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Supplementary Materials for

Maternal immunization confers protection against neonatal herpes simplex mortality and behavioral morbidity

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/11/487/eaau6039/DC1)

Data file S1 (Microsoft Excel format). Primary data.

Supplementary Materials:

Materials and Methods

Cells and viruses

The strains used in this study were HSV-1 17syn+ *(85)* and HSV-2 G *(86)*. Immunization studies were carried out using HSV-2 *dl*5-29, which lacks *UL5* and *UL29* and is derived from HSV-2 186 syn+ *(33)*. Virus stock preparation and the plaque assay were performed using Vero cells or V5-29 cells as described previously *(87, 88)*.

Neutralization Assays

We used two different neutralization assays for our experiments; plaque reduction neutralization (PRN) is a highly reproducible assay and was used for mouse samples. As the human samples were limited in quantity, we utilized a serum neutralization assay (SN) which requires less volume. SN assays were conducted by heat-inactivating cord serum samples at 56°C for 30 minutes and pre-incubating serial dilutions with 50 plaque-forming units (PFU) for one hour at 37°C. Samples were then incubated on a monolayer of Vero cells for 48 hours and assessed for cytopathic effect (CPE). Similarly, PRN assays involved incubation of serial dilutions of serum or 250 mg of tissue protein that were titrated in a standard plaque assay as previously described *(88)*.

Mice and animal procedures

All procedures were performed in accordance with federal and university policies. C57BL/6 (B6) and muMT (B6.129S2-*Ighm*^{tm1Cgn}/J) mice were purchased from The Jackson Laboratory and bred in the barrier facility in the Center for Comparative Medicine and Research at the Geisel

School of Medicine at Dartmouth. Blood was collected from naïve and latently infected mice during cardiac perfusion with 1-2 ml of cold PBS. Pooled blood samples were allowed to clot by stasis for at least 15 min. at room temperature and then were spun at 2000 x *g* for 10 min. at 4°C. The serum supernatant was collected and stored at −80°C. Sera treatments were given in a volume of 1 ml at indicated time points during or after gestation via intraperitoneal injection (i.p.). Dams were given 1 mg or 2 mg purified IgG i.p. for viral titer or survival experiments respectively. Sera from *dl*5-29 and mock immunized dams and their respective offspring were harvested at the same time (P4) and collected from the mandibular vein with a 5mm lancet (Medipoint). For fostering experiments, dams were bred simultaneously and monitored closely for birth after which litters were immediately tattooed and swapped to foster dams. Foster dams and litters were observed daily for 7 days for weight change and litter acceptance.

IgG Purification

IgG purification was performed on AKTA FPLC system via Protein G Agarose (Thermo Scientific Pierce) affinity chromatography. Briefly, 5 ml murine serum was equilibrated (1:2) with 100mM sodium acetate, pH 5, before the sample was applied to the column with a flow rate of 0.250 ml/min. The protein elution was achieved in a 15 ml volume with 100 mM glycine, pH 2.7, and quickly neutralized with 100mM Tris-HCL, 0.5M NaCl, pH 8.5. Elution fractions were then concentrated, buffer exchanged to PBS and filter sterilized. Purity was assessed via protein gel electrophoresis and concentration was determined via Nanodrop spectrophotometer.

Immunization

Eight-week old B6 female mice were immunized twice intramuscularly with 10^5 PFU of extracellular *dl*5-29 virus, mock cell lysate, or PBS in a 25 µl volume. Injections were carried out 21 days apart and while mice were under isoflurane anesthesia.

Viral Challenge

For adult corneal infections, corneas were scarified with a 25-gauge needle and inoculated with 2 x 10^5 PFU/eye in a volume of 5 µl as previously described *(88)*. Neonatal pups postpartum day one to day two (P1-2) were infected intranasally (i.n.) with indicated amounts of HSV-1 in a volume of 5 µl under isoflurane anesthesia.

