Generation of avian influenza A DIVA vaccines with chimeric hemagglutinin recombinant viruses

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Abstract

In order to produce a dually effective vaccine against H9 and H5 avian influenza viruses that aligns with the DIVA (differentiating infected from vaccinated animals) strategy, we generated a chimeric H9/H5N2 recombinant vaccine that expressed the whole HA1 region of H9N2 and the HA2 region of recent highly pathogenic avian influenza (HPAI) H5N8 viruses. The chimeric H9/H5N2 virus showed *in vitro* and *in vivo* growth properties and virulence that were similar to those of the LPAI H9 virus. An inactivated vaccine based on this chimeric virus induced serum neutralizing (SN) antibodies against both H9 and H5 viruses but induced cross-reactive HI antibody only against H9 viruses. Thus, this suggests its compatibility for use in the DIVA strategy against H5 strains. Furthermore, each HA1- and HA2 stalk-specific antibody response was sufficient to inhibit viral replication and protect the chimeric virus immunized chicken and mice from lethal challenge with both H9N2 and HPAI H5N1 viruses. Taken together, these results demonstrate that the novel chimeric H9/H5N2 recombinant virus is a low pathogenic virus, and this chimeric vaccine is suitable for a DIVA vaccine with broad spectrum neutralizing antibody against H5 avian influenza viruses.

Introduction

Current influenza virus killed vaccines predominantly induce anti-hemagglutinin (anti-HA) antibodies that are commonly strain specific in that the antibodies have potent neutralizing activity against homologous strains but do not cross react with HAs of other influenza virus subtypes. In contrast, the HA2 stalk domain is relatively well conserved among subtypes, and recently, broadly neutralizing antibodies against this domain have been isolated. Therefore, in light of the need for a vaccine strain that applies the DIVA strategy utilizing an HI assay and induces broad cross-protection against H5N1 and H9N2 viruses, we generated a novel chimeric H9/H5N2 virus that expresses the entire HA1 portion from the H9N2 virus and the HA2 region of the heterosubtypic H5N8 virus. The chimeric H9/H5N2 recombinant vaccine protected immunized hosts against lethal challenge with H9N2 and HPAI H5N1 viruses with significantly attenuated virus shedding in immunized hosts. Therefore, this chimeric vaccine is suitable as a DIVA vaccine against H5 avian influenza viruses.

Methods & Results

1. Plasmid constructions

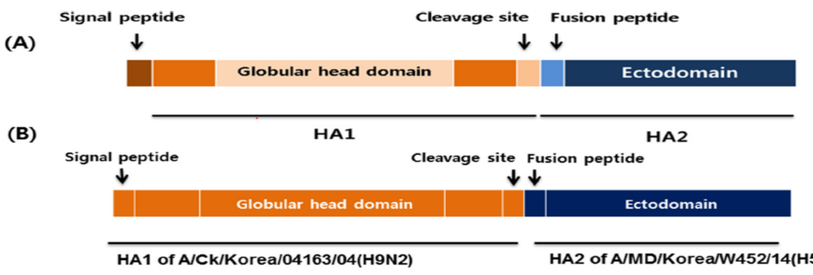

Fig 1. Construction strategy for the chimeric cHA H9/H5N2 plasmid and alignment of HA2 regions.

2. Vaccination and Challenge

A/CK/Korea/04163/04 (H9N2), cHA H9/H5N2 (H9N2) were used for whole inactivated vaccine strains twice with two weeks interval.

Fig. 2: Two weeks after the second vaccination, HI, SN or ELISA titers were observed.

Fig. 3: Two weeks after last vaccination, wild type H9N2 or HPAI H5N8 viruses were inoculated to chickens.

Fig. 4 and 5: Infected viral virus titers were measured in various specimens in vaccinated birds

Fig. 6 and 7. cHA H9/H5N2 vaccine efficacy was confirmed in mouse model.

