

Modified Glycoprotein D DNA vaccines protect against challenge in an HSV-2 infection model



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Introduction

- Herpes Simplex Virus type 2 (HSV-2) is the main causative agent of genital herpes. HSV lesions are believed to enhance the transmission of Human Immunodeficiency Virus and can result in neonatal herpes, a rare but serious condition. While there have been a number of clinical trials of HSV-2 vaccines, none have yielded an effective vaccine.
- The main aim of this study was to assess the potential of novel HSV-2 glycoprotein D (gD2)-based DNA vaccines in a mouse HSV-2 challenge model. The vaccines utilize unique codon optimization and ubiquitination technology designed to enhance antibody responses and to induce a strong cellular immune response, respectively. Specifically, two vaccine constructs were tested. One construct encodes full-length gD2 protein, while the other encodes amino acids 25-331 of gD2 with a single ubiquitin attached at the N-terminal. The vaccine constructs were tested alone or mixed and the findings are presented below.

Materials and Methods

Constructs

- Codon-optimized inserts encoding full-length gD2 and N-terminally ubiquitinated and gD2₂₅₋₃₃₁ were designed using immune Coricode codon preferences (Frazer, 2009), synthesized, and inserted into pcDNA3 (Invitrogen) to make pcDNA3-O2-gD2 and pcDNA3-O2-UgD2₂₅₋₃₃₁, respectively.

Infection study

- 20 female BALB/c mice per vaccine group: half challenged at 50xLD₅₀ dose and half at 500xLD₅₀ dose of HSV-2 via the vaginal route.
- Intradermally immunized 3 times at 2 week intervals with 20µg of vaccine.
- Serum was collected on days -1, 28 and 42 & vaginal swabs were taken on days 1, 3 and 5 post-challenge.
- Survival of the mice was monitored for 21 days.

- The ELISA and HSV-2 vaginal swab PCR methods are described in Kask et al., 2010.

IFN-γ ELISPOT

- Balb/c mice were immunized at days 0, 14 and 28, and the spleens collected on day 42.
- 96-well filter plates (Millipore) were coated with Mab (AN18; Mabtech), washed then blocked. Spleen cells (10⁶/well) were added to medium supplemented with recombinant hIL-2 (ProSpec-Tany TechnoGene Ltd) and peptide to a final concentration of 10IU/well and 10µg/mL, respectively. 13mer gD2 peptides were used in the ELISPOT (Auspep & Mimotope, Melbourne; Muller et al., 2009). Medium containing hIL-2 without peptide was added to control wells. Plates were incubated for 16-20 hours at 37°C in 5-8% CO₂. For detection of IFN-γ secreting cells, cells were lysed and detected using Biotinylated detection mAb (R4-6A2; Mabtech), horseradish peroxidase (HRP)-conjugated streptavidin and DAB tablets (Sigma). Spots were counted using an automated ELISPOT plate counter.

Results

- Three different vaccine combinations, along with a negative empty control vaccine, were tested: pcDNA3-O2-gD2 (O2-gD2) alone, pcDNA3-O2-UgD2₂₅₋₃₃₁ (O2-UgD2₂₅₋₃₃₁) alone and a 1:1 mix of the two constructs.
- All three active vaccines gave 90-100% protection at day 21 after viral challenge at the 50xLD₅₀ dose
- At the higher challenge dose, O2-gD2 and the mixed vaccine continued to give 90-100% survival, however the survival rate for the ubiquitinated construct alone dropped to 50%.

