAATCCGTTTGGA-fluorescein; Probe 2, 5′-LC Red 640-CTTCGTGGTGTTCCTGTTCTGCGT-Phosphate. DNA was extracted from whole blood or (neonatal) liver/spleen homogenates using Roche MagNa Pure LC Total Nucleic Acid Isolation Kit according to manufacturer's instructions. The PCR reaction mixtures were prepared using the Lightcycler Fast Start Master hybridization probes (Roche Applied Sciences) supplemented with 2.5 mM $MgCl_2$, 0.5 μM primers and 0.2 μM probes. PCR was performed in the Lightcycler instrument. The limit of detection of the assay was consistently between 5 and 10 copies/reaction.

2.7. Statistical analyses

Statistical analyses used SAS software (SAS Institute Inc., NC, USA). The incidence of viremia in the dams was analyzed using Fisher's exact test. The incidence of mortality in the pups was analyzed using a generalized linear mixed model with binomial distribution (SASGLIMMX) because pup mortality events within a litter were considered as dependent events. Significance was ascribed to p-values < 0.05/3 (=0.017) using a Bonferroni correction to account for multiple comparisons with the control group. Parametric analyses of immunogenicity and PCR variables were performed on log_{10} transformed data. The Shapiro-Wilk test, Skewness and Kurtosis calculations were used to specify limits of acceptable normality. Differences were identified by ANOVA followed by Tukey's test. All comparisons were two-tailed. Significance was ascribed to p-values <0.05 (and in the case

of antibody titers and concentrations, to 2-fold differences). Titers and concentration values are described to two-significant figures in the text. Mean and standard deviation pup-weight calculations considered pup weights in a litter as dependent variables.

3. Results

3.1. Disposition

In Study 1, 52 guinea pigs completed the 3-dose vaccination course; 18 animals received gB/FA, 16 received gB/AS02$_V$, and 18 received gB/AS01$_B$. All vaccinated animals and eighteen unvaccinated controls were mated. The majority of animals in each group became pregnant (Figure 1A) and these animals were challenged with GPCMV, after ~45 days of the anticipated 65 day gestation period. Two animals miscarried in the gB/FA group and one in the gB/AS01$_B$ group. These two animals were not included in the pregnancy outcome analyses since miscarriage <7 days after viral challenge is not considered to be related to viral infection [17] and is compatible with the relatively high (~8.5%) spontaneous miscarriage rate in guinea pigs [9]. One animal in the gB/AS01$_B$ group died of an unexpected bowel perforation. This animal had GPCMV viraemia (1.9×10^5 GPCMV genome copies/mL) and carried four fetuses, all of which had no signs of GPCMV infection. For the remaining pregnant animals (9–14 per group), parturition occurred at least 7 days after GPCMV challenge.

In Study 2, 28 guinea pigs completed the two-dose vaccination course; three groups of eight animals received unadjuvanted HCMV gB (hgB) vaccine, hgB/AS01$_E$ and hgB/AS01$_B$, respectively, and four animals received PBS (Figure 1B).

3.2. Immunogenicity of GPCMV gB vaccines (Study 1)

GPCMV-specific antibody and neutralizing responses were detected in all vaccinated animals. GPCMV-specific antibody GMTs were (11–75-fold) higher in each vaccine group after Doses 2 and 3 compared with after Dose 1 (Figure 2). After Dose 1, the antibody GMTs in the gB/AS01$_B$ and gB/AS02$_V$ groups were lower than that in the gB/FA group ($p<0.05$), whereas after Dose 2, the GMT in the gB/AS01$_B$ was higher than that in the gB/FA group ($p=0.005$). Antibody GMTs were not significantly different between the three vaccine groups after Dose 3, nor were the GMTs significantly different when compared with those after Dose 2.