Immunization and experimental infection of chickens

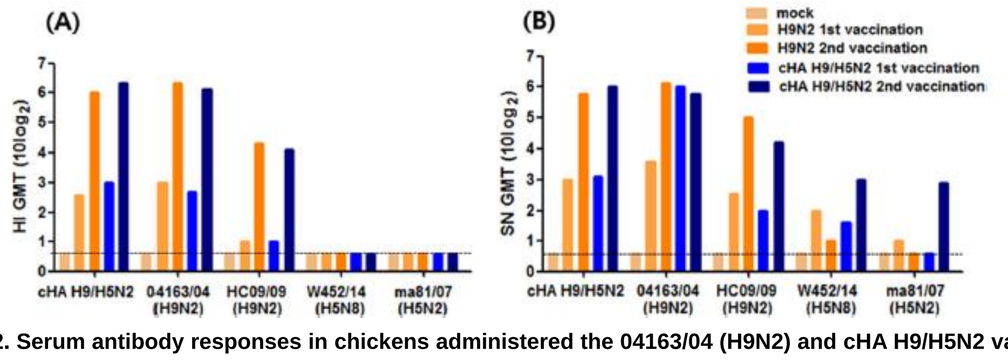

Fig 2. Serum antibody responses in chickens administered the 04163/04 (H9N2) and cHA H9/H5N2 vaccines.

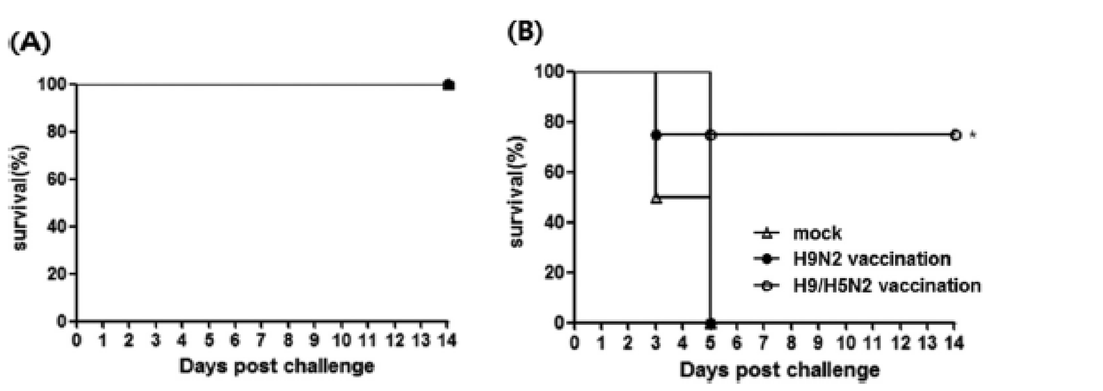

Fig 3. Survival rates of H9N2- and cHA H9/H5N2-vaccinated chickens against H9N2(A) and HPAI H5N8 virus (B)

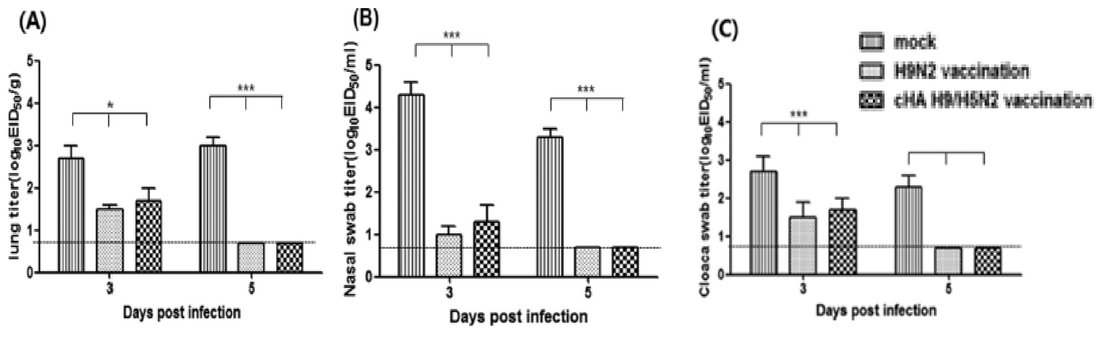

Fig 4 .Viral titrations in H9N2- and cHA H9/H5N2-vaccinated chickens following challenge with the H9N2 virus. Virus titers were measured with chicken embryonated eggs with lungs(A), nasal swabs (B) and cloaca swabs(C). The limit of virus detection was set to 0.7 log10 EID50/g or 0.7 log10 EID_{50}/ml. Statistical significance compared to mock vaccination was determined by a *t test (*, P 0.05; **, P 0.001; ***, P 0.0001).*

