Comparative efficacy of adjuvants MPL, MTP-PE, LT1, and CDN in gD3pep liposomes against intravaginal Herpes Simplex Virus Type-1 Infections

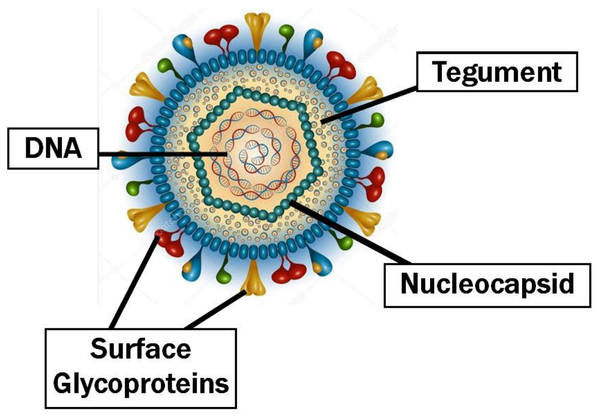
Khadija Shafiq and Dr. Jill Adler-Moore

Department of Biological Sciences, California State Polytechnic University, Pomona

Abstract

Herpes Simplex Virus Type 1 (HSV-1) infects 48% of individuals ages 14 to 49 in the United States and is responsible for nearly half of genital herpes infections [1]. While antiviral treatments reduce the symptoms of infection, there are no vaccines that can prevent this infection. To test the efficacy of a liposomal HSV-1 vaccine containing three epitopes of the HSV-1 gD protein (L-gD3pep), we first developed an intravaginal HSV-1 (Strain F) infection in BALB/c mice by challenging the mice with 20 uL of varying dilutions of virus (1.88 x 10^6 PFU/mL). Mice were observed for morbidity for 11 days and the challenge dose of 1:4 dilution HSV-1 F-strain was selected for further testing as it produced morbidity in all mice by day 6. Other groups of mice (n=21/gp) were then subcutaneously vaccinated d0, d14, and d28 with L-gD3pep containing different adjuvants including MPL (monophosphoryl lipid A), MTP-PE (muramyl tripeptide phosphatidylethanolamine), LT1 (lipidated tucaresol), or CDN (cyclic dinucleotide) with phosphate buffered saline (PBS) as the control. Serum was harvested d31 (n=7 mice/gp) to assess HSV-1 neutralizing antibody titers (NAb). On d35, 14 mice/gp were challenged Ivag with HSV-1 and monitored for morbidity (mucosal and neurological symptoms, weight loss) to d57. Viral burden was determined in spinal cords collected from viral challenged mice on d42 (n=7/gp), using a PFU assay. To determine the sensitivity of the HSV-1 PFU assay, we collected spinal cords from non-vaccinated mice (n=5/gp), homogenized the samples with 300uL of a known amount of HSV-1 virus (2560 PFU/mL) and performed a PFU assay on the homogenates. We found that 99% of the added virus was detectable indicating that the assay was a reliable method for analyzing viral burden in HSV-1 infected tissues. In the vaccine study with the different adjuvants, survival was 86% with CDN, 71% with MPL, 57% with MTP-PE, and 14% with both LT1 and PBS. Weight loss and symptom scores paralleled survival. CDN and MPL groups had significantly lower viral burden in their spinal cords ($p \leq 0.05$ vs. other gps). In conclusion, our results showed that protection against HSV-1 genital infection correlated with the type of adjuvant in the L-gD3pep with the CDN, targeting to TLR9, and MPL, targeting to TLR2. providing the most protection.

Herpes Simplex Virus Structure



References

[1] D. Jaishankar and D. Shukla. "Genital Herpes: Insights into Sexually Transmitted Infectious Disease." *Microbial Cell* 3 (2016): 438-450

[2[Rubio, Jennifer. (2017). Efficacy Against Vaginal Herpes Infection using adjuvants in a gD Tripeptide Liposomal Vaccine. *California State Polytechnic University, Pomona.* Master's Thesis.

[3] Keiff, Eliiot, et al. (1972). "Genetic Relatedness of Type 1 and Type 2 Herpes Simplex Viruses. *Journal of Virology* (3): 738-745.

[4] Cox, John C., and Alan R. Coulter. "Adjuvants —a classification and review of their modes of action." *Vaccine* 15.3 (1997): 248-256.

[5] Danilchanka, & Mekalanos. (2013). Cyclic Dinucleotides and the Innate Immune Response. *Cell, 154*(5), 962-970.

[6] Meyers, Paul A. "Muramyl tripeptide (mifamurtide) for the treatment of osteosarcoma." *Expert review of anticancer therapy* 9.8 (2009): 1035-1049.

[7] Fernández-Tejada, et al. "Design, Synthesis, and Immunologic Evaluation of Vaccine Adjuvant Conjugates Based on PRA and Tucaresol." *Bioorganic &Amp; Medicinal Chemistry*, vol. 22, no. 21, 2014, pp. 5917–5923.

