Dose-ranging study of a self-amplifying RNA and LNP-carrier vaccine in mice and pigs

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Background

The pandemic of SARS-CoV2 led to the first-time authorization of messenger ribonucleic acid (mRNA) based vaccines that employ synthetic mRNA molecules. Although the approved mRNA vaccines have proven their prophylactic efficacy, a regular booster vaccination is required due to modest longevity of neutralizing antibodies and T cell responses. Additionally, these vaccines have shown to elicit various side effects ranging from local reactogenicity to rare but serious disease. This has revealed a need for the development of vaccine platforms that not only provide the flexibility of the mRNA technology but are also able to balance vaccine immunogenicity and reactogenicity to provide a broad and enduring protection.

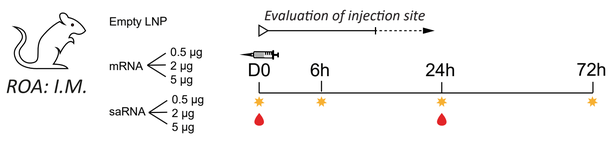
The self-amplifying RNA (saRNA) platform encodes an alpha-viral derived replicase complex and allows the incorporation of multiple antigens by insertion of multiple sub-genomic promoters. Upon cytoplasmic delivery, this replicase amplifies the original RNA-strand and generates multiple copies of sub-genomic RNA. This mechanism leads to a higher and more persistent antigen load inside the host cell at a lower vaccine dosage. This guarantees a better antigen expression timeframe and threshold required for the induction of a potent adaptive immune response. Altogether, these features provide the possibility to use lower dosages and/or less frequent administrations, which in turn provides an advantage in its production cost and risk for side effects.

In the work demonstrated here, we investigated how different doses of our proprietary in-house developed saRNA constructs affect the innate and cellular immune response in outbred mice and pigs.

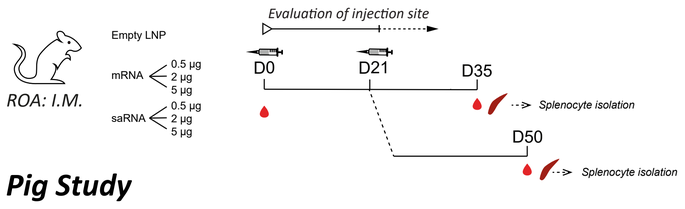
Methodology

Mouse Study

Experiment 1: Innate immune responses with a luciferase reporter in outbred mice.

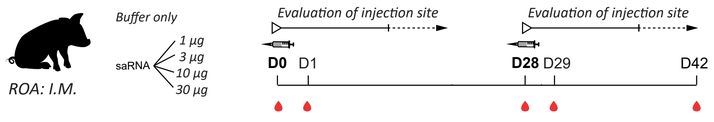


Experiment 2: Adaptive immune responses with a model antigen in outbred mice.



Pig Study

Experiment 3: Innate and adaptive immune responses with a model antigen in outbred pigs.



Results

Mouse study

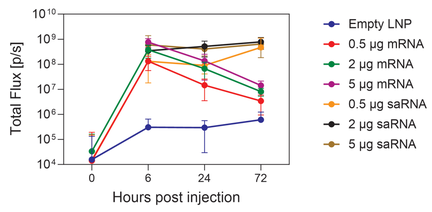


Figure 1 *Luciferase expression after intramuscular injection of different doses of LNP formulated saRNA or conventional mRNA constructs expressing luciferase as a gene of interest in outbred SWISS mice (in vivo imaging, n=6).*

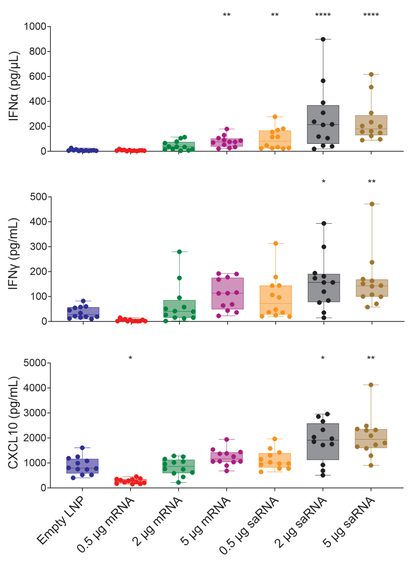


Figure 2 *Interferon alpha (IFNα), IFN gamma (IFNγ) and CXCL10 concentrations as determined in the serum of SWISS mice 24 hours after intramuscular injection of different doses of LNP formulated saRNA or conventional mRNA constructs expressing luciferase as a gene of interest. Constructs that show statistically significant differences (Kruskal-Wallis test) with the empty LNP are indicated with an asterisk (*) p ≤ 0.05, (**) p ≤ 0.01, (***), p ≤0 .001, (****) p ≤ 0.0001 (n=12).*