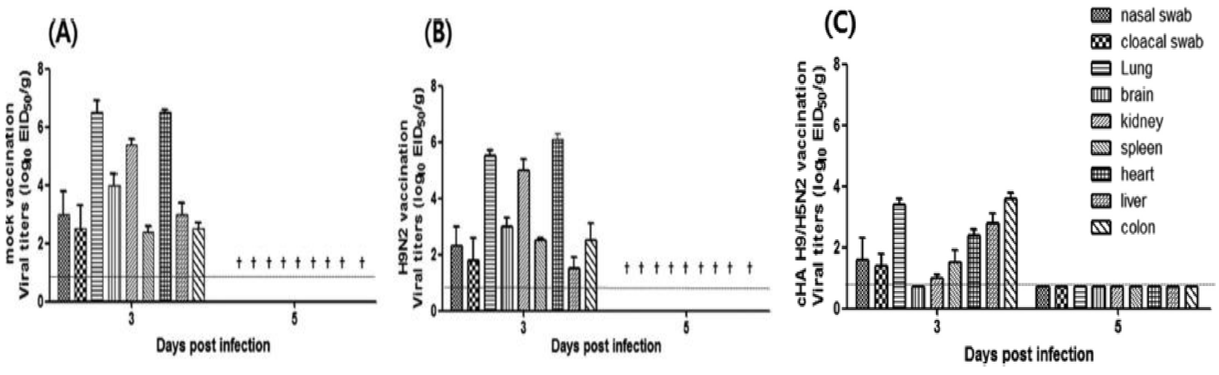

Fig 5. Viral titers in H9N2- and cHA H9/H5N2-vaccinated chickens following challenge with HPAI H5N8 virus of PBS-alum (A), H9N2 (B), and cHA H9/H5N2 (C) vaccine groups. The limit of virus detection was set to 0.7 $\log_{10}$ EID_{50}/g. † indicates that there were no samples collected because the chickens in this group died.

Immunization and experimental infection of chickens

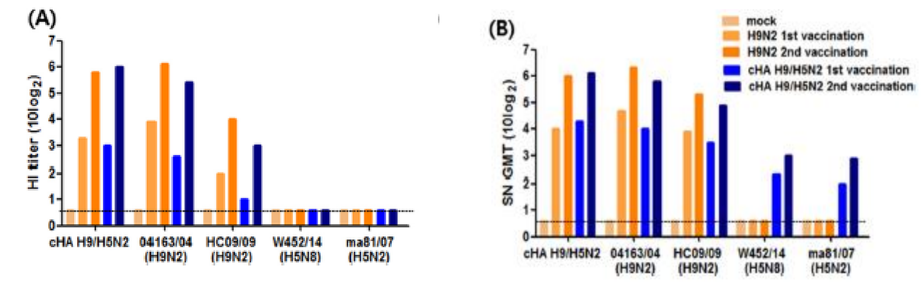

Fig 6. Serum antibody responses in mice administered the 04163/04 (H9N2) and cHA H9/H5N2 vaccines.