Geometric mean avidity indices (AIs) were 2.0–2.7-fold higher in each vaccine group after Doses 2 and 3 compared with after Dose 1, and suggested a degree of antibody affinity maturation after the second dose. The peak AIs were similar to the AI of 70% found with antibodies induced by GPCMV infection [25]. Geometric mean AIs were not significantly different between the three vaccine groups after each of the three doses.

GPCMV-neutralizing GMTs were 2.8–4.2-fold higher in each vaccine group after Dose 3 compared with after Dose 2 (Figure 2). After Dose 2, the GPCMV-neutralizing GMT in the gB/AS02$_V$ was higher than in the gB/FA group ($p=0.014$) whereas after Dose 3, the GMTs were not significantly different between the three vaccine groups.

3.3. Vaccine efficacy (Study 1)

Vaccine efficacy was estimated from two outcomes: protection against GPCMV-lethality in the offspring and protection against GPCMV-viremia in the dams (Tables 1 and 2).

Sixty four percent (9/14) of litters born to control (GPCMV-infected) dams included dead pups (Table 1). In total, 65% (37/57) of these pups were dead. In the three vaccine groups overall, 41% (12/29) of litters included dead pups. In the gB/FA, gB/AS02$_V$ and gB/AS01$_B$ groups, 36% (12/33), 10% (4/39) and 24% (8/34) of pups were dead, giving estimates of vaccine efficacies against pup mortality as 44% (p=0.014), 84% (p<0.001) and 64% (p<0.001), respectively (Table 1).

One live pup and one dead pup (both from the control group) could not be retrieved for PCR analysis. Hence, a total of 101 live pups and 60 dead pups were evaluated for congenital GPCMV transmission (see Table 1). In control and vaccinated dams, GPCMV was detected (by PCR in the liver or spleen) in a higher proportion of dead pups (82%; 49/60) than of live pups (45%; 45/101), suggesting that congenital GPCMV transmission was associated with pup mortality.

At Day 10 post-challenge, 93% (13/14) of the control group dams had GPCMV viremia, whereas in the gB/FA, gB/AS02$_V$ and gB/AS01$_B$ groups, 70% (7/10), 30% (3/10) and 11% (1/9) of the dams had viremia (Table 2). Hence gB/AS02$_V$ and gB/AS01$_B$ efficacies against viremia were estimated at 68% (p=0.002) and 88% (p<0.001), respectively (Table 2). However, gB/FA efficacy estimate of 25% was not significantly different from control (*p*=0.272).

In the dams with viremia, the geometric mean concentration (GMC) of GPCMV genomes (i.e. viral load) in the combined three vaccine groups was 1.1×10^5 copies/mL (0.9, 1.7 and 0.9 copies/mL in the gB/FA, gB/AS02$_V$ and gB/AS01$_B$ groups, respectively; Table 2) and was 6.4-fold lower (p<0.001) than that in the control group (6.8×10^5 copies/mL). In the combined three vaccine groups, the GPCMV-neutralizing geometric mean titer (GMT) in the dams with viremia was 1800 but was not significantly different (*p*=0.125) from that (3000) in the dams without viremia (Supplementary Figure 1). No differences were identified between vaccine groups in antibody GMTs or geometric mean antibody avidities (Supplementary Figure 1).

In vaccinated dams without viremia, 78% (14/18) of litters included at least one GPCMV+ pup (Table 2) and 33% (6/18) of the litters included at least one dead pup. In vaccinated dams with viremia, all 11 litters included GPCMV+ pups (Table 2) and 55% (6/11) of the litters included dead pups. However, only 69% (9/13) of the litters from the control dams with viremia included GPCMV+ pups.

3.4. Immunogenicity of HCMV (h)gB vaccines (Study 2)

In Study 2, AS01$_B$ and AS01$_E$ (which contains half the quantities of MPL and QS-21 than AS01$_B$) were evaluated in hgB vaccines in light of the GPCMV efficacy results and of other results of AS01-adjuvanted vaccines in clinical development [18]. HCMV (hgB)-specific responses were observed in all vaccinated animals (Figure 3). Both hgB/AS01$_E$ and hgB/

$AS01_B$ induced higher human hgB-specific antibody GMCs after each of the two doses than those induced by hgB alone ($p<0.001$). $hgB/AS01_B$ also induced higher HCMV-neutralizing GMTs after Dose 2 than that induced by hgB alone ($p<0.05$). The antibody GMCs and neutralizing GMTs induced by $hgB/AS01_E$ were not significantly different from those induced by $hgB/AS01_B$.

