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POST-CHALLENGE



Figure 1. rPmpD-specific cytokine responses following immunization with different vaccine formulations in C57BL/6 mice. Splenocytes were harvested and stimulated with rPmpD two weeks after the final immunization (**A**, **C**, **E**) or six weeks post-challenge (**B**, **D**, **F**), and assessed for IFN_Y, IL-5 or IL-10 secretion. SLA formulation-specific differences in cytokine profiles are observed. The results are expressed as the mean \pm the standard deviation for groups of 5 mice measured in triplicate from two experiments (*P≤0.05, **P≤0.01, ***P≤0.001 relative to PBS-immunized mice).

PRE-CHALLENGE

POST-CHALLENGE







Day	35	56	35	56	35	56	35	56
Formulation	rPmpD		SLA-AF rPmpD		SLA-SE rPmpD		SLA-LSQ rPmpD	



Figure 2. rPmpD-specific IgG1 and IgG2c responses following immunization with different rPmpD vaccine formulations in C57BL/6 mice. Anti-rPmpD serum IgG1 and IgG2c titres were measured two weeks following penultimate and final immunisations (**A**, **C**) or six weeks post-challenge (**B**, **D**). Results are expressed as the geometric mean \pm the standard deviation for groups of 5 mice measured in triplicate, and are representative of two separate experiments. (* P≤0.05, **P≤0.01).



В





Figure 3. Immunization with rPmpD elicits robust cervico-vaginal antigen-specific IgG titres. C57BL/6 mice were immunized with all rPmpD formulations and mucosal anti-rPmpD IgG1 (A) and IgG2c (B) titres were measured at the indicated time points prior to Ct challenge. Results are expressed as the geometric mean + the standard deviation for groups of 5 mice measured in triplicate, and are representative of two separate experiments. (C) Data are also shown as IgG2c:IgG1 titre ratios two weeks following final immunization, and are representative of two separate experiments (*P≤0.05). Data shown are for the same animals as in Fig.2.



Figure 4. Reactivity of anti-rPmpD and anti-UVEB serum. Serum from mice immunized with rPmpD (A) or UVEBs (B) was tested for reactivity against each antigen in western blots. (C) AntirPmpD serum reacts with UVEBs in an indirect ELISA (inset shows reactivity against rPmpD). (D) Anti-UVEB serum failed to react with rPmpD (inset shows reactivity against UVEBs). Dotted lines represent the OD₄₅₀ cut-off value equivalent to the mean plus two standard deviations of control pre-immune serum.



С

D



Days post challenge

Days post challenge

25

Figure 5. Infection curves showing bacterial shedding following challenge of vaccinated mice. C57BL/6 mice were infected intra-vaginally with *Ct* serovar D/UW3/Cx three weeks post final immunization with either rPmpD or *Ct* serovar E/Bour UVEBs (positive control). Individuals were swabbed on days 1, 3, 7, 14 and 22 post infection, and recoverable cervico-vaginal IFU assessed on Hak cell monolayers. Culture-negative mice were assigned a cut-off value of <10 IFU. Formulation- and antigen-specific differences in time to complete resolution of infection are observed. Results are expressed as the mean \pm the standard deviation for groups of 5 mice.





Figure 6. Quantification of vaccine elicited protection against *Ct* challenge. (**A**) Resistance to infection was assessed by swabbing individuals on day 1 and determining recoverable IFU on Hak cell monolayers. (**B**) Total bacterial load over the 22 day time period was quantified by integrating the area under the shedding curves (depicted in Fig.5) for individual mice. Data were analysed using a one-way analysis of variance (ANOVA) for groups of five mice. Significance values displayed are relative to PBS-immunized mice (*P≤0.05, **P≤0.01, ***P≤0.001).