Influenza Group 2 HA stem-only nanoparticles induce heterotypic immune response

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Introduction

The influenza virus is responsible for a disease burden of 25-30 million cases leading to 150,000 hospitalizations and 30,000-40,000 deaths in the United States each year.[1] Currently, the composition of the influenza vaccine must be modified each season due to the antigenic drift of the head region of surface glycoprotein hemagglutinin (HA). Current vaccines, which target the HA head, only provide immunity against matching influenza strains.[2]

In order to design a vaccine that would protect across HA type to combat both seasonal antigenic drift and pandemic strains that result from antigenic shift, our lab has devised a HA stabilized-stem nanoparticle. We have previously demonstrated that group 1 HA-stem alone presented on a self assembling 24-mer nanoparticle, ferritin can provide broad heterosubtypic protection[3]. Here, we have designed, stabilized and expressed group 2 HA-stem on a 60-mer lumazine synthase nanoparticle.

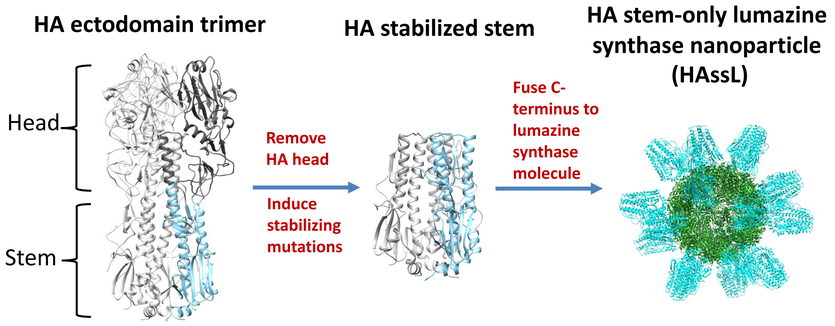


Figure 1. Ribbon diagram depicting construction of HAssL (right). Lumazine synthase is shown in green.[4]

Goals

- To assess antigenicity and immunogenicity of H7 (group 2) HA-stem lumazine synthase nanoparticles (H7ssL)

Methods

H7ssL: Expression and purification:
HA stem fused to lumazine synthase nanoparticles were expressed in mammalian Expi293 cells. Nanoparticles were purified using a lectin affinity column followed by size exclusion chromatography. SDS-PAGE gel electrophoresis was performed on purified particles.

Antigen characterization:
Antigens (H1, H3, H5, and H7) were expressed with His-Tag in Expi293 cells and purified using a nickel affinity column. Proteins were eluted with 300mM imidazole and then subjected to size exclusion chromatography. SDS-PAGE gel electrophoresis was performed on purified proteins.

Immunizations:
Female BALB/c mice were given intramuscular injections of 2 µg H7ssL, 2 µg H7ssF (ferritin np control), or empty np. Groups were boosted at 4 and 8 weeks. Sera was collected at 2, 6, and 10 weeks.

Antibody response analysis:
To measure the antigenicity of H7ssL, purified nanoparticles were tested for binding to FI6, CT149, C6261, and C8020 monoclonal Abs through ELISA. To measure the immunogenicity of H7ssL, purified influenza antigens(H1, H3, H5, and H7) were coated on plate and used to quantitatively measure immune response in immunized mice. Immune response was characterized using endpoint ELISA.