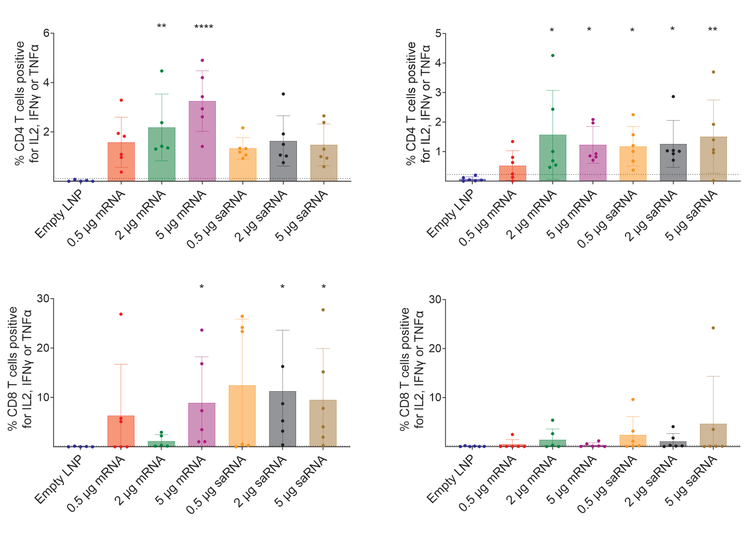


Figure 3 *CD4+ (top graphs) or CD8+ (bottom graphs) T cells isolated 35 (left graphs) or 50 (right graphs) days after the primary intramuscular vaccination with different doses of LNP formulated conventional mRNA or saRNA constructs expressing Influenza hemagglutinin and stimulated with overlapping peptides of the hemagglutinin protein for 2 hours. Subsequent cytokine secretion was blocked by a 4 hour brefeldin A and monensin treatment and stained for flowcytometry. CD4+ or CD8+ T cells expressing either IL2, IFNγ or TNFα were quantified with flow cytometry. The responder threshold (dashed line) was set as the average of the empty LNP + 2xSD. Constructs that show statistically significant differences (Kruskal-Wallis test) with the empty LNP are indicated with an asterisk (*) p ≤ 0.05, (**) p ≤ 0.01, (***), p ≤0 .001, (****) p ≤ 0.0001 (n=6).*

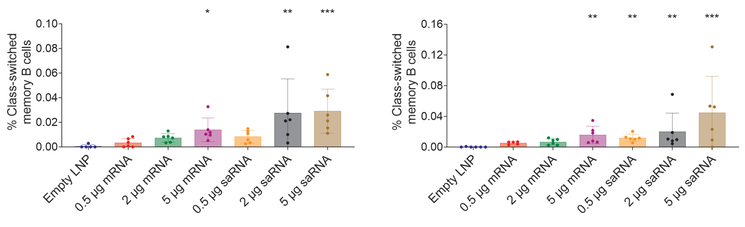


Figure 4 *35 (left graph) or 50 (right graph) days after the primary intramuscular vaccination with different doses of LNP formulated conventional mRNA or saRNA constructs expressing Influenza hemagglutinin, mouse splenocytes were isolated and hemagglutinin-specific, class-switched memory B cells (CD19+CD38+IgD-IgM-CD80+CD273+CD73+Hemagglutinin+) were quantified using flow cytometry. Constructs that show statistically significant differences (Kruskal-Wallis test) with the empty LNP are indicated with an asterisk (*) p ≤ 0.05, (**) p ≤ 0.01, (***), p ≤0 .001, (****) p ≤ 0.0001 (n=6).*