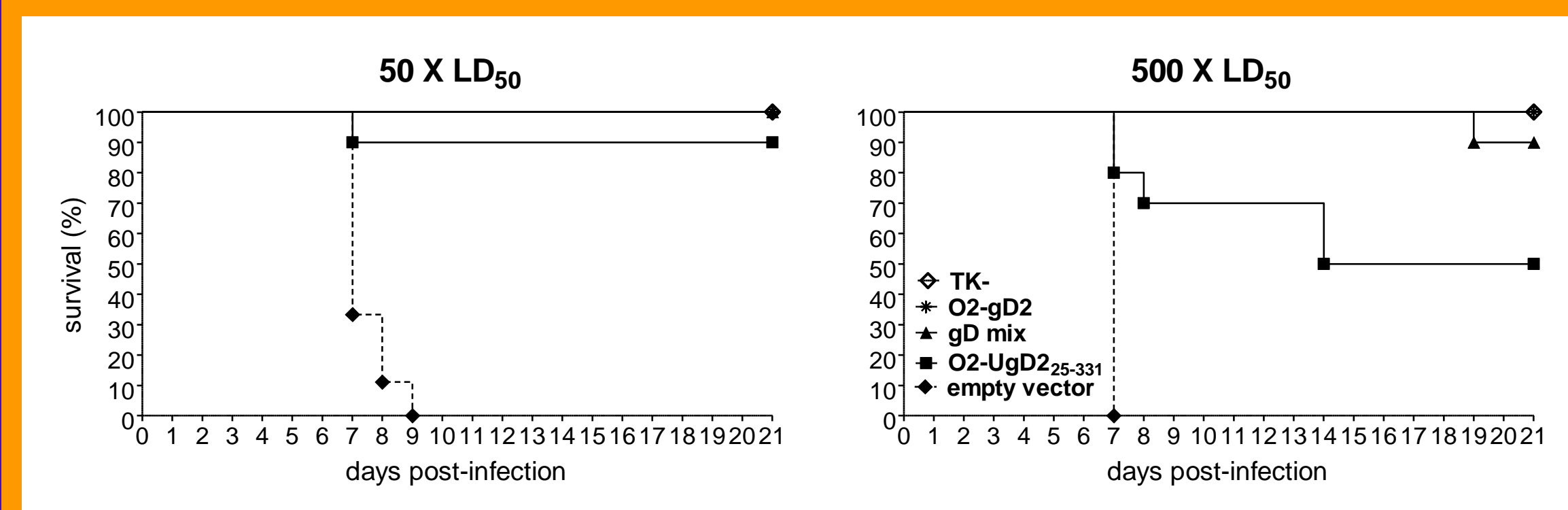


Figure 1. Survival of mice immunized with HSV-2 gD2 constructs after challenge with live HSV-2. Balb/c mice (n =10 per group) were immunized with gD2 constructs and challenged intravaginally with HSV-2. The survival of mice over 21 days following 50 x LD₅₀ or 500 x LD₅₀ viral challenge is graphed. Tk- refers to the positive control replication-competent attenuated HSV-2 vaccine.

- All of the active vaccines gave rise to antibodies specific for gD (Figure 2).

