Neuraminidase inhibition and viral neutralization mediated by anti-NA antibody induced by recombinant vaccinia virus harboring *NA* gene derived from H5N1 and 2009 pandemic influenza viruses

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Objective:

This study aimed to investigate the functional activities of antibodies to neuraminidase (NA) by enzyme-linked lectin assay (ELLA) for inhibition of NA enzymatic activity, and by microneutralization (microNT) assay using antisera obtained from mice immunized with recombinant vaccinia viruses harboring *NA* gene derived from a highly pathogenic avian influenza (HPAI) H5N1 virus or a 2009 pandemic H1N1 (H1N1pdm) virus.

Methods:

- A/Thailand/1(KAN-1)/2004(H5N1) virus
- A/Thailand/104/2009 (H1N1) virus

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Cloning of *NA* gene into pSC11 plasmid

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Construction of recombinant vaccinia viruses
- rVac-H5N1 NA
- rVac-H1N1pdm NA

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Characterization of recombinant NA by
- Confocal immunofluorescence
- MUNANA assay

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BALB/c mice immunization

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Determination for specificity and function of NA antibody by IFA, ELLA and microNT

Results:

Confocal microscopy showed that NA protein expressed in TK- cells infected by rVac-H5N1 NA or rVac-H1N1pdm NA localized in the cytoplasm and cytoplasmic membrane (Figure 1). The recombinant viruses contained NA enzymatic activity (Figure 2), and induced NA specific antibody in the immunized mice as demonstrated by IFA (Figure 3).

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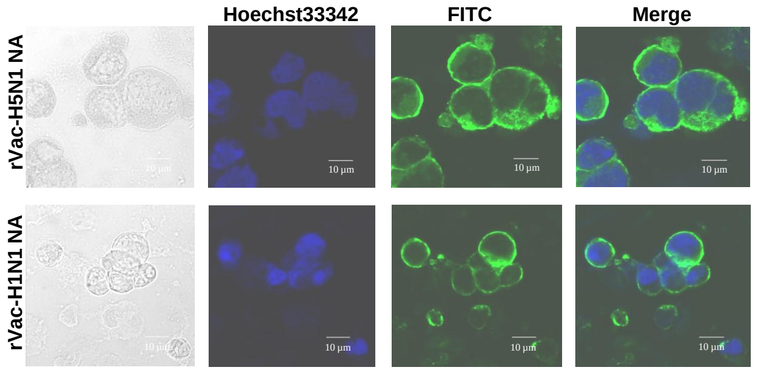

Figure 1: Expression and localization of NA protein in the infected TK- cells

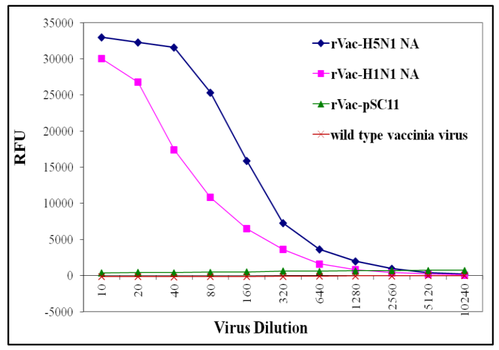

Figure 2: NA enzymatic activity of recombinant NA in lysates of the infected TK- cells by MUNANA assay

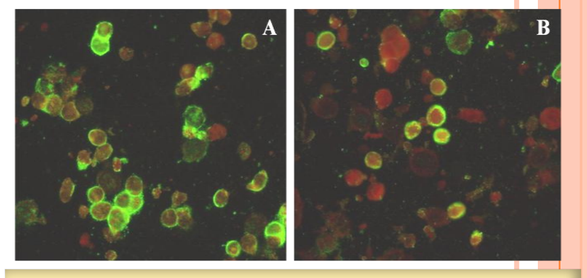

Figure 3: IFA for NA antibody in sera from mice immunized with rVac-H5N1 NA (A) or rVac-H1N1pdm (B) using MDCK cells infected with the rgHANA H5N1 or A/Thailand/104/2009 (H1N1) virus as the test antigen. (rg-reverse genetic virus)