Results

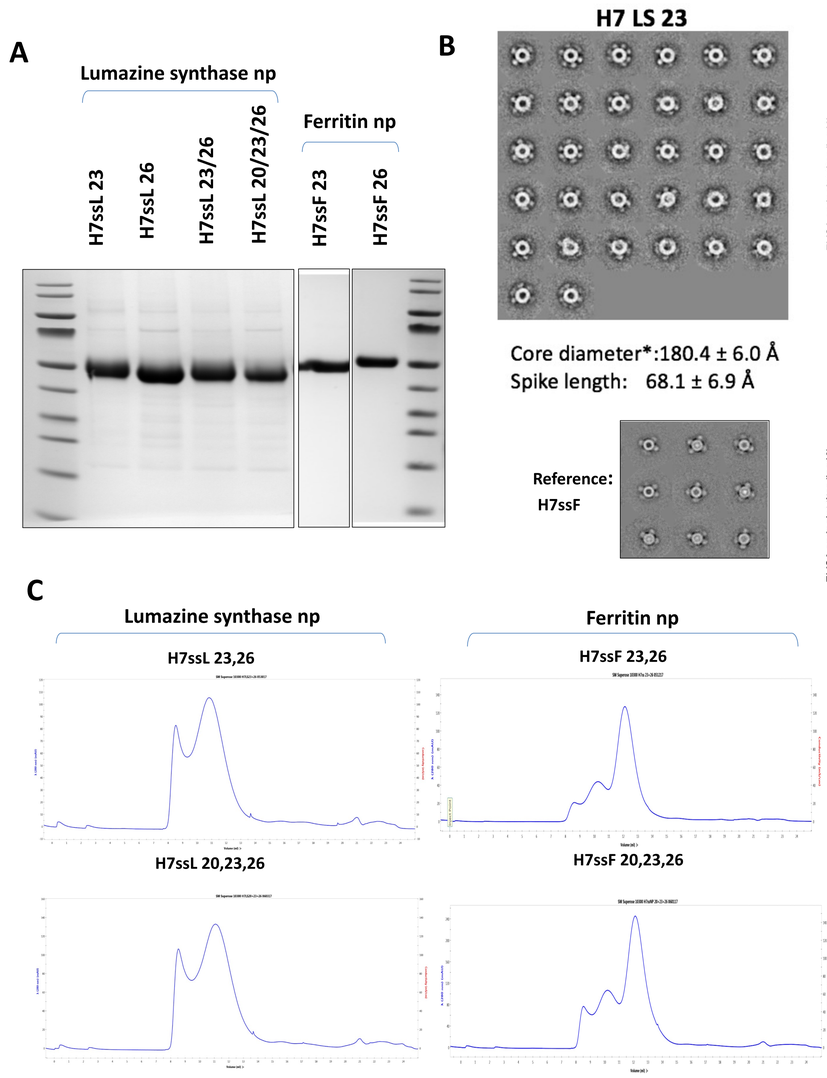
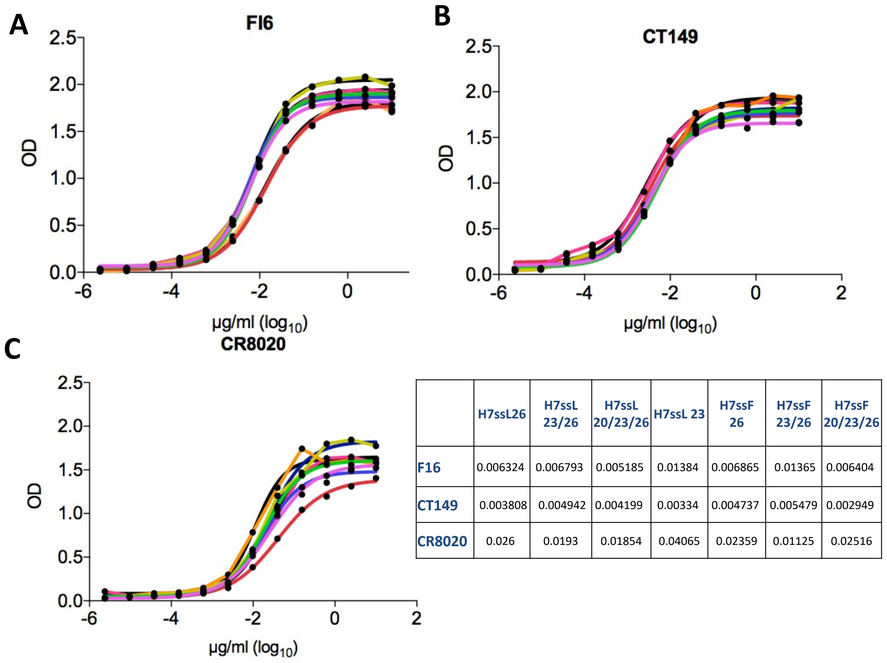


Figure 2. Purification and Expression of H7-LS-np. (A) SDS-PAGE showing purified H7-LS-np compared to H7ssF (ferritin-based np). **(B)** Electron micrograph (EM) analysis of H7ssL compared to reference H7ssF **(C)** Size exclusion profiles of H7ssL compared to H7ssF using Fast Protein Liquid Chromatography.



