Immunogenicity and Protective Efficiency in Mice of a Smallpox DNA Vaccine Candidate

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membrane protein L1. For example, mice have been fully protected by a vaccine containing a combination of vaccinia L1, A33, and B5

stained with 1 ml of staining solution (0.1% crystal violet in 20% ethanol). After

BALB/c mice (n=5) were immunized by intramuscular injection with subsequent electroporation. Mice were vaccinated three times at 2-week intervals with the IgM-tL1R plasmid. A control group received the pcDNA 3.1 vector with no insert. ELISA was used to test for the presence of anti-tL1 IgG antibodies in serum samples obtained one week afer each vaccination. As shown in Figure 2A, mice that were vaccinated with a 50 µg dose of IgM-tL1R had a dramatic increase in anti-tL1 IgG over the three weeks following the frst injection. However, no signifcant anti-tL1R IgG levels were detected in the serum samples collected from control mice. As shown in Figure 2B, when 50 µg of DNA was delivered by electroporation, high antibody titers were observed compared to the control group ($p<0.001$). Taken together, these results indicate that electroporation-based vaccination with IgM-tL1R plasmid DNA rapidly induces a tL1-specifc antibody response. In addition, DNA vaccination did not result any weight loss indicating that the mice tolerated the vaccination (Figure 2C). T ese results suggest that our DNA vaccine is safe and immunogenic in an animal model.

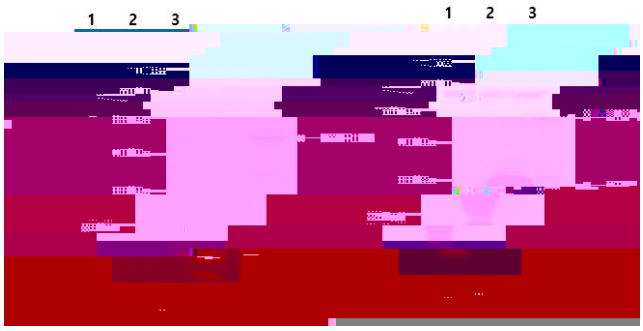

Figure 1B: Immunoblot analysis of EGFP-tL1 fusion proteins. Cells were transfected with plasmids expressing either EGFP-tL1 protein or EGFP. Immunoblots were analyzed with anti-GFP antibody

from mice prior to vaccination. Based on these results, it is clear that a neutralizing antibody response is elicited when mice are vaccinated with IgM-tL1R plasmid.

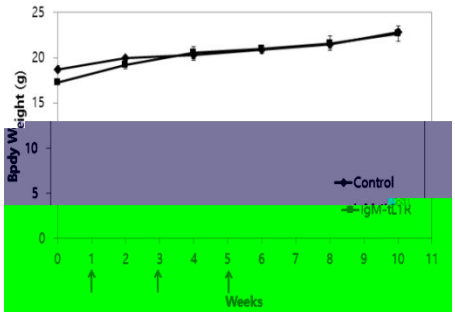

Figure 2C: Body weight change after DNA vaccine administration. Values represent the mean ± standard deviation.

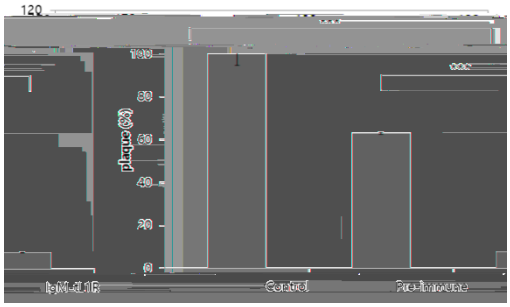

Figure 3: Vaccinia virus-neutralizing antibody response elicited by immunization with IgM-tL1R DNA vaccine. The serum samples (1:10 dilution) were obtained at five weeks post vaccination and evaluated for the presence of neutralizing antibodies against vaccinia virus by a plaque reduction neutralization test. Control group cells were infected but were incubated without serum samples. Values represent the mean ± standard deviation. Significant results are marked with asterisks: *** $p<0.001$.

Antibody isotype profiles elicited by DNA vaccines

To screen the antigen-specific response provided by immunization with IgM-tL1R, both T1 and T2 type immune response were evaluated in immunized mice. The subclass distribution of serum IgG antibodies was analyzed over the course of immunization and was used as an indicator of T1 or T2 bias immune response induced (Figure 4A). It is known that IgG1 is associated with a T2-like response, while a T1 response is associated with the induction of other subclasses [24]. Thus, IgG1/IgG2a ratio was used as indicators of T1 or T2 polarized responses [25,26]. As shown in Figure 4B, IgG1/IgG2a ratios were relatively small in the immunized group (<1.0) compared to those of the pre-immunized group (>1.0). These results illustrate that vaccination with IgM-tL1R induces T1-skewed immune with antibody.

DNA-based vaccines using a truncated form of L1, consisting of the

responses. Both IgG1 and IgG2 were detected after immunization indicating that both T1 and T2 immune systems were stimulated. However, the resulting IgG1/IgG2a ratio was <1.0, suggesting that the T1 response was more prominent, which indicates that IgM-tL1R elicited a cell-mediated immune response. Indeed, induction of both T1- and T2-

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32. Sakhatskyy