Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Short communication

Protection against herpes simplex virus type 2 infection in a neonatal murine model using a trivalent nucleoside-modified mRNA in lipid nanoparticle vaccine

Philip C. LaTourette II, Sita Awasthi, Angela Desmond, Norbert Pardi, Gary H. Cohen, Drew Weissman, Harvey M. Friedman

Infectious Disease Division, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104-6073, USA
University Laboratory Animal Resources, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104, USA
Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104, USA
The Children’s Hospital of Philadelphia, Infectious Disease Division, University of Pennsylvania Department of Pediatrics, Philadelphia, PA 19104, USA
Department of Basic and Translational Sciences, University of Pennsylvania School of Dental Medicine, Philadelphia, PA 19104, USA

abstract

Neonatal herpes is a dreaded complication of genital herpes infection in pregnancy. We recently compared two vaccine platforms for preventing genital herpes in female mice and guinea pigs and determined that HSV-2 glycoproteins C, D and E expressed using nucleoside-modified mRNA in lipid nanoparticles provided better protection than the same antigens produced as baculovirus proteins and administered with CpG and alum. Here we evaluated mRNA and protein immunization for protection against neonatal herpes. Female mice were immunized prior to mating and newborns were infected intranasally with HSV-2. IgG binding and neutralizing antibody levels in mothers and newborns were comparable using the mRNA or protein vaccines. Both vaccines protected first and second litter newborns against disseminated infection based on virus titers in multiple organs. We conclude that both vaccines are efficacious at preventing neonatal herpes, which leaves the mRNA vaccine as our preferred candidate based on better protection against genital herpes.

1. Introduction

Neonatal herpes is an uncommon but potentially devastating infection [1]. The annual global incidence of neonatal herpes is approximately 14,000 cases, or 1 case per 10,000 births of which HSV-1 comprises 4000 and HSV-2 10,000 [1]. HSV-1 contributes more cases than HSV-2 in the Americas, Europe and Western Pacific [1]. Transmission to newborns most often occurs during labor and delivery from mothers with active genital herpes. Over half the infants with neonatal herpes have disseminated infection or encephalitis, with 60% mortality if untreated, and severe neurologic disease in two-thirds of survivors despite antiviral treatment [1,2]. Currently, no vaccine is available to prevent genital or neonatal herpes. Efforts to prevent neonatal herpes include visual inspection for lesions at delivery, cesarean section when lesions are present, and behavioral messaging to reduce risk of transmission late in pregnancy. These measures are often ineffective because of asymptomatic infection.

An effective method of protection is through maternal antibodies that develop after infection and pass transplacentally or through breast milk to newborns [3,4]. Vaccination of women is another approach to develop antibodies that can protect newborns until they are old enough to be vaccinated on their own, with influenza as an example [5]. An important issue for candidate herpes vaccines is whether maternal antibodies produced by immunization will provide sufficient protection to a newborn that is exposed to HSV either because of breakthrough infection in the pregnant woman or because of postnatal exposure. Murine models of neonatal herpes infection have addressed vaccine protection of newborn pups. Maternal immunization of female mice with an HSV-2 replicant defective live virus vaccine, d5-29, protected newborn pups against neonatal HSV-1 and HSV-2 infection [6]. A follow up study
was performed by these same investigators in a collaboration with our laboratory using an HSV-2 subunit protein vaccine consisting of glycoproteins C, D and E (gC2, gD2, gE2) administered with CpG and alum as adjuvants. Our rationale for using the three immunogens in the trivalent vaccine is that antibodies to gC2 and gD2 neutralize virus, antibodies to gD2 and gE2 block cell-to-cell spread, and antibodies to gC2 and gE2 block immune evasion from antibody and complement [7–10]. The trivalent protein vaccine also provided excellent protection against HSV-1 and HSV-2 neonatal herpes [11].