Tissue Analysis

For neutralization and multiplex, TG tissue was perfused, extracted, and homogenated with a tissue blender (Omni International) in PBS containing cOmplete protease inhibitor (Roche). Homogenate was sonicated six times, 15 seconds each, and spun at 14,000 rpm for five min. at 4°C. The protein concentration for each sample was determined using the Pierce BCA assay kit (Thermo Fisher Scientific). In neutralization assays, TG extracts were normalized for protein concentration. For viral titers of organs, tissue was harvested after cardiac perfusion with at least 5 ml of cold PBS per animal. All tissues and organs were collected in 1.5 ml tubes containing 100 µl of 1 mm diameter glass beads and 1 ml of Dulbecco's modified Eagle's medium (DMEM) containing 2% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% amphotericin B. Sample homogenates were prepared by mechanical disruption in a Mini-Beadbeater-8 (BioSpec Products) and then sonicated for 1.5 minutes in an ice bath. Viral titers of homogenates were determined by plaque assay on Vero cells.

Open Field Test (OFT)

Test boxes were fabricated in-house and sanitized with Clidox before and after each test subject. Five to 7-week-old B6 mice were placed into the open field arena (30 cm x 30 cm) and allowed to habituate for 10 minutes. The following 10 min. were recorded (Canon Vixia HFM52) and quantified with open-source software *(83).* Both male and female mice were included in the study. Test room lighting, temperature and noise levels were kept consistent and the researcher was not present in the room during the recorded time.

Multiplexed Antigen-specific Antibody Assay

A customized microsphere assay was developed using a panel of HSV-1 proteins (gifts from Gary Cohen, Roselyn Eisenberg, and Neal Deluca), HSV-1 gD ectodomain (gD') (Immune Tech. Corp.), gG1 and gG2 (Abcam), goat anti-IgG (Southern Biotech), HIV gp120 (made in-house in HEK 293 cells cells), and albumin (RPI). Proteins (6.5 μg) were conjugated to carboxylated magnetic fluorescent microspheres (MagPlex-C Microspheres, Luminex Corp.) in an adaptation of a previously described method *(89).* For fluorescent signal detection, we used black, clear bottom 384-well plates. Serum samples were incubated with HSV protein microspheres (500 beads/well) overnight at 4°C and washed in PBS with 1% BSA, 0.05% Tween-20, and 0.1% Sodium Azide. Anti-mouse IgG PE or anti-human IgG PE (Southern Biotech) was incubated at 0.65 μg/ml for 45 min in PBS-TBN. The microspheres were washed and resuspended in 50 μl of sheath fluid (Luminex) and read using a Bio-plex array reader (FlexMap 3D, Bio-Plex Manager 5.0, Bio-Rad). The median fluorescence intensity (MFI) of PE signal was determined for each bead set in each well at 1/1000 dilution for mouse samples and 1/5000 for human samples. For

human data, we mean-centered and scaled to a 0-1 range (1 referring to maximum binding and 0 referring to minimum binding) for each bead type. Human cord samples were categorized into two tertiles by mean antibody binding of all HSV-specific beads. If the mean antibody binding was above the second tertile, it was categorized as a high responder and a low responder if below the first tertile.

Immunofluorescence

Tissue was collected from perfused mice and fixed with 4% formaldehyde and cryopreserved in 30% sucrose. Sections were prepared and imaged as previously described *(23)*. Fluorescence intensity was quantified using ImageJ with 3 biological replicates and was calculated using: corrected total fluorescence = integrated density – (area x mean fluorescence of background).

Wire Hang Test

Neuromuscular motor function and strength was assessed using the Wire Hang Test mice at 5 weeks post infection. Each mouse was placed on a wire platform which was then inverted and suspended over a cage. The latency from the beginning of the test until the mouse fell was recorded.

