Influenza A Virus Internal Proteins as Vaccine Targets

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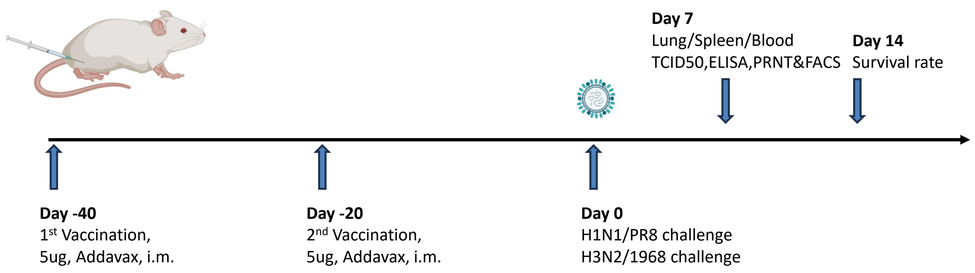
Background

Influenza is a constant threat to public health and significant measures have been taken to prepare for the potential emergence of a novel pandemic strain. However, seasonal vaccination has partial efficacy with the continued need for annual vaccination due to immune waning and antigenic drift for the vaccine to be updated to representative strains. Thus, there is a dire need for a universal influenza vaccine that utilizes more conserved antigen targets. Compared with the canonical vaccine target influenza A viruses (IAV) haemagglutinin (HA), proteins like the nucleoprotein (NP), non-structural proteins (NS) and polymerase acidic (PA) are highly conserved and may provide even greater immune mediated protection against not only homologous but also heterologous infection through vaccination. We conducted a **S**ingle IAV Recombinant **P**roteins **I**mmunized **M**ice **S**tudy (SPIMS) to investigate the question. Findings of the study will provide valuable information for the development of vaccines for the prevention of future possible influenza virus spill over infection.

Methods



5 mice per group were vaccinated with 5ug H1N1/PR8 virus protein (HA/NP/NS1/PA/PB1/PB2, PBS as control) with Addvax at volume ratio of 1:1 through intramuscular injection. 2 doses of vaccinations were given before infection with H1N1/PR8 or H3N2/1968 virus at 10 times of LD50 of the naïve mice. 7 days after infection, the lung, spleen, and blood were collected for TCID50(50% tissue culture infectious dose), ELISA(enzyme-linked immunosorbent assay) , PRNT(Plaque reduction neutralization test) & ICS(Intracellular cytokine staining). Another parallel group of mice with the same vaccination and infection conditions were monitored for 14 days after infection for survival rate

Results

- **H1N1/PR8 HA and NP protein vaccination showed higher survival probability upon H1N1 and H3N2 infection, respectively**
- **HA protein vaccinations can limit homologous H1N1/PR8 replication in the lung, but not H3N2/1968**

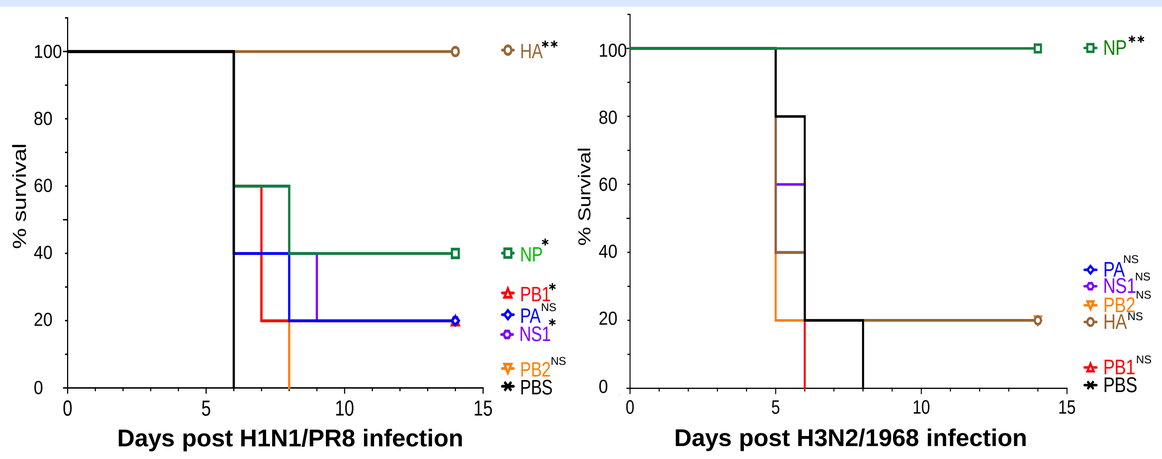


Figure 1. Survival of vaccinated mice infected with homologous and heterologous influenza viruses. Statistical comparison with the PBS vaccinated group with Log-rank (Mantel-Cox) test and Gehan-Breslow-Wilcoxon test, **P value<0.01, * P value<0.05, NS not significant. N=5 mice/group. Virus intranasally inoculated at 10LD50.

