Novel immunogen design elicits increased protection against avian H7N9 influenza that is associated with mobilization of seasonal influenza T cell memory

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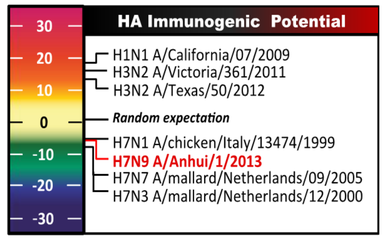
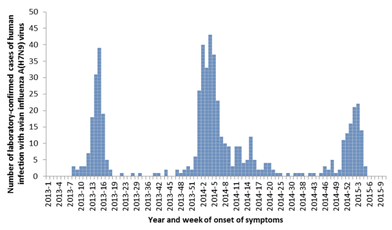
Introduction

A new avian-origin influenza virus emerged near Shanghai in February 2013. Human-to-human transmission of avian-origin H7N9 influenza A has been limited to a few family clusters, but the high mortality rate (27%) associated with human infection has raised concern about the potential for this virus to become a significant human pathogen.

Previously, H7 HA-containing vaccines have been poorly immunogenic.

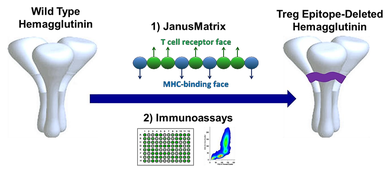
- We used well-established immunoinformatics tools to estimate the immunogenic potential of H7N9 proteins.
- HA proteins derived from human-derived H7N9 strains contain fewer T cell epitopes than most other circulating strains of influenza.
- Conservation of T cell epitopes with other strains of influenza was very limited.
- Based on our analysis, avian-origin H7N9 2013 appears to be a "stealth" virus.
- Improved vaccines for emerging influenza are urgently needed.





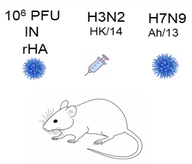
Methods

- Sequence analysis of the two faces of H7N9 T cell epitopes revealed sequences with potential to activate Tregs.
- Immunoassays confirmed an H7N9 Hemagglutinin (HA) sequence that increases the frequency of Tregs and suppresses effector responses (Liu *et al*. (2015) *Human Vaccines & Immunotherapeutics*, 11:2241-2252).



- ***Site-specific modifications were engineered in H7N9 HA to delete this Treg-activating epitope and to harness memory CD4 effector T cells.***
- OPT1 (three amino acid substitutions replaced a reported regulatory T cell (Treg) epitope with a highly conserved and broadly reactive CD4+ T cell epitope from H3-HA) and OPT2 (integrates six H3-HA CD4+ T cell epitopes, one Treg epitope removed).

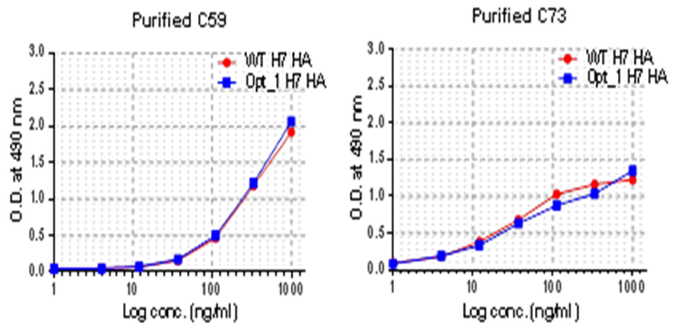
Immunization



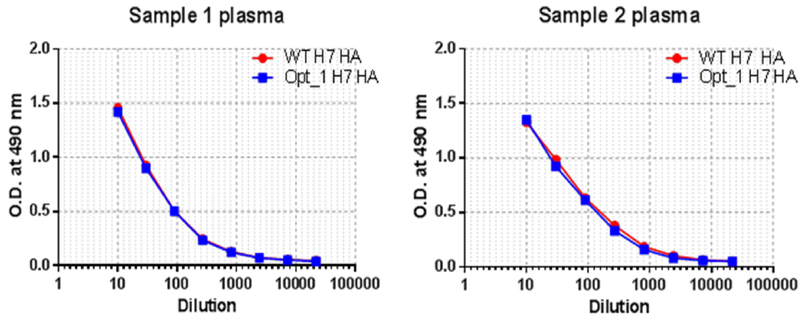
Vaccination studies were carried out in HLA-DR3 transgenic mice pre-immune to H3N2 (A/Hong Kong/4108/2014).

