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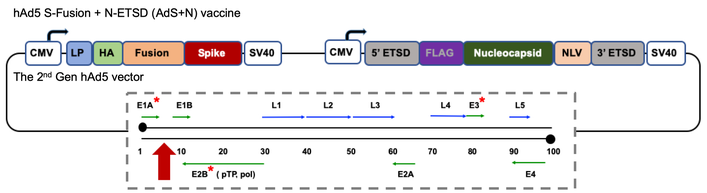
S saRNA PRIME, S+N DNA HETEROLOGOUS VACCINATION ELICITS ROBUST IMMUNE RESPONSES IN MICE

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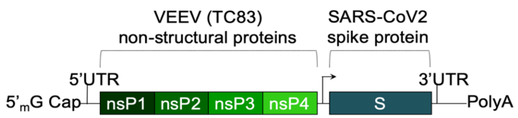
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BACKGROUND

To address the need for a vaccine regimen that is efficacious against SARS-CoV-2 variants, we assessed effects of heterologous vaccination [1] with an adenovirus(Ad)-vectored DNA-based vaccine and a self-amplifying RNA-based (saRNA) vaccine. The human Ad serotype 5 (hAd5)-vectored dual-antigen vaccine (below) [1] encodes Wuhan strain or 'wild type' (wt) SARS-CoV-2 spike (S) protein modified with a fusion sequence to enhance cell-surface expression and nucleocapsid (N) protein with an enhanced T-cell stimulation domain to increase the potential for MHC class I and II stimulation.



The saRNA vaccine is the Infectious Disease Research Institute's S(wt) SASA S vaccine (below) delivered in a nanostructured lipid carrier. The S sequence is codon-optimized with a D614G mutation, a di-proline modification for stabilization in the pre-fusion conformation, and a tri-glutamine (3Q) mutation inactivating the furin cleavage site.



METHODS

Outbred CD-1 mice received homologous or heterologous Day 0 prime > Day 21 boost vaccination with the AdS+N and/or SASA S vaccines as shown below. Sera and splenocytes were collected on Day 35. Serum was screened by ELISA for IgG subclasses raised against N and S (wt and Delta) variants, and for pseudovirus neutralization versus Wuhan (wt), Beta, and Delta variants. T-cells were screened by ELISpot and ICS for cytokine production.



N #	Code	Prime	Dose (route)	Boost Dose	Dose (route)
4	UnTx	Untreated		untreated	
7	AdS+N > AdS+N	AdS+N	$1x10^{10}$ VP (SC)	AdS+N	$1x10^{10}$ VP (SC)
6	SASA S > SASA S	SASA S	10 µg (IM)	SASA S	10 µg (IM)
7	AdS+N > SASA S	AdS+N	$1x10^{10}$ VP (SC)	SASA S	10 µg (IM)
7	SASA S > AdS+N	SASA S	10 µg (IM)	AdS+N	$1x10^{10}$ VP (SC)

Blood collection
Vaccination
Euthanasia: isolation of serum; collection of splenocytes

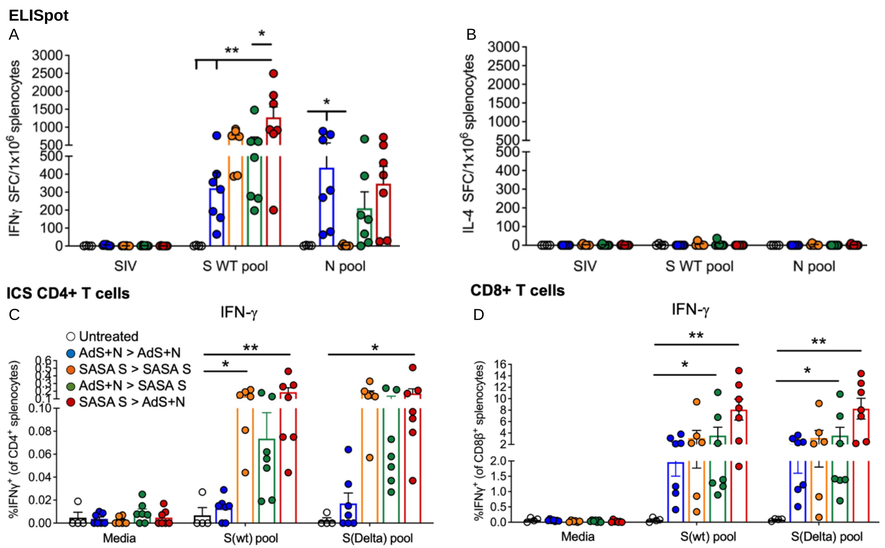
Heterologous hAd5 spike (S) + nucleocapsid (N) & S saRNA (SASA S) vaccination generated IgG against N & both wild type & Delta S1.