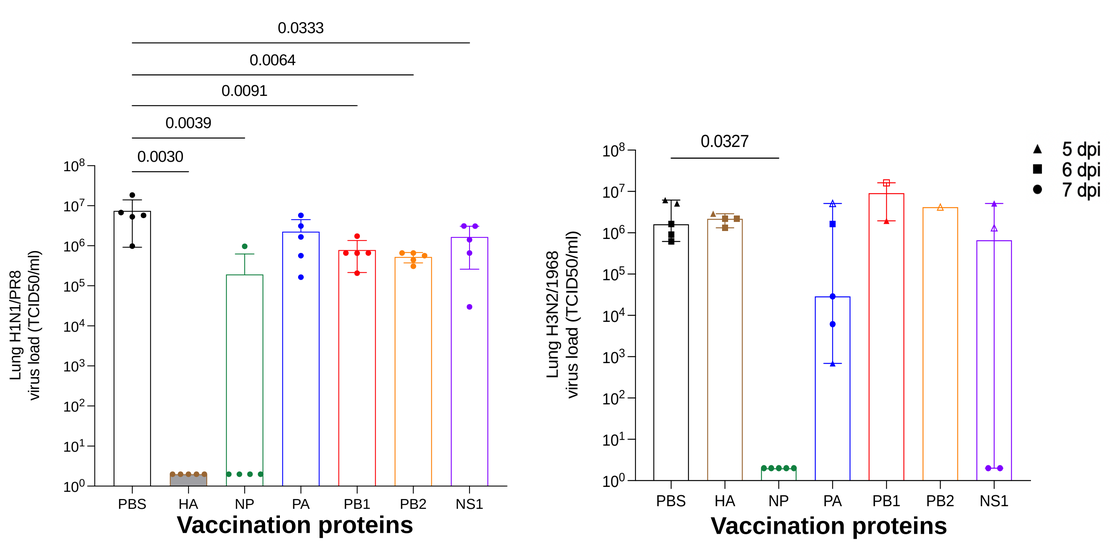


Figure 2. Lung viral loads. N=5 mice/group, 7dpi for H1N1/PR8 infection; Different numbers of mouse in each group and different time point (as per legend) for H3N2/1968 infection due to high lethality. See the labels. One-way ANOVA versus PBS.

- **HA and NS1 vaccination were best at eliciting IgG to homologous and heterologous virus challenge.**
- **NP vaccination contributed certain levels of Ab protection to heterologous virus challenge.**

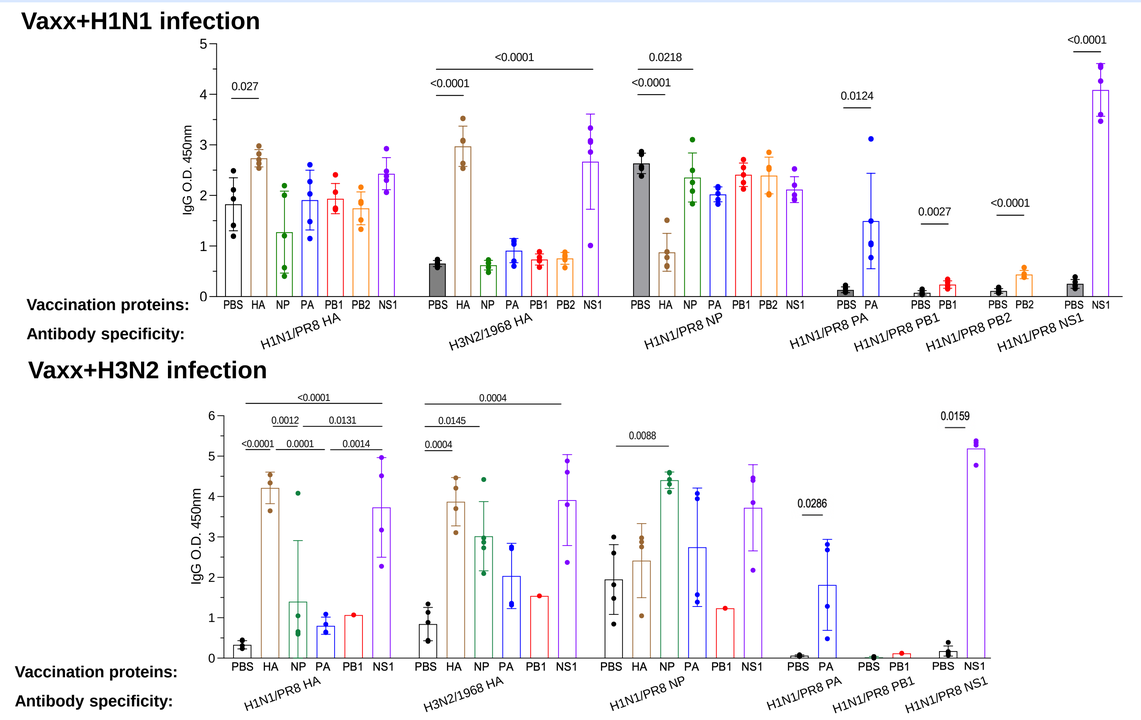


Figure 3. IgG antibody responses following infection of vaccinated mice. Serum samples at 1 week post infection with H1N1/PR8 or H3N2/1968, were tested from vaccinated mice by ELISA for IgG specific to HA, NP and matching proteins to their vaccination. N=1 to 5 mice per group. Data shown in mean ± SD, Two-way ANOVA versus PBS.