4. Discussion

In Study 1, three-dose administration of $gB/AS01_B$ or $gB/AS02_V$ before conception protected against guinea pig pup mortality and maternal viremia in response to GPCMV challenge during pregnancy, and gB/FA protected against pup mortality alone. The three vaccines were similarly immunogenic, all inducing relatively high avidity gB-specific antibody responses after two doses and GPCMV neutralization responses after three doses. Although less frequently observed in the vaccine groups than in the control group, viremia was detected in animals with gB-specific antibody responses. Other studies of gB vaccines in guinea pigs have suggested that high neutralization responses are associated with protection [16;17]. This association was also suggested but not clearly identified in this study, perhaps because the sample sizes were too small. Vaccine-induced cellular immunity may have also contributed to the protective vaccine efficacies [26] but this parameter was not assessed.

Congenital GPCMV infection appeared to be associated with pup mortality, although approximately one-half of the infected pups were alive at birth. Moreover, congenital GPCMV infections occurred in 78% of the vaccinated dams that did not develop detectable viremia. The assessment of viremia in dams at only one time point (Day 10 post-inoculation) is one possible limitation of this study. Nevertheless, immune responses in some of the vaccinated dams may not have been sufficient to clear the relatively high load of injected virus in time to prevent a transmission event from occurring. To what extent neutralizing antibodies were involved in this response is difficult to judge because neutralization of GPCMV maternal-fetal transmission may be more appropriately assessed with epithelial- or endothelial-cell based assays in addition to the fibroblast-based assay [27;28].

The demonstration that protection can be achieved with $gB/AS01_B$ and $gB/AS02v$, extends the previous observation that immunostimulatory adjuvants are required in a gB and AS02v in subunit vaccine formulation [17]. Moreover, the use of $AS01_B$, $AS01_E$ clinical trials of candidate malaria, hepatitis B and HIV vaccines has revealed that both antigen-specific humoral and cell-mediated immune responses are induced [29–32], and in the case of the candidate malaria vaccine, both types of response are associated with protection [31;33]. However, the evaluation of protective efficacy against HCMV is not feasible in the guinea pig challenge model, given the inability of HCMV to infect these animals. Immunogenicity was evaluated in the guinea pig though, and both HCMV-derived gB vaccines were immunogenic. Vaccines formulated with either $AS01_B$ or $AS01_E$ were more immunogenic than the unadjuvanted vaccine, supporting the combined use of HCMV-derived gB antigen and AS01 in clinical development. Moreover, $hgB/AS01_E$ was not significantly less immunogenic than $hgB/AS01_B$ suggesting that $AS01_E$, with its lower quantities of

immunostimulants, could be a more suitable formulation in humans. Indeed, a clinical trial with the candidate gB/AS01$_E$ subunit vaccine is now underway in healthy volunteers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

HCMV   human cytomegalovirus

GPCMV   guinea pig cytomegalovirus

gB   glycoprotein B antigen

hgB   human CMV-derived gB antigen

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Highlights

Cytomegalovirus (CMV) infection in pregnant women can cause birth defects.

Three gB antigen vaccines were evaluated in a pregnant-guinea pig CMV-challenge model.

Two vaccines also contained clinically-relevant Adjuvant Systems AS01 and AS02.

gB/AS01 and gB/AS02 offered protection against viremia in dams and mortality in pups.