S1

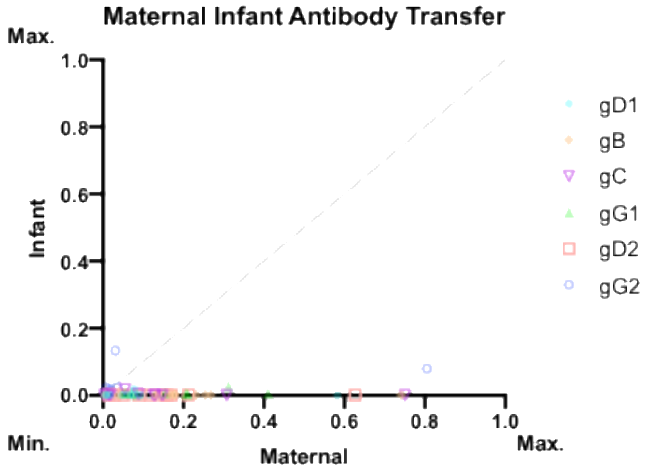

Fig. S1. Maternal antibody is not present in 18-month-old children.

Matched maternal and 18-month infant samples were probed for HSV-1/2 specific antibodies and plotted by glycoprotein binding (maximum binding = 1, minimum binding = 0). Dotted line refers to line of identity, n= 10 mothers and infants.

S2

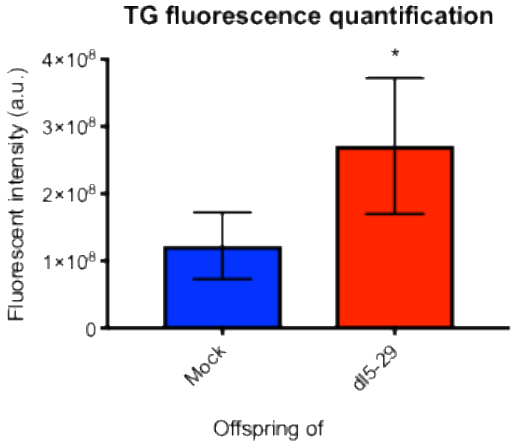

Fig. S2. Measurement of IgG staining in TG tissues from Fig. 4D.

Fluorescence intensity of anti-mouse IgG was measured in TG tissue sections (10 µm) using ImageJ. A.U. = arbitrary units. Error bars are SD. n= 6 mice in each group. Statistical significance was determined by unpaired t-test; *, $P < 0.05$.

S3

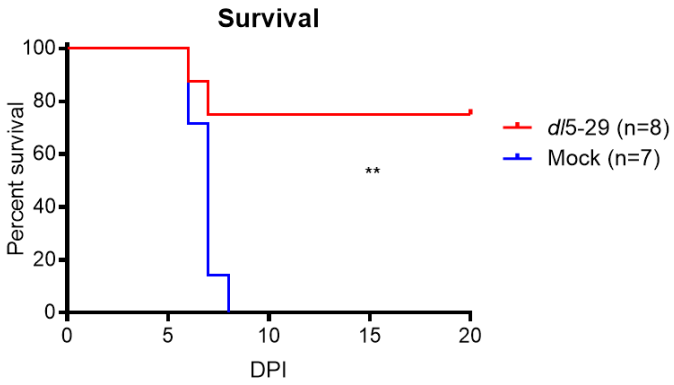

Fig. S3. Long-term (≥245 days) protection by maternal immunization with *dl*5-29.
B6 females were immunized and boosted as shown in Figure 4A. Following 5 pregnancies, (245 days post immunization) P1-2 neonates from dams immunized with *dl*5-29 (red) or from mock immunized dams (blue) were challenged with 10^3 PFU HSV-1. Statistical significance was determined by log-rank test; **, $P < 0.01$.

