Accelerated kinetics of transient lung pathology with a pronounced Th2 immune response following SARS-CoV-2 infection in hamsters vaccinated with formalin-inactivated, Alum-adjuvanted whole virus

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Background

The COVID-19 pandemic induced a global effort to develop SARS-CoV-2 vaccines. One of the concerns regarding vaccine safety is the potential manifestation of vaccine-associated enhancement of disease (VAED). Here, the potential of the Syrian hamster as a model for VAED was investigated. Post SARS-CoV-2 challenge, clinical, virological, pathological and immunological outcomes were assessed in hamsters vaccinated with a formalin-inactivated, alum-adjuvanted SARS-CoV-2 vaccine.

In a first study, four different vaccine regimens (single vs prime-booster vaccination with either 5 or 0,5 µg vaccine preparation) were compared with a non-vaccinated (Placebo) group. Results revealed:

- Partial to no protection of clinical disease, minimal reduction of viral loads in lungs and no evidence for disease enhancement in vaccinated groups;
- Enhanced histopathology in all vaccination groups as compared with the vaccinated group at DPI5, which resolved by DPI13;
- Undetectable neutralizing antibodies in all but 2 (out of 20) hamsters prior challenge;
- Upregulation of Th2 cytokines in lungs at DPI5 (IL-4 and IL-13).

In a second experiment (presented here), the pathology kinetics and immunological signatures of vaccinated and non-vaccinated hamsters were evaluated. A group of re-challenged hamsters was used a naturally-protected control.

Methods

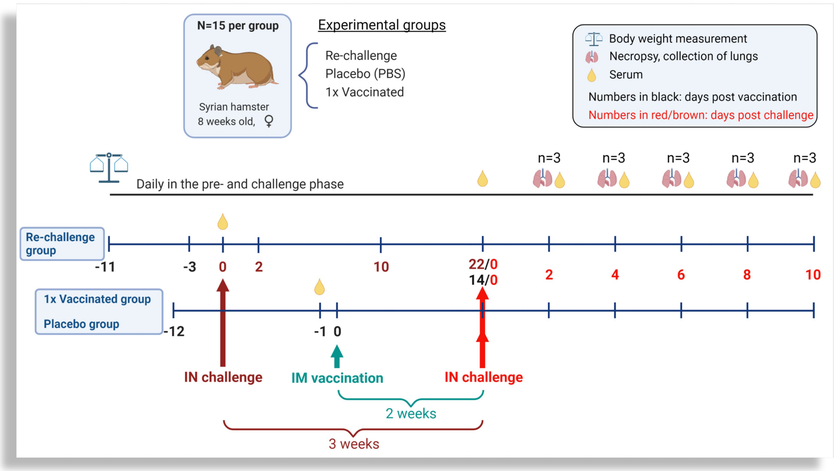

Figure 1. Experimental setup. Vaccine – purified formaldehyde-inactivated SARS-CoV-2 (prototype, D614G) formulated with 1% Alum, dose - 5 µg. Challenge virus – prototype SARS-CoV-2 (D614G)

Conclusions

- Following challenge infection with a homologous strain, hamsters vaccinated with formalin-inactivated SARS-CoV-2 adjuvanted with Alum developed lung pathology of similar magnitude but with accelerated kinetics as compared to non-vaccinated hamsters
- The lung pathology of vaccinated hamsters is characterized by prolonged and more pronounced perivascular infiltration with inflammatory cells
- Th2 cytokines are transiently upregulated in the lungs of vaccinated hamsters
- The hamster model and the assays we developed can be used as a benchmark for assessing vaccine-associated pathology when testing the safety of novel SARS-CoV-2 vaccines

Results

Clinical and virological outcomes

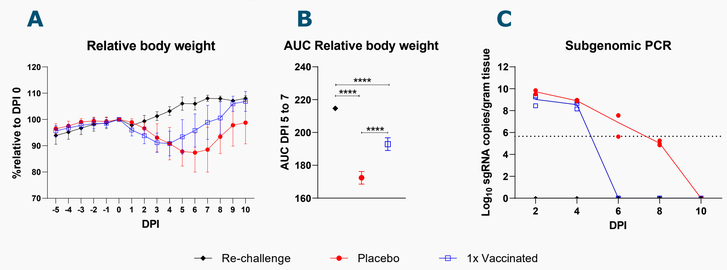

Figure 2. Body weight loss and viral loads in lungs following SARS-CoV-2 challenge infection. A) Relative body weight over time expressed as percentage of body weight at the day of challenge (days post infection (DPI) 0) (n=15 up to DPI 0 and decreasing with 3 hamsters on every even DPI from DPI 2 onwards). **B)** Area under the curve (AUC) of DPI 5 to DPI 7 (lowest relative body weight of the Placebo group) (n=6). **C)** Viral subgenomic RNA loads in lungs (indicative of viral replication). **A and B)** Symbols show group means. **C)** Symbols show individual values and lines illustrate group means. Error bars in plot **A** show SD (standard deviation), and in plot B - 95% CI (confidence intervals). The dotted line represent test detection limit. Significant differences between groups are shown with asterisks (p< 0.0001 - ****).