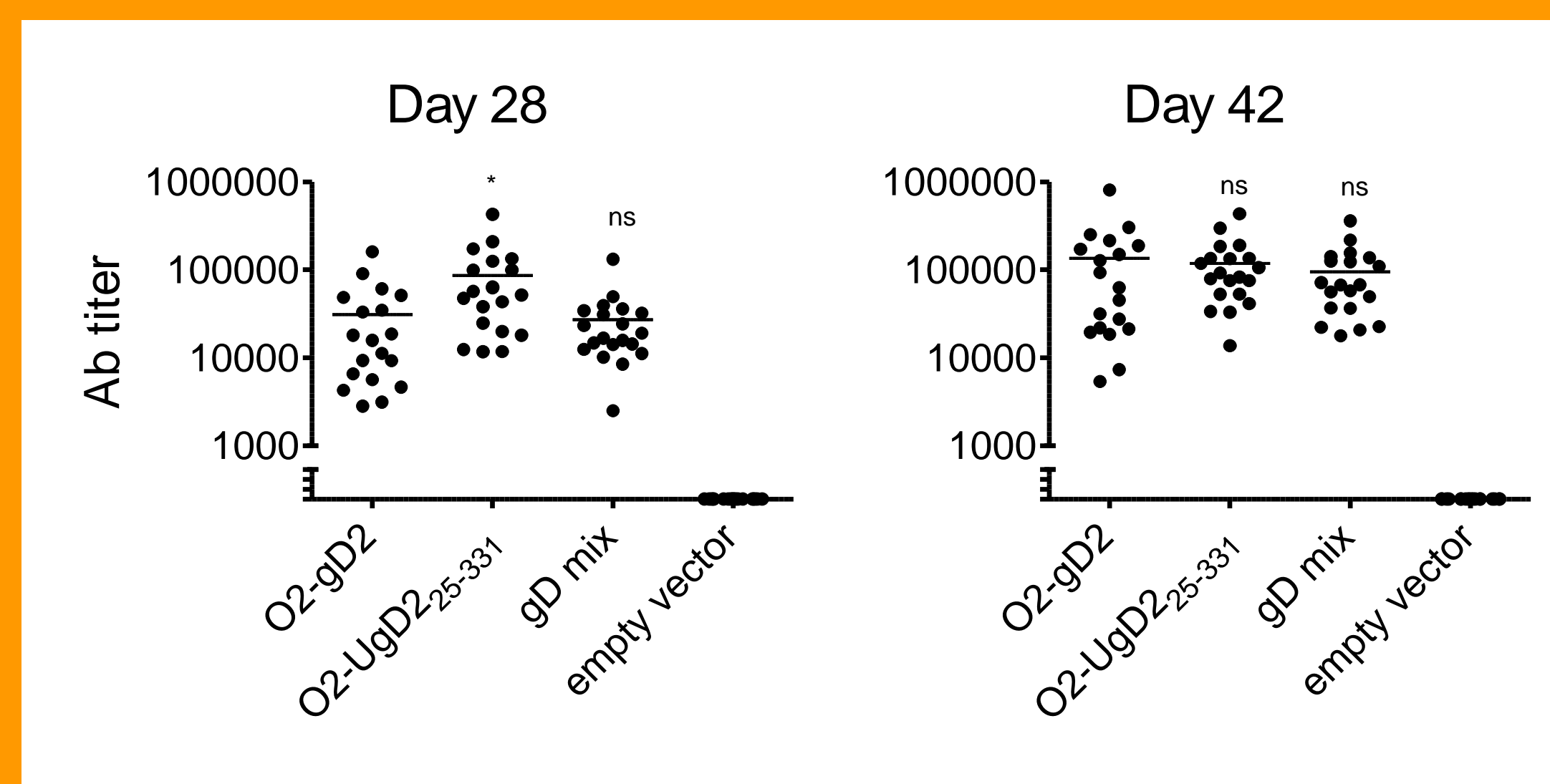


Figure 2. HSV gD IgG antibody responses following immunization with various gD2 constructs. Sera from Balb/c mice immunized with the gD2 expression constructs as in Fig 1 were assayed by ELISA. One-way ANOVA followed by Tukey's Multiple Comparison test was used to compare O2-gD2, O2-UgD2₂₅₋₃₃₁ and the mix. * P<0.05 compared to O2-gD2 alone; ns not significantly different from O2-gD2 alone.