Efficacy and immunogenicity results support clinical testing of gB/AS01 and gB/AS02.
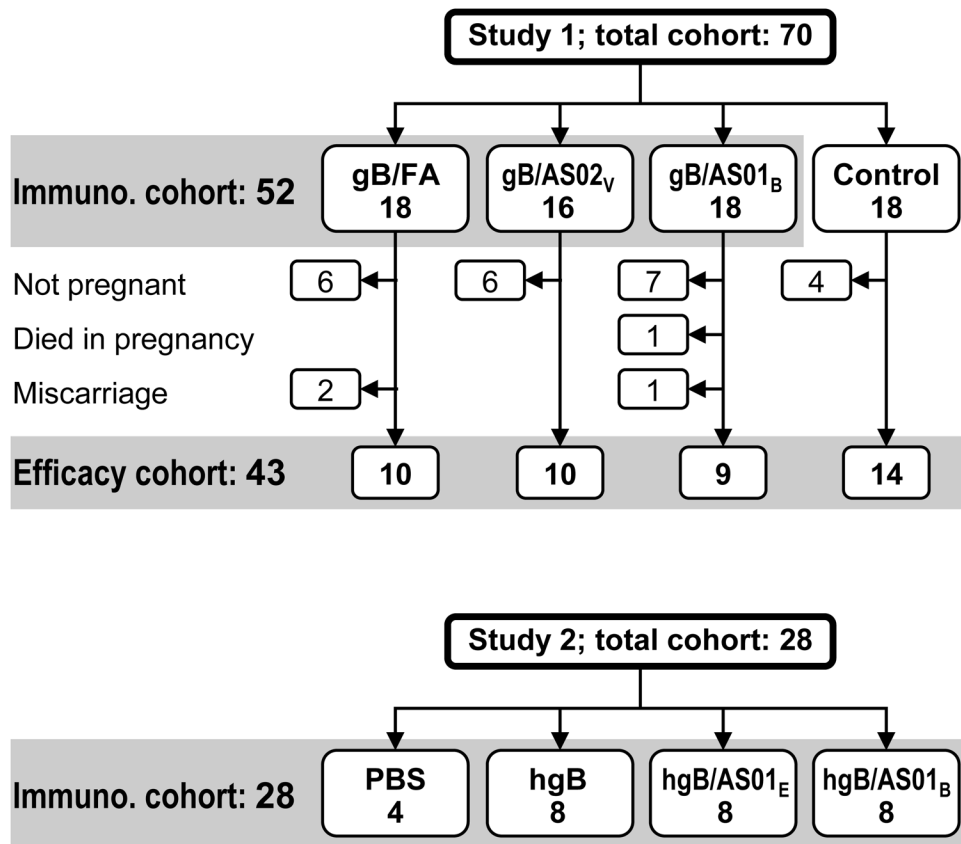
Figure 1. Animal allocation to different treatment groups and analysis cohorts in Study 1 and Study 2

In Study 1, the reason for the loss/removal of animals from the Efficacy cohort is described on the left side of the schematic.
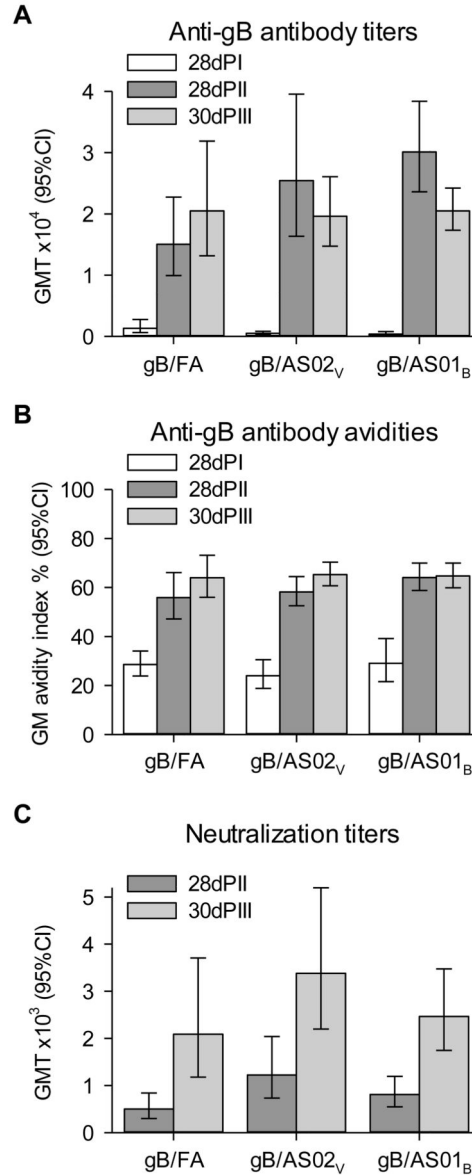
A