We recently reported that immunization of female mice and guinea pigs with gC2, gD2, and gE2 administered as nucleoside-modified mRNA encapsulated in lipid nanoparticles (LNP) outperformed the protein formulation in preventing genital herpes infection [7]. Immunization with nucleoside-modified mRNA-LNP has gained considerable recognition in response to COVID-19, and is a highly promising approach for infectious disease vaccine development [12–14]. The nucleoside-modified mRNA immunogens have been particularly potent in producing high titer antibody responses, likely related to potent CD4+ T follicular helper cell and germinal center B cell responses [15]. Three years ago, we began transitioning from a trivalent baculovirus protein vaccine to a nucleoside-modified mRNA vaccine for prevention of genital herpes [7,8]. Here, we compared protection provided by immunization of mothers (dams) with the baculovirus protein vaccine or the nucleoside-modified mRNA vaccine against intranasal (IN) HSV-2 infection in pups.

2. Materials and methods

2.1. Mice

Protocol 805187 was approved by the University of Pennsylvania Institutional Animal Care and Use Committee following guidelines of National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

2.2. Vaccines

Female 6–8-week-old BALB/c mice (Charles River) were immunized intramuscularly (IM) prior to mating using trivalent mRNA-LNP, trivalent protein CpG/alum, or Poly(C) RNA-LNP (control) as immunogens [7]. The control immunogen contained 10 µg of Poly(C) RNA, while the nucleoside-modified mRNA vaccine contained 10 µg each of gC2, gD2 and gE2 at a ratio of 1 µg RNA to 20 µg LNP. The protein vaccine contained 5 µg each of gC2, gD2 and gE2 with 50 µg CpG/mouse and 25 µg alum/µg protein. Two immunizations one month apart were performed with mRNA or Poly(C), while three immunizations two weeks apart were administered with the protein vaccine, each in a volume of 50 µl.

2.3. Breeding and infection (see graphic abstract)

Immunizations with mRNA or Poly(C) were on days −70 and −42, or with protein on days −70, −55, and −42. Two weeks after the last immunization, on day −28, females were paired with males. Postnatal day 0 (PND 0) was the day the litter was detected. Neonatal pups were infected IN on PND 3 with 1 × 103 HSV-2 strain MS on PND 3 [9]. Three days later, brain, heart, lung, spleen, liver, kidney and colostrum (milk) were harvested. Some neonates were sacrificed to assess serum antibody levels on PND 3 or PND 6–7, and some dams were bred for a second litter.

2.4. Serological testing

Serum was simultaneously collected from dams and their pups on PND 3 or 6–7. Serum from dams was obtained by lancet piercing of a submandibular vein, while pup serum was obtained by decapsulation and pooling samples from the same litter. Serum endpoint neutralizing antibody titers were determined without complement and reported as the highest dilution to reduce the number of virus plaques by ≥50% compared to PBS control [9]. Serum IgG binding (ELISA) endpoint titers for gC2, gD2 or gE2 were performed by coating ELISA plates with 50 ng of gC2, gD2 or gE2 antigen and adding serial two-fold dilutions of serum at an initial dilution of 1:500, followed by HRP-conjugated anti-mouse IgG [7]. To determine whether dams that nursed HSV-2-infected pups seroconverted, ELISA plates were coated with HSV-2 glycoprotein G (gG2) and serum was evaluated for a four-fold or greater rise in titer [16].

Nursing neonates were infected IN with 1 × 103 PFU HSV-2 MS on PND 3. Three days later, approximately 25 µg of colostrum was harvested by scooping the gelatinous contents from the excised stomach and stored at −80 °C. The colostrum was slowly thawed on ice, and 400 µl of PBS was added to each sample because of its viscosity and fatty content. The colostrum was homogenized and diluted 1:20 in PBS for neutralizing antibody assays or 1:125 for IgG gD2 ELISA.

2.5. Virus titers in organs

Neonatal brain, lung, liver, spleen, and kidney were placed in 300 µl of DMEM containing 5% fetal bovine serum and vancomycin (25 µg/ml). The organs were minced with scissors, homogenized, and 10-fold serial dilutions added to Vero cells to determine virus titers by plaque assay [9].

2.6. Statistical analysis

Significance tests were two-sided and were calculated by the Mann-Whitney test using GraphPad Prism version 8.3, or Fisher’s Exact test.