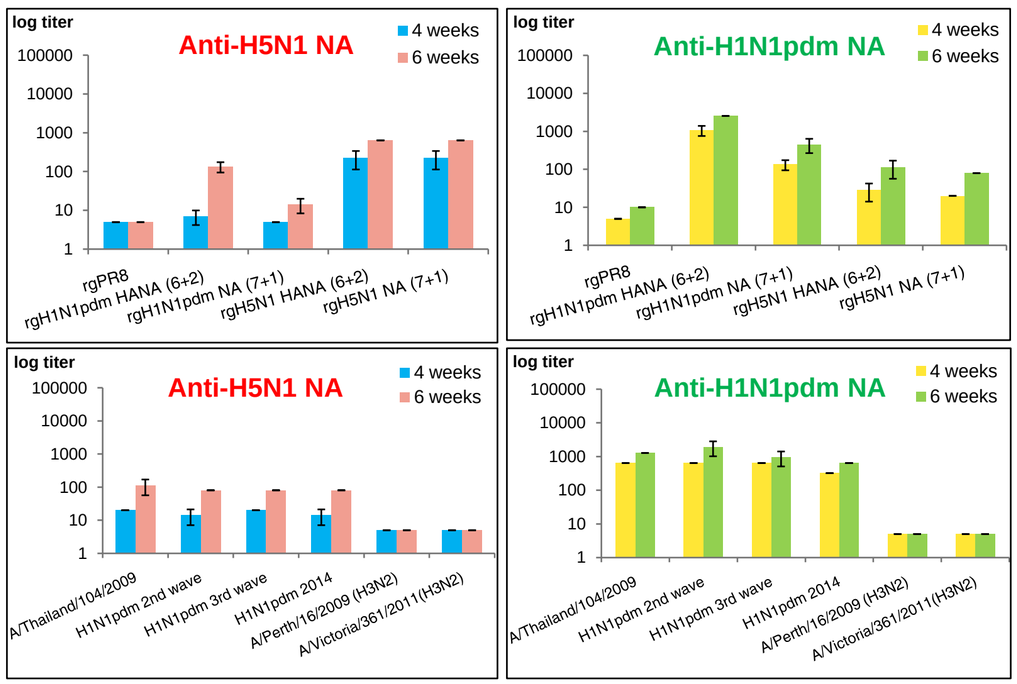

Figure 4: ELLA demonstrated that sera from mice immunized with rVac-H5N1 NA or rVac-H1N1pdm NA has higher NA inhibition antibody titer against the viruses harboring homologous NA subtype and lower titer against the heterologous NA subtype. None of both immune sera had NA inhibition activity against H3N2 viruses.

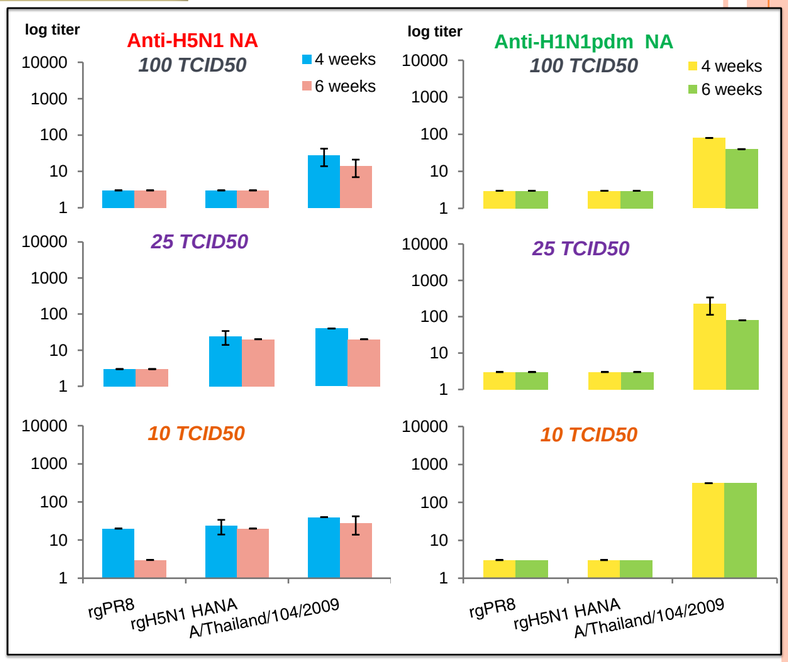

Figure 5: The immune sera to rVac-H5N1 NA exhibited neutralizing (NT) antibody activity against rg-H5N1 HANA virus, and cross NT activity against wild type pandemic H1N1 virus at 25 and 10 TCID50, but not at 100 TCID50. In contrast, sera from mice immunized with rVac-H1N1pdm NA exhibited NT activity against H1N1pdm virus but not against the rg-H5N1 HANA virus.

Conclusion: It is suggested that NA antibody might exhibit protection against influenza virus infection by blocking NA enzymatic activity needed for viral progeny release and partial neutralize the virus infectivity. Cross NA inhibition antibody was found across H5N1 and H1N1pdm NA, but not PR8 NA (All had N1 subtype.) and N2 NA as showed by ELLA. Anti-H5N1 NA has broader NT activity than anti-H1N1pdm NA as shown by microNT assay.