S4

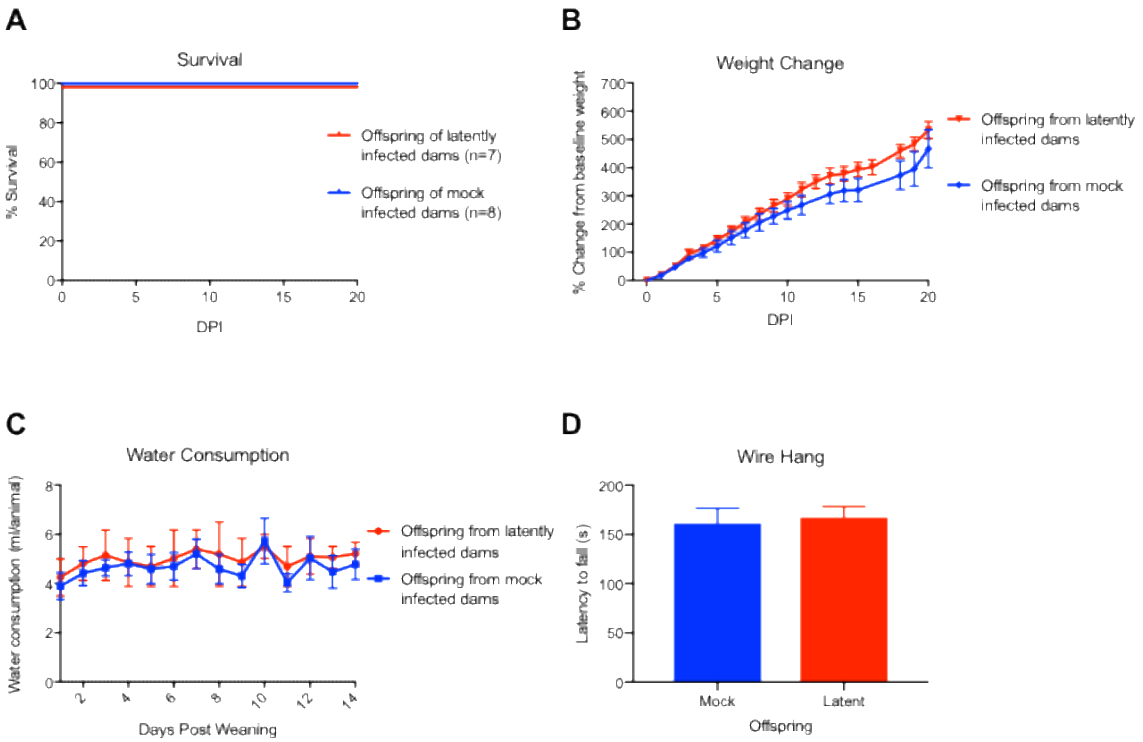

Fig. S4. Neonatal mice do not show signs of infection with a low HSV-1 dose.

P1-2 mice from latently or mock infected dams were challenged with 100PFU of HSV-1 and monitored for **A)** survival, **B)** weight change, and **C)** daily water consumption after infection. **D)** Neuromuscular motor function was assessed using the Wire Hang Test on these mice at 5 weeks post infection.. Error bars indicate SD.

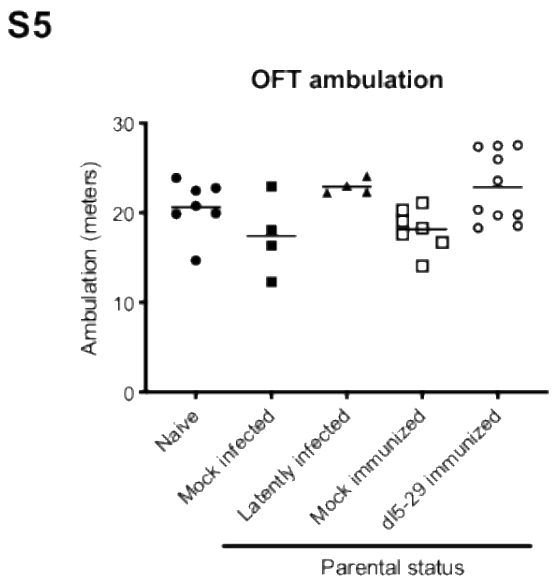

Fig. S5. Total ambulation of HSV-challenged and naïve mice in the OFT.

Total distance (meters) was quantified from video recordings of the OFT from offspring of latently infected, mock-infected, *dl*5-29 vaccinated, mock vaccinated dams and naïve age-matched controls.