3. Results

3.1. HSV-2 IN dosing determination

Newborn pups were infected IN on PND 3 with 1 × 102, 1 × 103 or 1 × 104 PFU of HSV-2. The LD50 was calculated as 15 PFU/mouse (Fig. 1A). 1 × 103 PFU (67 LD50) was selected for subsequent neonatal infections.

3.2. Serological testing

Pups from first litters of immunized dams were infected IN with 1 × 103 HSV-2 on PND 3 and colostrum was collected on PND 6. Pups born to dams immunized with trivalent mRNA-LNP or trivalent protein CpG/alum had gD2 IgG detected in their colostrum by ELISA (binding antibodies), while pups in the Poly(C) group had no gD2 IgG (Fig. 1B). The mean gD2 endpoint titer for pups in the mRNA group was 1:3667 and in the protein group was 1:2615 (P = 0.1074). Siblings had gD2 IgG titers that were either identical or varied by ≤four-fold, suggesting that siblings acquired comparable levels of antibodies from their mothers. Neutralizing antibody titers were also measured in colostrum. Neutralizing antibody was absent in Poly(C) and protein pups, but was somewhat higher in the mRNA pups in that 2 of 8 neonates had neutralizing antibody
We measured IgG titers to gC2, gD2 and gE2 in serum from dams and their first litters on PND 3 or PND 6–7. One dam immunized with Poly(C) and her two pups had no detectable gC2, gD2 or gE2 IgG. In the mRNA group, two dams and their seven or nine pups had IgG antibodies to gC2, gD2 and gE2 that were either the same titer or within one or two dilutions of each other. In the protein group, one dam and her four pups had IgG antibodies to gC2, gD2 and gE2 that were identical in the dam and pups (Fig. 1D). IgG titers were generally higher in the mRNA than protein groups, but the small number of mother-pup pairs precluded statistical analysis.

Serum neutralizing titers were collected on PND 3 or PND 6–7 from six dams (2 in Poly(C), 2 in mRNA and 2 in protein groups) and their first litter of pups, and from four dams (2 in mRNA and 2 in protein group) and their second litter of pups (Fig. 1E). The average interval between first and second litters was 80 days for mRNA and 49 days for protein pups. One dam was evaluated over two generations of pups (denoted by an open circle in the mRNA group, Fig. 1E), while the other nine dams were assessed at the time of either their first or second litter. Eight of the 10 litters of pups were uninfected at the time serum was collected, while serum from two second generation litters born to dams immunized with trivalent protein was harvested three days post-infection at the same time that organs were harvested. These two dams did not subsequently seroconvert to HSV-2 despite nursing infected pups for three days. No neutralizing antibodies were detected in the Poly(C) dams or their pups. Neutralizing antibody titers in dams and their pups were identical in 4 mother-pup pairs, varied by 2-fold in 3 pairs (higher in dams than pups in 2 of 3), or 4-fold in one pair (higher in pups). The higher ELISA IgG and neutralizing titers in some newborns than dams likely represent the combined contribution of transplacental and colostrum antibodies [6,17]. The need to dilute the colostrum with PBS because of its viscosity and fatty content makes it difficult to compare colostrum titers with serum titers. We conclude that high titers of neutralizing antibodies are detected in neonates during first and second litters providing evidence of durable transplacental and/or milk transfer of antibodies from dams to their pups.

### 3.3. Virus titers in neonatal tissues

Neonates were infected IN on PND 3 with 1 × 10^3 PFU HSV-2. Brains, lungs, livers, spleens and kidneys were harvested three days later (Fig. 2A). Most organs from neonates in the Poly(C) group were positive for virus, while very few were positive in the mRNA or protein groups. The few positive samples were from brains in the mRNA and protein groups (Fig. 2A and Table 1).