[8] Kruppenbacher, J.P. & Kläss, R & Eggers, H.J.. (1994). A rapid and reliable assay for testing acyclovir sensitivity of clinical herpes simplex virus isolates independent of virus dose and reading time. Antiviral research. 23. 11-22.

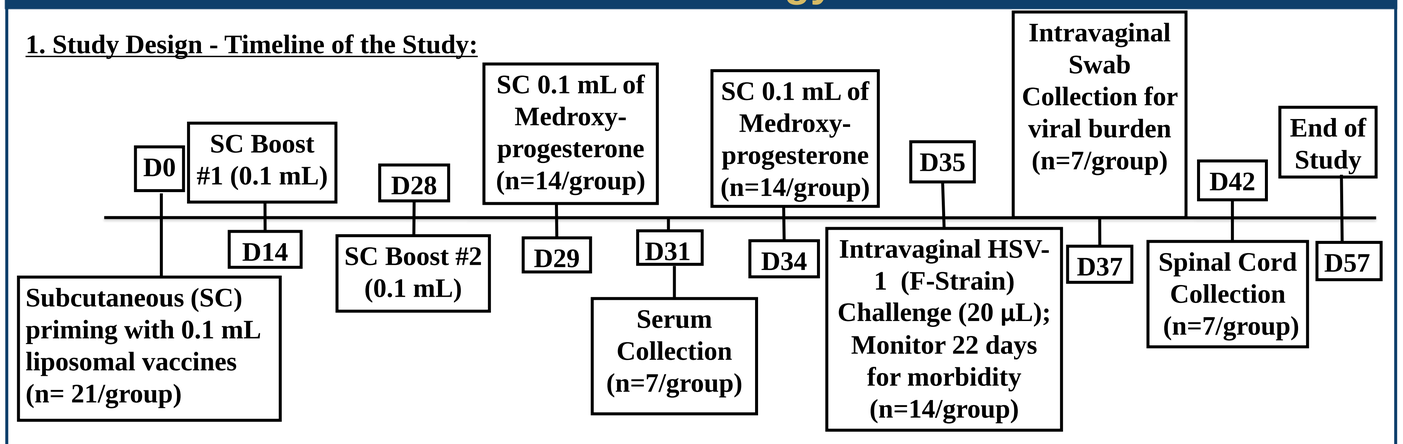
[9] Ludlow M., Kortekaas J., Herden C., Hoffmann B., Tappe D., Trebst C., Griffin D.E., Brindle H.E., Solomon T., Brown A.S., et al. Neurotropic virus infections as the cause of immediate and delayed neuropathology. *Acta Neuropathologica*. 2016; 131:159–184.

Acknowledgements

Thanks to all of the individuals that helped with this project, including Edgar Gonzalez and other lab mates in Dr. Adler's lab at California State Polytechnic University Pomona. Special thanks to the NSF – LSAMP Fellowship, NIH MBRS-RISE Program, Molecular Express Incorporated, and the Kellogg Honors College for supporting this study.

Methodology

1. Study Design - Timeline of the Study:



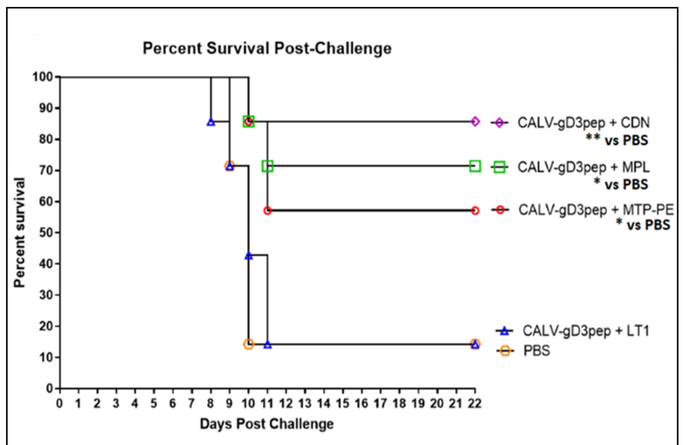
2. Viral Challenge: Intravaginally challenged with HSV-1 (F-strain), observed daily for mucosal signs (scale=0 no lesions-4 lesions, rectal impaction, inactivity), neurological symptoms (scale = 0 no tail effects -2 tail lagging) and weight change.

3. Viral Burden in Spinal Cords: PFU assay of homogenized spinal cords with Vero Cells

4. Determine Sensitivity of the PFU Assay = $\frac{\text{Detected PFU} \times 100\%}{\text{PFU from Virus Stock}}$ Detectable virus = 100% - Sensitivity