Pig study

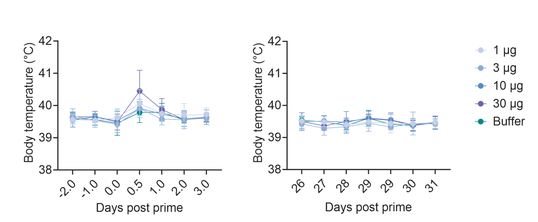


Figure 5 *Pigs were vaccinated with an saRNA construct expressing the SARS-CoV2 spike protein at 4 different doses or buffer-only as a negative control. The rectal body temperature of each animal was determined two days before and three days after the primary (day 0, left graph) and four days after secondary injection (day 28, right graph). SaRNA vaccine doses that exhibit statistically significant differences (2-way ANOVA) with the buffer-only group are indicated with an asterisk (*) p ≤ 0.05, (**) p ≤ 0.01, (***), p ≤0 .001, (****) p ≤ 0.0001 (n=8).*

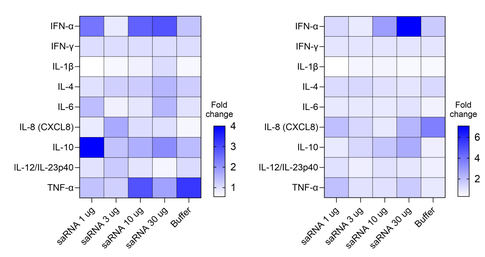


Figure 6 *Serum cytokines were determined right before and 24 hours after primary (left graph) or secondary (right graph) vaccination with an saRNA construct expressing the SARS-CoV2 spike protein at 4 different doses or buffer-only as a negative control. Average fold changes were determined as the cytokine titer after vaccination divided by the titer right before vaccination (n=8).*

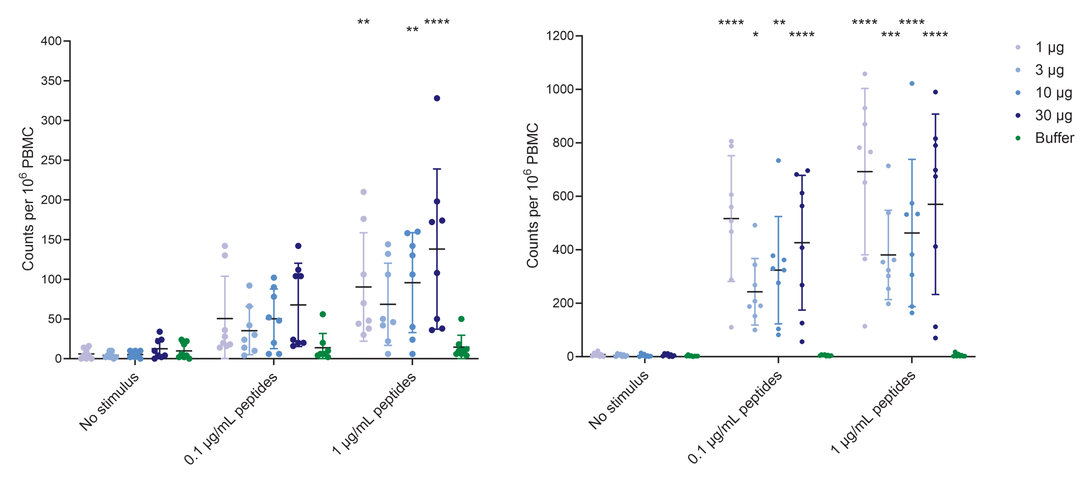


Figure 7 *Following vaccination with an saRNA construct expressing the SARS-CoV2 spike protein at 4 different doses or buffer-only as a negative control, T cell responses were determined by IFNγ ELIspot. PBMC isolated at 28 days (left graph) or 42 (right graph) days post primary injection were incubated with no stimulus (medium only), 0.1 or 1 µg/mL of overlapping peptides of the SARS-CoV2 spike protein for 16 hours. Cells expressing IFNγ were quantified using an IFNγ ELISpot assay and plotted as IFNγ spots per 10^6 PBMC. SaRNA vaccine doses that exhibit statistically significant differences (2-way ANOVA) with the buffer-only group are indicated with an asterisk (*) p ≤ 0.05, (**) p ≤ 0.01, (***), p ≤ 0.001, (****) p ≤ 0.0001 (n=8).*

Conclusion

These proof-of-concept data in mice and pigs confirm that our novel saRNA-based vaccines maintain their immunological potency at lower doses and even provide potential for further dose reduction. By continuing to optimize our saRNA vaccine platform, we aim to develop a new generation of vaccines able to balance immunogenicity and reactogenicity and as such helping us to be prepared for emerging pathogen outbreaks and potential future pandemics.