

H5N1 Avian Influenza Recombinant Protein Vaccine is Immunogenic in Mice and Induces HAI and Neutralizing Antibodies

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Abstract #799

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Abstract

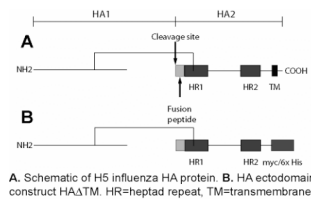
Background. The emergence of highly pathogenic H5N1 avian influenza viruses in human populations that lack significant levels of pre-existing immunity constitutes a public health emergency. Traditional inactivated vaccine production methods limit the capacity to produce enough vaccine for the general population. A recombinant protein subunit vaccine could overcome many of these obstacles. We sought to develop a novel method for producing highly pure recombinant H5 influenza hemagglutinin (HA) that would be immunogenic in a mouse model. Methods. We constructed a DNA plasmid that exhibited high levels of expression of H5N1 HA in mammalian cells. This construct was used to develop a novel strategy to produce a highly pure, soluble H5N1 HA (HADTM). We then immunized mice with adjuvanted HADTM protein. Results. Recombinant HADTM was greater than 95% pure and formed trimers similar to native H5 HA of the correct predicted molecular weight. HADTM was glycosylated and was resistant to trypsin digestion, showing that it formed stable trimers. HADTM was detected in immunofluorescent and immunoblot assays by human serum obtained from a recipient of H5N1 vaccine, suggesting that HADTM retained elements of native conformation. Mice immunized with a single dose of HADTM mounted serum antibody responses that specifically reacted with HADTM in both ELISA and immunoblot assays. The antibody titer of HADTM-immunized mice was greater than that of human H5N1 vaccinees in both assays, suggesting that HADTM is highly immunogenic. Conclusion. Biochemical and immunologic analysis of HADTM protein showed that it retained important features of native H5 HA protein. The next experiments we will perform will be to determine serum virus neutralizing hemagglutination-inhibition titers and to determine cell-mediated immunity in the HADTM-vaccinated mice. HADTM protein may be a potential vaccine candidate for human studies.

Methods

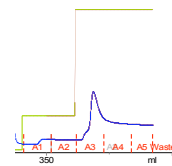
- Sequence-optimized native H5 HA gene
- Generated transmembrane-truncated construct (HA Δ TM)
- Cloned into pcDNA3.1/myc/his expression vector
- Transfected into 293 Freestyle cells
- Purified by IMAC chromatography
- Concentrated and dialyzed

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Transmembrane-truncated construct (HA Δ TM)



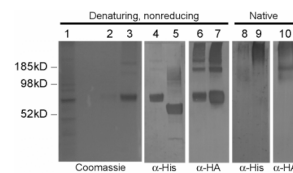
HA Δ TM expresses efficiently in mammalian cells and is secreted



Chromatogram of IMAC purification of HA Δ TM. Imidazole concentration is plotted over UV absorbance of eluted fractions.

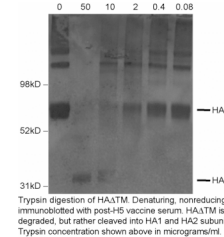
- HA Δ TM elutes as a highly pure single peak
- Yield 0.5–1 mg purified HA Δ TM per 30 ml culture supernatant

HA Δ TM forms monomers under denaturing conditions and trimers under native conditions



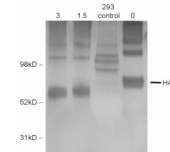
Analysis of H5 HA Δ TM by PAGE (Lanes 1-3) and immunoblot (Lanes 4-10). 1, mock-transfected 293F supernatant; 2 and 3, successive elution fractions of column-purified H5 HA Δ TM; 4, H5 HA Δ TM immunoblot with anti-His; 5, hMPV F Δ TM protein control; 6 and 7, successive eluted fractions of H5 HA Δ TM immunoblot with anti-H5 HA Mab; 8, H5 HA Δ TM immunoblot with anti-His Mab; 9, hMPV F Δ TM protein control; 10, H5 HA Δ TM immunoblot with anti-H5 HA Mab.

HA Δ TM forms trypsin-resistant stable trimers



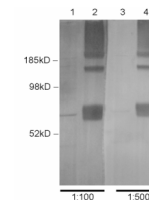
Trypsin digestion of HA Δ TM. Denaturing, nonreducing gel immunoblotted with post-H5 vaccine serum. HA Δ TM is not degraded, but rather cleaved into HA1 and HA2 subunits. Trypsin concentration shown above in micrograms/ml.

HA Δ TM is N-glycosylated



Peptide N-glycosidase F digestion of HA Δ TM. Denaturing, nonreducing gel blotted with post-H5 vaccine serum. PNGase F concentration shown in units.

Human serum from H5N1 vaccinee detects HA Δ TM



Denaturing, nonreducing gel immunoblotted with serum from 80-year old subject vaccinated with 2 doses of inactivated H5N1 vaccine. Lanes 1 and 3, mock-transfected 293 cell supernatant. Lanes 2 and 4, H5 HA Δ TM.

Intramuscular HA Δ TM is immunogenic in mice

Animal	Homagglutination inhibition Titer	Reciprocal neutralizing titer	ELISPOT	
			Virus	H5 Δ TM
1	3	<25	13	3
2	12	<25	3	20
3	24	<25	13	3
4	192	437	20	37
5	<3	<25	47	57
6	192	188	7	30
7	<3	<25	27	13
8	3	<25	10	10
9	3	<25	27	40

HAI, serum neutralizing antibody titers and ELISPOT reactivity of BALB/c mice to H5N1 virus. Mice were immunized intramuscularly with H5 HA Δ TM adjuvanted with Titermax Gold (Sigma) on day 0 and 14. Serum and splenocytes were harvested on day 28 and tested for HAI and in vitro neutralizing activity by standard methods, using the live-attenuated vaccine strain. ELISPOT was performed by standard methods using stimulation with either live virus or H5 HA Δ TM and is expressed as spot-forming cells/million cells. Control unimmunized animals showed no reactivity.

Intraperitoneal HA Δ TM is immunogenic in mice

Animal	Reciprocal neutralizing titer
1	<25
2	<25
3	400
4	80
5	75
6	125
7	110
8	<25
9	<25

Serum neutralizing antibody titers of BALB/c mice to H5N1 virus. Mice were immunized intraperitoneally with H5 HA Δ TM adjuvanted with Monophosphoryl Lipid A (MPL) on day 0. Serum was obtained on day 14 and tested for in vitro neutralizing activity against the live-attenuated H5 vaccine strain. Control unimmunized animals showed no activity.

Conclusions

- HA Δ TM is highly expressed in mammalian cells, is glycosylated and forms trimers
- HA Δ TM appears to retain elements of native conformation
- HA Δ TM is immunogenic in mice and induces HAI and neutralizing antibodies
- HA Δ TM offers potential as a subunit vaccine for avian influenza