Anti-gB antibody titers

B

Anti-gB antibody avidities

C

Neutralization titers

Figure 2. Immunogenicity of gB vaccines

Guinea pigs were immunized with three doses of either gB/FA, gB/AS02$_V$ or gB/AS01$_B$ at monthly intervals. Geometric mean (GM) (A) anti-gB antibody titers (GMTs), (B) avidity indices and (C) GPCMV neutralizing titers in the immunogenicity cohort of Study 1. Immunogenicity was measured in samples taken at (A–C) 28 days post 1st immunization (28dPI); (A–C) 28 days post 2nd immunization (28dPII); and (A and B) 30 days post 3rd immunization (30dPIII). Error bars describe 95% confidence intervals (95% CIs). GPCMV-specific antibody GMTs were (11–75-fold) higher in each vaccine group after Doses 2 and 3 compared with after Dose 1, but were not significantly different between the three vaccine groups after Dose 3. GMTs were significantly different following Dose 3 than when compared with those following Dose 2 for each group.
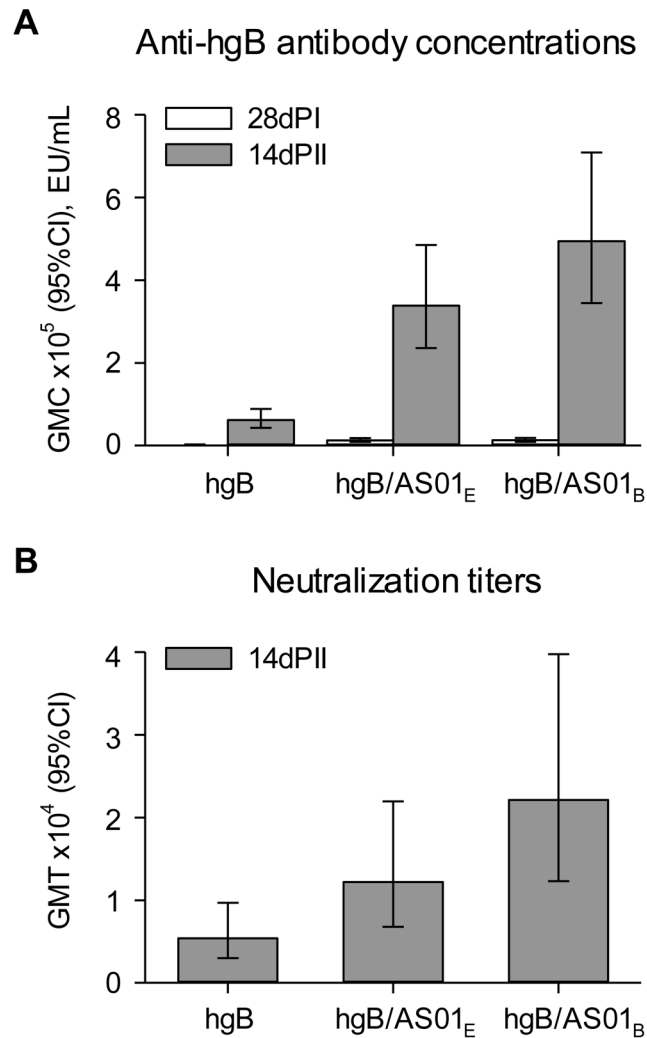
A