T-cell responses to both S (wt) & Delta were highest with the SASA S prime > AdS+N boost

Potent Wuhan, Beta & Delta strain pseudovirus neutralization was seen with sera from SASA S vaccinated mice

RESULTS

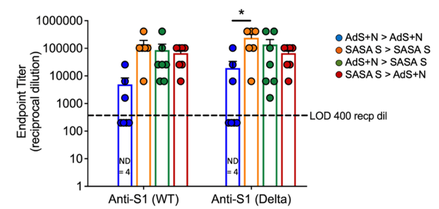
- In both ELISpot (A, B) and ICS (C, D), T-cell responses were the highest with the SASA S prime > AdS+N boost, and were skewed toward T_H1, as shown below.
- CD4+ and CD8+ T-cell responses to S(wt) and S(Delta) peptides in ICS were similar (C, D).



Statistical analyses performed using one-way ANOVA with Tukey's post-hoc comparison of groups; where *p ≤ 0.05 and **p < 0.01. Data graphed as the mean and SEM.

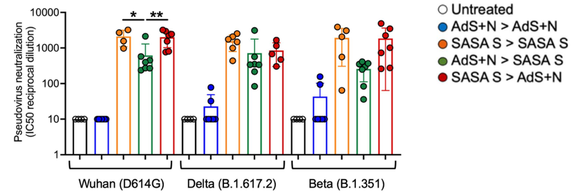
RESULTS

- Anti-S(wt) IgG levels were highest with any vaccine regimen that included the SASA S vaccine and only combinations that included the AdS+N vaccine elicited production of anti-N IgG. All humoral responses were T_H1 biased as reflected by the IgG1/IgG2a+ IgG2b+IgG3 ratios. (data not shown)
- Endpoint IgG titers of anti-S1(wt) and anti-S1 (Delta) were similar for each vaccine regimen (below).



Values were < LOD in 4 /7 AdS+N > AdS+N mice. Statistical analyses performed using one-way ANOVA with Tukey's post-hoc comparison of all groups; * p = 0.0333; sera with anti-S1 IgG < LOD were assigned a value of 200 (1/2 the LOD). Data graphed as the mean and SEM.

- Sera from mice vaccinated with any regimen including the SASA S vaccine were highly and similarly neutralizing of Wuhan, Delta, and Beta strain pseudoviruses (below).



Statistical analyses performed using one-way ANOVA with Tukey's post-hoc comparison of groups where *p ≤ 0.05 and **p < 0.01 (Statistical analysis for IC50 values < the LOD was not performed). Data graphed as the mean and SEM.

CONCLUSIONS

- The best overall immune responses were seen with SASA S prime followed by AdS+N boost, which displayed robust anti-S IgG generation with potent neutralization capacity, very high levels of S-specific T-cells, as well as N-specific IgG and T-cell responses [2].
- These data support the continued investigation of heterologous vaccination to confer protection against SARS-CoV-2.

ADDITIONAL KEY INFORMATION

References: [1] Gabitzsch E, et al. (2021) Front Immunol 12:10.3389/fimmu.2021.729837;
[2] Rice A, *et al.* bioRxiv:2021.11.29.470440, 2021

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