Second litter pups born to four previously immunized dams were evaluated for neonatal herpes. Three of the dams had nursed first litter pups between PND 3 and 6 that were infected IN with HSV-2; however, none of these dams seroconverted to HSV-2 as determined by ELISA IgG antibodies to gG2. Therefore, protection in pups of the second-generation litters is because of maternal immunization and not maternal HSV-2 infection. In the second-generation litters, all organs from two pups in the Poly(C) group
were positive, while very few were positive in the mRNA or protein groups (Fig. 2B and Table 1). Trivalent mRNA and trivalent protein were significantly different from Poly(C) RNA in first litters, second litters and overall (P < 0.0001 calculated by two-tailed Fisher’s Exact test), but not significantly different from one-another (Table 1).

### 4. Discussion

A goal of the current study was to assess whether the trivalent nucleoside mRNA-LNP vaccine is efficacious in preventing neonatal herpes, a feared complication of genital herpes. IgG ELISA antibodies and neutralizing antibody titers in mice immunized with trivalent mRNA or trivalent protein were similar to antibody titers in their newborns, and neutralizing antibodies were durable from first to second pregnancy after an interbirth interval of 80 days for mRNA and 49 days for protein. First litter pups were born approximately 40 days after the final immunization with mRNA or protein; therefore, immunity was durable in protecting second litter pups for at least 120 days for mRNA and 89 days for protein. We did not evaluate whether the pup antibody titers were derived transplacentally or from colostrum, although both are likely important contributors [6,17]. In addition to neutralizing antibodies, antibody dependent cellular cytotoxicity (ADCC) protects against HSV infection in newborn mice and humans [3,4,18,19]. Neonatal mice do not have effective ADCC effector cells by PND 3; therefore, vaccine protection was more likely mediated by neutralizing antibodies based on the age we infected and harvested organs [19]. The mRNA and protein vaccines were both highly efficacious in preventing disseminated infection to multiple organs over 2 generations of litters, while the Poly(C) control provided no protection. The mRNA vaccine protected 27/30 (90.0%) brains after IN infection over two generations, while the protein vaccine protected 29/32 (90.6%), and both vaccines prevented disseminated infection to lung, liver, spleen and kidney, except for a single pup in the protein group that had liver infection. Preventing disseminated infection and infection of the developing brain is an important goal for a vaccine in humans [4,20].

Several vaccine candidates have now been evaluated in the mouse neonatal herpes model, including replication-defective live virus, single-cycle live attenuated virus, trivalent protein, and in this report, trivalent mRNA [6,11,19]. Each vaccine provided strong protection, suggesting that more than one approach is likely to be effective. The mRNA vaccine outperformed the protein vaccine in preventing genital herpes in mice and guinea pigs [7]. Based on comparable protection by mRNA and protein vaccines against neonatal herpes reported here, we consider the nucleoside-modified mRNA vaccine to be our lead candidate for human trials.

### CRediT authorship contribution statement

**Philip C. LaTourette:** Investigation, Project administration, Methodology, Data curation, Writing - original draft. **Sita Awasthi:** Conceptualization, Investigation, Methodology, Validation, Formal analysis, Supervision, Project administration. **Angela Desmond:** Methodology, Investigation. **Norbert Pardi:** Resources. **Gary H. Cohen:** Resources, Writing - review & editing. **Drew Weissman:** Resources, Writing - review & editing. **Harvey M. Friedman:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - original draft, Writing - review & editing.

### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: HMF, SA, GHC and DW are inventors on patents held by the University of Pennsylvania for protein (HMF, SA) and mRNA (HMF, SA, GHC, DW) vaccines for genital herpes. NP is also named on a patent describing use of nucleoside-modified mRNA-LNP as a vaccine platform. The authors have disclosed their interests fully to the University of Pennsylvania, and have in place an approved plan for managing any potential conflicts arising from licensing of the patents.

### Acknowledgements

HMF is the recipient of R01 AI139618 from the National Institutes of Health, USA. HMF, GHC and DW are inventors on patents held by the University of Pennsylvania for protein (HMF, SA) and mRNA (HMF, SA, GHC, DW) vaccines for genital herpes. NP is also named on a patent describing use of nucleoside-modified mRNA-LNP as a vaccine platform. The authors have disclosed their interests fully to the University of Pennsylvania, and have in place an approved plan for managing any potential conflicts arising from licensing of the patents.