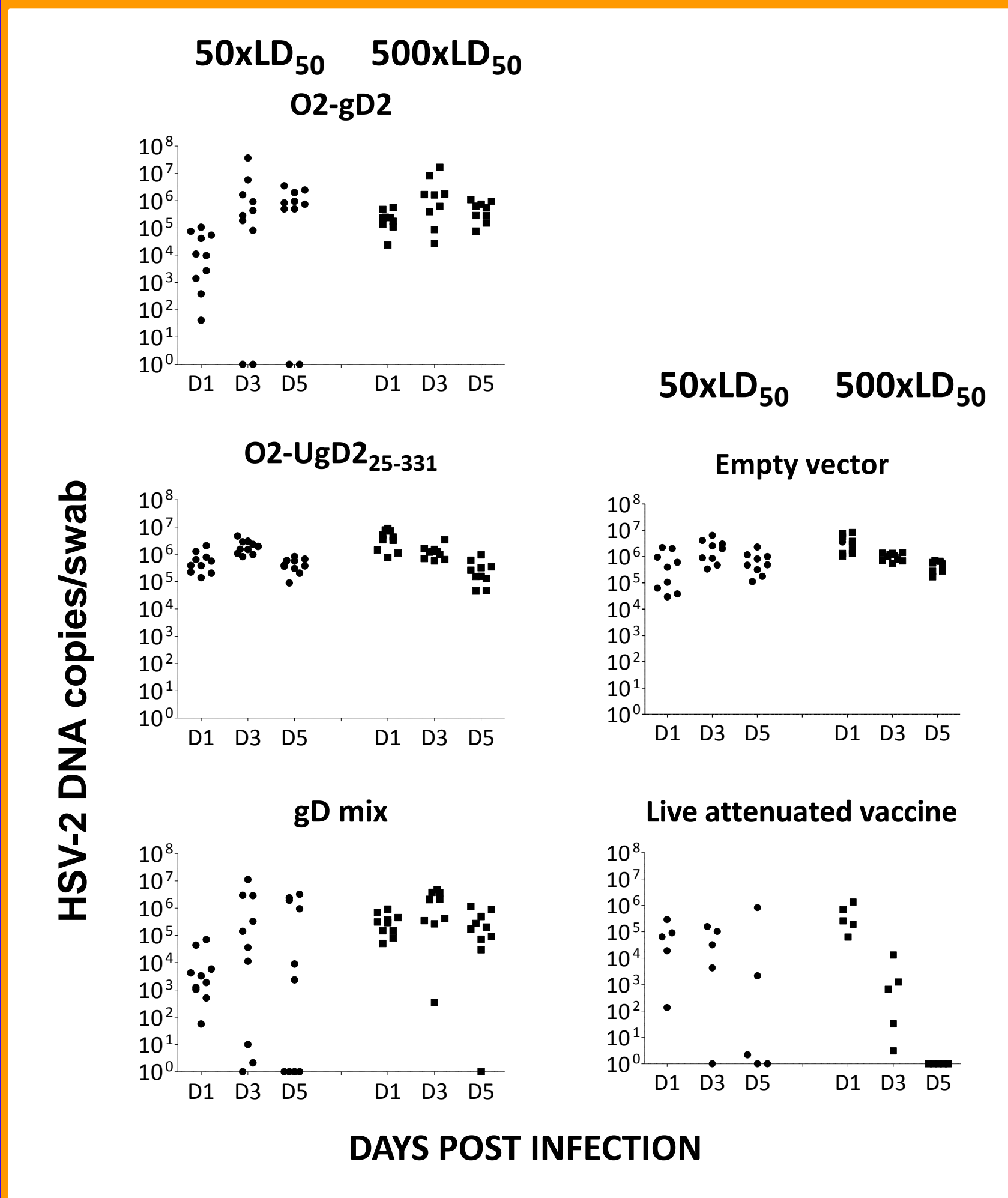
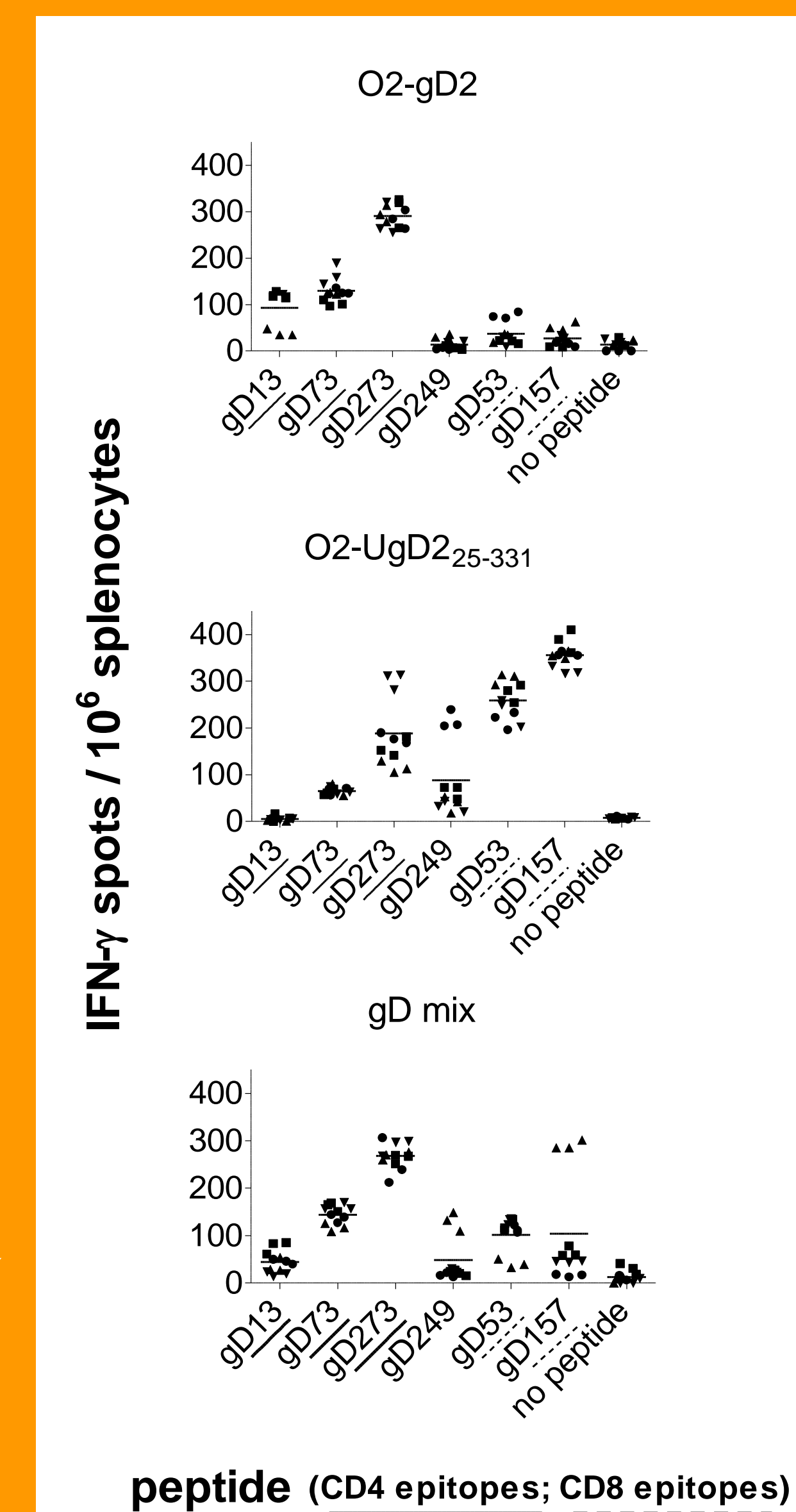