	H7ssL26	H7ssL 23/26	H7ssL 20/23/26	H7ssL 23	H7ssF 26	H7ssF 23/26	H7ssF 20/23/26
F16	0.006324	0.006793	0.005185	0.01384	0.006865	0.01365	0.006404
CT149	0.003808	0.004942	0.004199	0.00334	0.004737	0.005479	0.002949
CR8020	0.026	0.0193	0.01854	0.04065	0.02359	0.01125	0.02516

Figure 3. Antigenicity of H7ssL particles. The nanoparticle's antigenicity was assessed through binding to mAbs (A) FI6 (B) quaternary Ab CT149 and (C) HA Group 2-specific CR8020.

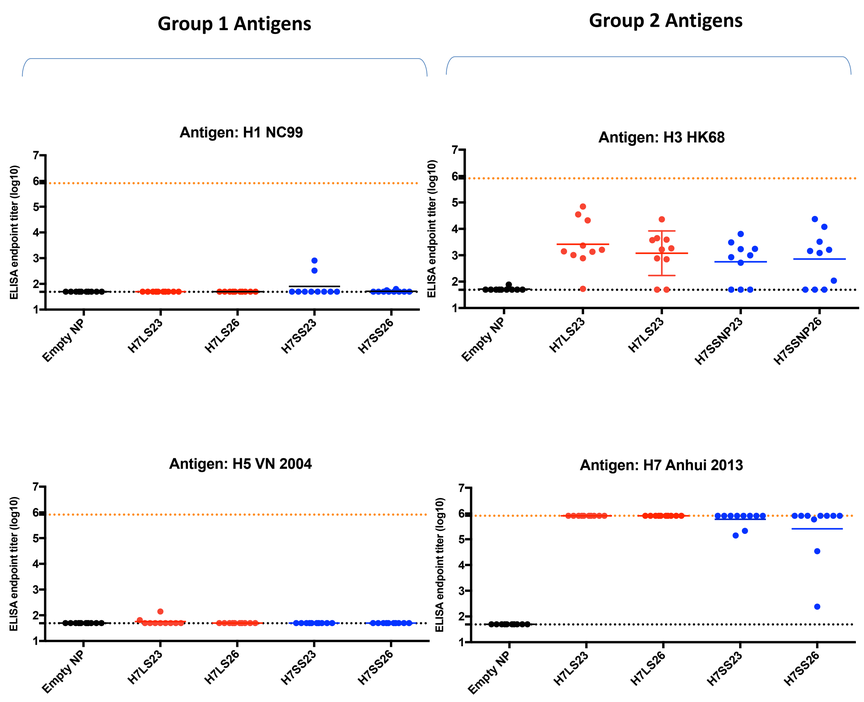


Figure 4. Immunogenicity of H7ssL in Balb/c mice after third immunization. H7ssL elicits strong Ab response against H7, and heterosubtypic response against H3 antigen. Response against Group 1 antigens was not observed.

Conclusion

- HA stem-only lumazine synthase molecules were expressed and purified
- EM images displayed spikes with a core diameter of 180.4 ± 6.0 Å and spike length of 68.1 ± 6.9 Å
- H7ssL displayed significantly more spikes on the np surface compared to H7ssF
- Antigenicity analysis demonstrated strong binding to monoclonal antibodies. Binding to quaternary antibody CT149 shows that the HA stem is in correct structural confirmation.
- H7ssL induced heterosubtypic immune response after three immunizations

Bibliography

1. "Disease Burden of Influenza," CDC (2017). Web.
2. Graham, B.S., "Universal Influenza Vaccines." (2015)
3. Kanekiyo, M. et Al., ""Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies." (2015)
4. Figure modified from Yassine, et. Al.,(2015) and Dr. Jeffrey Boyington.

Acknowledgements

I would like to thank all members of the Graham Lab at the VRC, the NIH Summer Internship Program, and the NIH Office of Intramural Training and Education.