### References

[1] Looker KJ, Magaret AS, May MT, Turner KM, Vickereman P, Newman LM, et al. First estimates of the global and regional incidence of neonatal herpes infection. Lancet Glob Health 2017;5:e300-9.
[2] Kimberlin DW. Neonatal herpes simplex infection. Clin Microbiol Rev 2004;17:1–13.

[3] Yeager AS, Arvin AM, Urbani LJ, Kemp 3rd JA. Relationship of antibody to outcome in neonatal herpes simplex virus infections. Infect Immun 1980;29:532–8.

[4] Prober CG, Sullender WM, Yasukawa LL, Au DS, Yeager AS, Arvin AM. Low risk of herpes simplex virus infections in neonates exposed to the virus at the time of vaginal delivery to mothers with recurrent genital herpes simplex virus infections. New England J Med 1987;316:240–4.

[5] Zaman K, Roy E, Arifeen SE, Rahman M, Raqib R, Wilson E, et al. Effectiveness of maternal influenza immunization in mothers and infants. N Engl J Med 2008;359:1555–64.

[6] Patel CD, Backes IM, Taylor SA, Jiang Y, Marchant A, Pesola JM, et al. Maternal immunization confers protection against neonatal herpes simplex mortality and behavioral morbidity. Sci Transl Med 2019;11.

[7] Awasthi S, Hook LM, Pardi N, Wang F, Myles A, Cancoo MP, et al. Nucleoside-modified mRNA encoding HSV-2 glycoproteins C, D, and E prevents clinical and subclinical genital herpes. Sci Immunol 2019;4:eaaw7083.

[8] Awasthi S, Lubiński JM, Shaw CE, Barrett SM, Cai M, Wang F, et al. Immunization with a vaccine combining herpes simplex virus 2 (HSV-2) glycoprotein C (gC) and gD subunits improves the protection of dorsal root ganglia in mice and reduces the frequency of recurrent vaginal shedding of HSV-2 DNA in guinea pigs compared to immunization with gD alone. J Virol 2011;85:10472–86.

[9] Awasthi S, Huang J, Shaw CE, Friedman HM. Blocking herpes simplex virus 2 glycoprotein E immune evasion as an approach to enhance efficacy of a trivalent subunit antigen vaccine for genital herpes. J Virol 2014;88:8421–32.

[10] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines – a new era in vaccinology. Nat Rev Drug Discov 2018;17:261–79.

[11] Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Phase 1/2 study to describe the safety and immunogenicity of a COVID-19 RNA vaccine candidate (BNT162b1) in adults 18 to 55 years of age: interim report. medRxiv 2020.

[12] Pardi N, Hogan MJ, Naradikian MS, Parkhouse K, Cain DW, Jones L, et al. Nucleoside-modified mRNA vaccines induce potent T follicular helper and germinal center B cell responses. J Exp Med 2019;215:1571–88.

[13] Wald A, Ashley-Morrow R. Serological testing for herpes simplex virus (HSV)-1 and HSV-2 infection. Clin Infect Dis 2002;35:573–82.

[14] Kohl S, Strynadka NC, Hodges RS, Pereira L. Analysis of the role of antibody-dependent cellular cytotoxic antibody activity in murine neonatal herpes simplex virus infection with antibodies to synthetic peptides of glycoprotein D and monoclonal antibodies to glycoprotein B. J Clin Investig 1990;86:273–8.

[15] Kao CM, Goymer J, Loh LN, Mahant A, Aschner CB, Herold BC. Murine model of maternal immunization demonstrates protective role for antibodies that mediate antibody-dependent cellular cytotoxicity in protecting neonates from herpes simplex virus type 1 and type 2. J Infect Dis 2020;221:729–38.

[16] Whitley R, Arvin A, Prober C, Corey L, Burchett S, Plotkin S, et al. Predictors of morbidity and mortality in neonates with herpes simplex virus infections. The National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. New England J Med 1991;324:450–4.