- The vaccine provided partial protection from body weight loss and virus replication in lungs.
- No evidence for clinical vaccine-associated enhancement was observed.
- Re-challenged animals were protected from body weight loss and no viral replication was detected in lungs.

Pathological outcomes

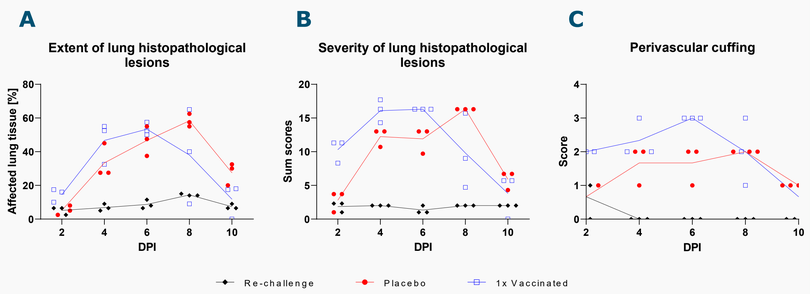

Figure 3. Histopathological lesions in lungs. A) Extent of histopathological lesions, expressed as percentage of the lung area of a slice of the whole left lung lobe. **B)** Severity of histopathological lesions, expressed as sum of 6 different individually scored parameters per hamster per DPI. **C)** Scores of the perivascular pro-inflammatory cell infiltration (cuffing) over time. Symbols show individual values and lines illustrate group means.

- Lung histopathology in vaccinated hamsters peaked earlier post infection (DPI 2 to 6) than in the placebo-treated hamsters and decreased at later time points.
- Histopathology of the placebo group reached the same magnitude at DPI 8.
- Perivascular infiltration with inflammatory cells (cuffs) was consistently more pronounced in vaccinated hamsters.

Immunological outcomes

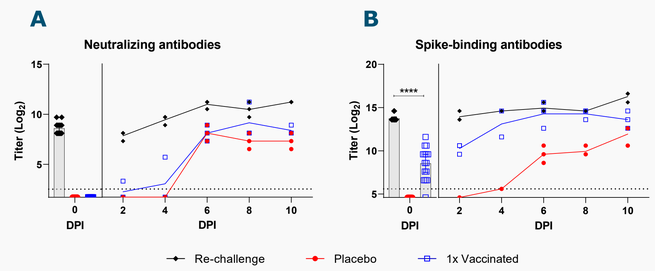

Figure 4. Serological responses. **A)** Neutralizing antibody titers, as determined by a microneutralization assay (MN50). **B)** Spike-binding IgG antibody titers as determined by an in-house ELISA. Symbols show individual values and lines illustrate group means. The dotted lines represent the detection limit of each test. Significant differences between groups are illustrated with asterisks (p < 0.0001 - ****).

- No neutralizing antibodies were detectable in the vaccinated hamsters before challenge, while 14 out of 15 animals had detectable Spike-binding antibodies.
- Re-challenged hamsters had high and homogenous titers of neutralizing antibodies and higher titers of Spike-binding antibodies than the vaccinated hamsters.

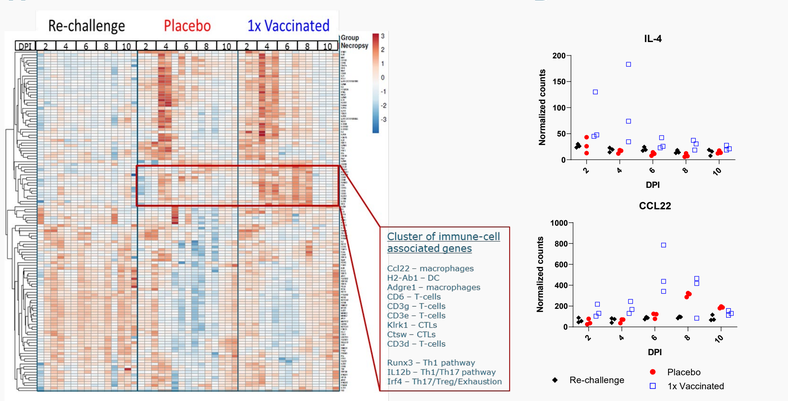

Figure 5. Gene expression profiles. A) Heat map of n=128 target genes. Gene expression was determined with Nanostring technology. Genes were selected based on relevant pathway annotations provided in the nCounter® CAR-T (Chimeric antigen receptor T-cell) Characterization Panel (mainly Th1, Th2, Th17, Treg) and annotations related to different immune cell types (based on human and/or mouse). **B)** Th2-related cytokine (IL-4) and chemokine (CCL22) expression as measured by Nanostring technology.

- Overall, gene expression profiles for placebo and vaccinated groups were similar, and both groups differed from the re-challenged group.
- A cluster of genes associated with immune cells showed prolonged upregulation in the vaccinated group (correlating with the perivascular cuffing in those animals, Fig. 3C).
- IL-4 and CCL22 were consistently upregulated in the vaccinated hamsters.

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