Anti-hgB antibody concentrations

B

Neutralization titers

Figure 3. Immunogenicity of hgB vaccines

Guinea pigs were immunized with two doses of either hgB (no adjuvant), hgB/AS01$_E$ or hgB/AS01$_B$ one month apart. Geometric mean anti-hgB antibody concentrations (GMCs) (A) and human CMV neutralizing titers (GMTs) (B) in the immunogenicity cohort of Study 2. Immunogenicity was measured in samples taken at, (A) 28 days post 1st immunization (28dPI); and (A and B) 14 days post 2nd immunization (14dPII). Error bars describe 95% confidence intervals (95% CIs).

Table 1

Vaccine efficacy evaluation with respect to pup mortality

| Group | No. litters | No. litters with dead pups | No. pups (N) | Live pups | | | Dead pups | | | % pup mortality (100 x nd/N) | Odds ratio (exact 95% CI) | VE[b], % | p-value |
				No.	Mean pup weight (SD), g	CMV+ (%)	No. (nd)	Mean pup weight (SD), g	CMV+ (%)				
control	14	9	57	20	107 (32)	6 (32[a])	37	98 (23)	30 (83[a])	65	-	-	-
gB/FA	10	5	33	21	115 (16)	14 (67)	12	88 (17)	10 (83)	36	0.31 (0.12–0.78)	44	0.014[c]
gB/AS02$_V$	10	3	39	35	116 (12)	16 (46)	4	114 (18)	3 (75)	10	0.06 (0.02–0.21)	84	<0.001[c]
gB/AS01$_B$	9	4	34	26	113 (14)	9 (35)	8	100 (24)	6 (75)	24	0.17 (0.06–0.45)	64	<0.001[c]

[a] One pup not included in the PCR analysis.

[b] Vaccine efficacy estimated from odds ratios.

[c] Significantly different from control (Generalized linear mixed model with binomial distribution followed by Student t with a Bonferroni correction for multiple comparisons, such that α=0.05/3=0.017).

No., number. N, total number of pups/group; nd, number of dead pups/group, CMV+, number of CMV-positive pups/group; CI, confidence interval; SD, standard deviation; VE, vaccine efficacy.

Table 2

Vaccine efficacy evaluation with respect to viremia

Group	No. dams, (N)	Viremia positive dams					Viremia negative dams			% viremia+ (100 x nv+/N)	Odds ratio (exact 95% CI)	VE, %	p-value
		No. (nv+)	GM, 10^5 CMV DNA copies/mL	Litters			No.	Litters					
				All live pups (≥ 1 CMV+ pup)	1 dead pup (≥ 1 CMV+ pup)			All live pups (≥ 1 CMV+ pup)	1 dead pup (≥ 1 CMV+ pup)				
control	14	13	6.8	5 (1)	8 (8)		1	0 (0)	1 (1)	93	-	-	-
gB/FA	10	7	0.9	3 (3)	4 (4)		3	2 (1)	1 (1)	70	0.180 (0.016–2.064)	25	0.272
gB/AS02$_V$	10	3	1.7	2 (2)	1 (1)		7	5 (5)	2 (1)	30	0.033 (0.0007–0.478)	68	0.002[a]
gB/AS01$_B$	9	1	0.9	0 (0)	1 (1)		8	5 (4)	3 (2)	11	0.0096 (0.0002–0.235)	88	<0.001[a]

[a] Significantly different from control (Fisher exact test with a Bonferroni correction for multiple comparisons, such that α=0.05/3=0.017).

No., number. N, total number of dams/group; nv+, number of viremia-positive dams/group; nv−, number of viremia-negative dams/group, 1 CMV+ pup, at least one CMV-positive pup/litter/group; viremia+, viremia-positive; GM, geometric mean; CI, confidence interval.; VE, vaccine efficacy.