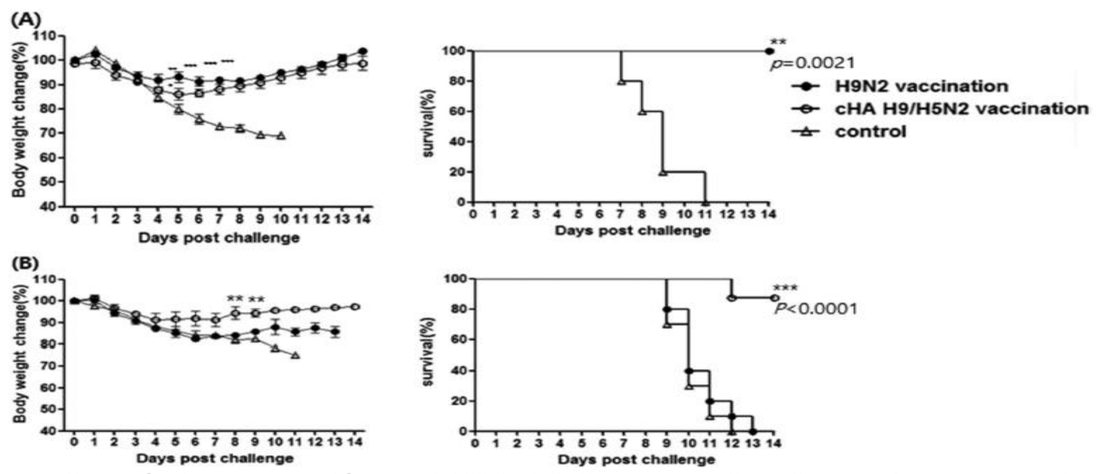

Fig 7 Survival rates and weight loss of 04163/04 (H9N2)- and cHA H9/H5N2-vaccinated mice against wild type H9N2 (A) and HPAI H5N8 (B)

TABLE 2 Comparison of tissue virus titers in mice immunized with each vaccine following lethal challenge with H9N2, H5N2, and H5N8 viruses[a]

		Mean virus titer (EID_{50}/g) ± SD at day postinfection														
		H9N2-vaccinated group					cHA H9/H5N2-vaccinated group					Mock group				
Challenge virus	Organ	1	3	5	7	9	1	3	5	7	9	1	3	5	7	9
ma163 (H9N2)	Lung	3.3 ± 0.3	1.6	—	—	—	3.3 ± 0.3	2.4	—	—	—	6.1	5.6	5.0	5.6	—[b]
W452/14 (H5N8)	Lung	3.6 ± 0.2	3.0 ± 0.2	3.8	4.1 ± 0.5	2.1 ± 0.3	3.0 ± 0.2	2.0 ± 0.2	1.8 ± 0.3	—	—	3.6 ± 0.2	3.3 ± 0.3	3.4 ± 0.5	4.4 ± 0.4	—[b]
	Brain	—	—	1.6	0.7	—[b]	—	—	—	—	—	—	—	2.0	1.2	—[b]
ma81/07 (H5N2)	Lung	5.5	5.1 ± 0.5	5.6 ± 0.2	6.8	—[b]	3.3 ± 0.3	3.2 ± 0.2	2.4 ± 0.5	—	—	5.1 ± 0.5	5.1 ± 0.5	5.0 ± 0.7	5.6	—[b]

[a]Dashes indicate that the tissue was negative for virus detection (lower limit of 0.7 $\log_{10}$ EID_{50}/g). Viruses were titrated in egg. Virus titrations are shown only for virus detected in multiple organs; except for W149 and W452, other viruses were not detected in multiple organs

Summary & Discussion

- We generated a chimeric HA (cHA) H9/H5N2 virus comprised of the entire HA1 domain from H9N2 and the HA2 region from a H5N8 (Fig 1) by using a reverse-genetics method.
- To test the immunogenicity and protective ability of cHA H9/H5N2 vaccines, groups of animals (SPF-chicken or mice) were immunized with the cHA H9/H5N2 vaccine, the H9N2 (04163/04) vaccine, or PBS-alum control.
- All vaccinated groups showed increases in mean HI titers following boosting vaccinations against the homologous strain. However, the H9N2 vaccine does not induce a cross-reactive HI titer against heterosubtypic H5 viruses (H5N8 and H5N2 viruses)
- The cHA H9/H5N2 vaccine immunized animals induced broad cross-protective SN titers against heterosubtypic W452/14 (H5N8) and ma81/07 (H5N2) strains.
- The cHA H9/H5N2 vaccine protects vaccinated animals against lethal H9N2 and H5N8 virus infection with reduced viral titers in the tissues in mice (fig 3, 4, 5 and 7).
- Taken together, our results suggest that the cHA H9/H5N2 DIVA vaccination strategy provides robust protection against homologous, heterologous, and heterosubtypic viruses of both subtypes.

References

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