Figure 3. Acute vaginal HSV-2 replication following gD2 vaccination and HSV-2 challenge. Vaginal samples were collected on days 1, 3 and 5 after HSV-2 viral challenge at 50 or 500 x LD₅₀ as shown, from mice immunized with the various HSV-2 gD constructs. HSV-2 DNA copy number in the samples was assessed using real-time PCR at each time point.

- 5 days after the 50x LD₅₀ challenge, the levels of HSV-2 DNA were below the level of detection in 2 of 10 mice in the O2-gD2 alone group and in 4 of 10 mice in the mixed vaccine group (Figure 3). At the higher challenge dose, HSV was not detected in 1 of 10 mice in the mixed vaccine group.

- A mirror immunogenicity experiment was also carried out. The IFN-γ ELISPOT (Figure 4) showed that O2-gD2 induces a strong CD4 response and that O2-UgD2₂₅₋₃₃₁ induces a strong CD8 response. The mixture gave an intermediate profile, that was more similar to the O2-gD2 result than the response induced by the ubiquitinated construct.

Figure 4. HSV-2 peptide specific T cell responses induced in mice immunized with various gD2 constructs. Spleen cells from Balb/c mice immunized with the gD2 expression constructs as for Figure 2 (n=4 per group) were assayed by ELISPOT for IFN-γ secretion in response to gD2 peptides. Peptides including gD2 CD4 epitopes (13, 73, 273) are marked with a solid line. Peptides including gD2 CD8 epitopes (53, 157) are marked with a dashed line. Peptide 249 incorporates CD4 and CD8 epitopes (Muller 2009). Each mouse was run in triplicate with each peptide, and each mouse has a unique geometrical symbol. Control wells with media had < 20 IFN-γ spots/million splenocytes.



Conclusions

- The survival data combined with the HSV-2 vaginal swab data indicate that the O2-gD2 vaccine alone and the mixed vaccine offer the best protection of the three active vaccines.
- Expression of the HSV-2 protein as a fusion with ubiquitin increases CD8 responses
- Correlation of antibody with the survival and vaginal swab outcomes suggests that antibody responses are associated with survival and lower replication. Addition of the ubiquitin construct further reduced vaginal replication with no antagonism for antibody levels or survival.

References

- Kask et al. Vaccine 28:7483 (2010)
Muller et al. J Gen. Virol. 90:1153 (2009)