5. Immunological Assay: Serum anti-HSV-1 neutralizing antibody titers

Results



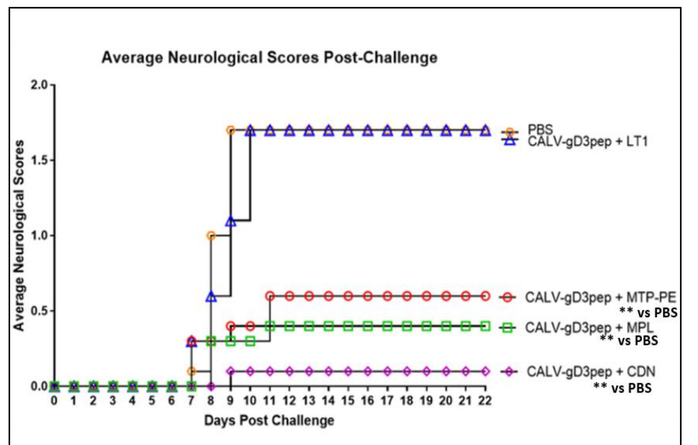
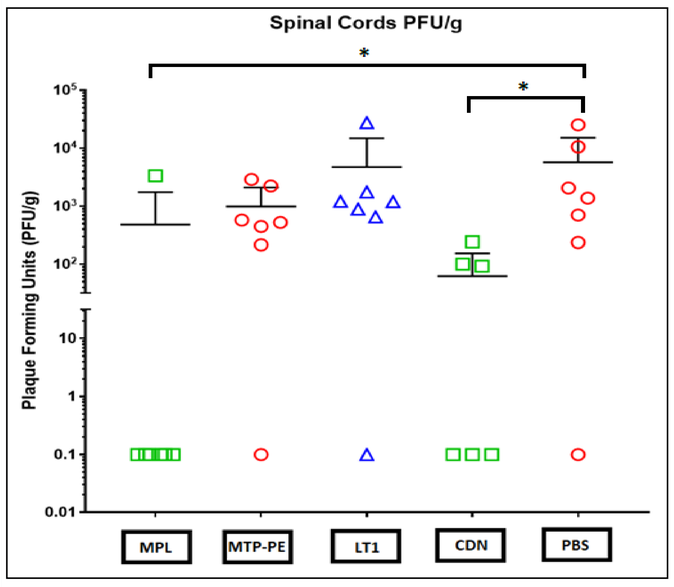


Figure 1. Percent Survival Post-Challenge & Figure 2. Neurological Scores Post-Challenge: gD3pep liposomal vaccines with CDN, MPL, and MTP-PE were protective against intravaginal HSV-1 challenge, with CDN and MPL demonstrating the most protection, based on significantly prolonged survival ($p < 0.0273$) and decreased neurological disease symptoms post-challenge ($P < 0.0291$) compared to liposomes with LT1 adjuvant and the PBS group.



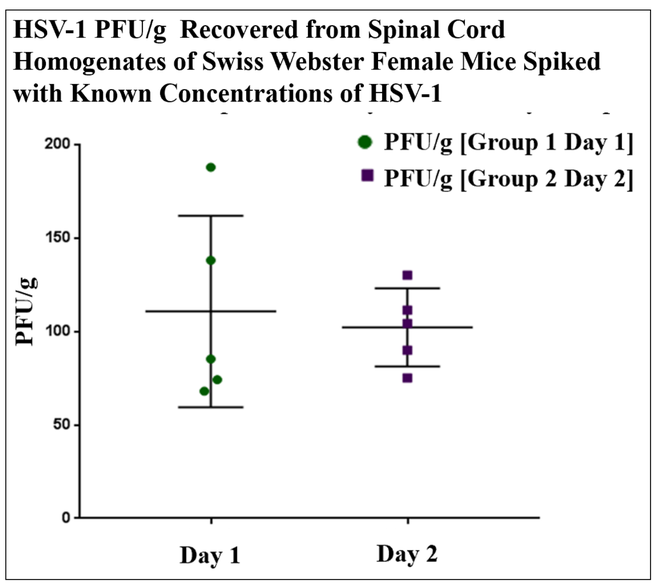


Figure 2. Spinal Cords PFU/g and Figure 3. HSV-1 PFU/g Recovered from Spinal Cord Homogenates of Swiss Webster Female Mice Spiked with Known Concentrations of HSV-1. Mice in the CDN and MPL groups had significantly lower viral burden in the spinal cords compared to the LT1 adjuvant and PBS control groups ($P < 0.0373$). This procedure was performed on two groups of mice (n=5/group) on two different days. The average PFU/g was not significantly different between the two groups of mice on the two different days. The assay sensitivity was 0.4% in Group 1 and 0.3% in Group 2. 99.6% of the virus from Group 1 was detected and 99.7% of virus was detected from Group 2.

Conclusions

- The VesiVaX® gD3pep liposomes containing the CDN or MPL adjuvant were the most protective against HSV-1 vaginal infection.
- LT1 adjuvant in the VesiVaX® gD3pep liposomes protected mice against intravaginal HSV-2 infection in previous studies in our laboratory but did not protect against HSV-1 infection, highlighting differences between the two virus infections and the immune response to the viruses.
- The HSV-1 PFU assay is a reliable, reproducible method for evaluating the viral burden/g spinal cord and when used in combination with morbidity, weight loss, and disease signs, this combination of endpoints can be used effectively to determine the protective efficacy of vaccine formulations for HSV-1 infection.
- Using combinations of MPL and CDN gD3pep vaccine formulations to determine if the combinations would enhance or decrease the protection generated by the vaccines against HSV-1 and HSV-2 infection in mice.