- **H1N1/PR8 protein vaccinations prevented the virus production by interfering the H1N1/PR8 virus entrance, replication and release.**
- **Vaccination with H1N1/PR8 NP protein elicited Abs that can limit the entrance of H3N2/1968 virus.**

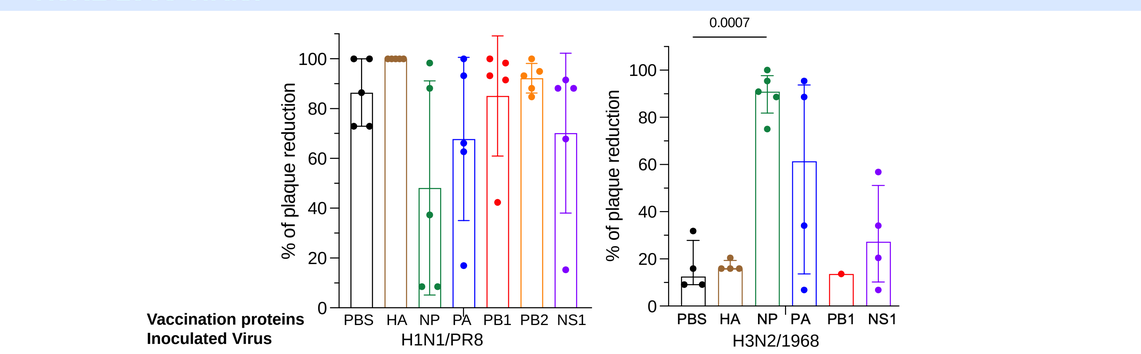


Figure 4. Plaque reduction by vaccine antibodies. Serum from vaccinated and infected mice 1 week post infection. Incubated with 200TCID50/30 µL of virus on MDCK cells and counted 2 days after. One-way ANOVA. The replication and release stages of both virus were suppressed by all groups with the existence of the serum by PRNT. Data not shown here.

- **NP protein vaccination produced significant IFNγ CD4⁺ and CD8⁺ T cell response magnitude upon H1 stimulation and CD8⁺ T cells upon H3 stimulation**
- **High CD8⁺ IL2 and TNF expression to H1 and H3 stimulation.**

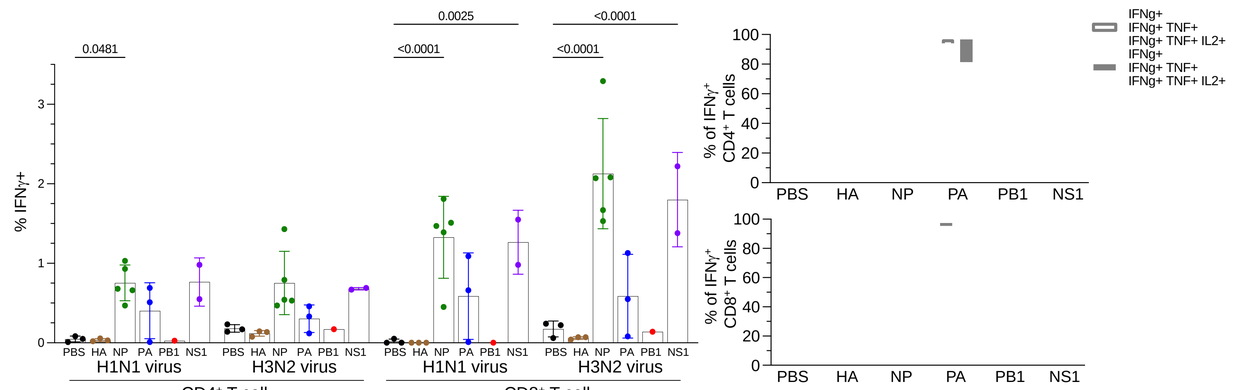


Figure 5. Spleen T cell cytokine response to H1N1/PR8 and H3N2/1968 virus stimulation. Splencoytes from 1 week post infection were stimulated with the same and different challenge virus to assess cytokine production by T cells by intracellular cytokine staining and acquired by flow cytometry. H1 shown in open bar. H3 in closed bar. Data shown in mean ± SD, Two-way ANOVA versus PBS.

Summary

1. Surface HA protein provides highest homologous protection, and remains our best target compared to other viral targets tested here.
2. H1N1/PR8 NP proteins vaccination reduced heterologous H3N2/1968 viral load and is an attractive vaccine target due to high conservation.

➔ Internal proteins due to high conservation are attractive vaccine targets and showed antibody boosting upon infection and T cell responses for NP and NS1.

Acknowledgements

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