- Mice received two H3N2 exposures (mucosal and systemic), followed by three rHA vaccinations.
  Mice were primed and boosted twice IM with wildtype or engineered H7N9 rHA without adjuvant eight weeks post-H3N2 exposure.
- Mice were challenged IN with H7N9 virus (A/Anhui/1/2013) four weeks following boost and followed for weight loss and survival.

Engineered H7-HA maintained antigenic structures

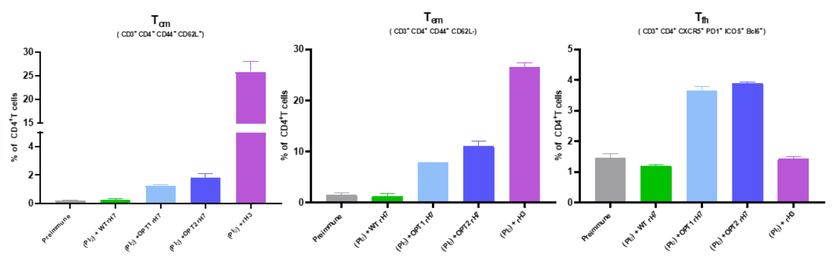


Previously identified monoclonal antibodies against WT rH7-HA also recognize Opt_1 rH7-HA



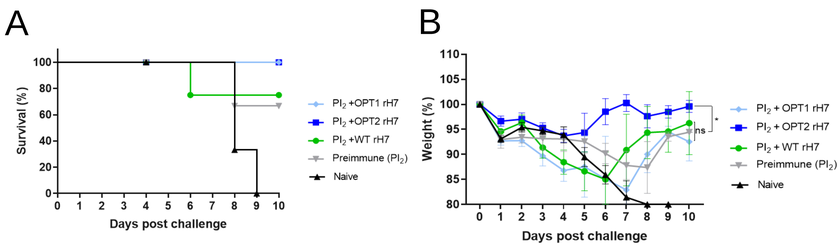
Antibodies from two patients with previously identified anti H7-HA antibodies also recognize Opt_1 rH7-HA

OPT1 and OPT2 rH7 enhance memory T cell response



Analysis by flow cytometry uncovered that, compared to preimmune controls, OPT1 and OPT2 vaccinations upregulated the population of Tcm (left), Tem (middle) and Tfh (right).

OPT1 and OPT2 vaccines protect against lethal H7N9



OPTimized vaccines protected against lethal H7N9 virus infection while in wildtype vaccinated animals had a survival rate similar to unvaccinated pre-immune controls. OPT1 and OPT2 lowered average weight loss post-infection with OPT2 animals maintaining >95% of their weight.

Conclusions

- Treg epitope deletion preserves H7N9 HA antigenicity.
- Epitope-driven approaches to vaccine design that involve careful consideration of the T cell subsets primed during immunization are a promising means of enhancing vaccine efficacy.
- Collectively, we find increased vaccine-induced protection against a pandemic influenza strain is associated with enhanced T cell immunity elicited by a novel immunogen design strategy that harnesses seasonal influenza CD4+ T cell memory.
- Structure-guided T cell epitope modification may also be useful to develop more effective vaccines against seasonal influenza strains.

References

1. Liu R, Moise L, Tassone R, Gutierrez AH, Terry FE, Sangare K, Ardito MT, Martin WD, De Groot AS. H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance. Hum Vaccin Immunother. 2015;11(9):2241-52.
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