Induction of Influenza (H5N8) Antibodies by Modified Vaccinia Virus Ankara H5N1 Vaccine

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To the Editor: Aquatic birds form a natural reservoir of avian influenza viruses from which new human and animal influenza viruses originate. After initial detection in 2010 in China, a new highly pathogenic avian influenza (HPAI) virus of the H5N8 subtype reemerged in ducks in South Korea in 2014 (*1*,*2*). The hemagglutinin gene of this virus was distantly related to those of H5N1 subtypes that have caused infections in humans since 1997 (*3*). The World Health Organization/World Organisation for Animal Health/Food and Agriculture Organization of the United Nations H5N1 Evolution Working Group has assigned this new H5 to clade 2.3.4.4. Several poultry farms in the Netherlands, Germany, United Kingdom, and Italy were recently affected by infection with H5N8 virus closely related to the strains circulating in Asia (*4*), leading to implementation of preventive measures to restrict viral spread. Human infections with this new HPAI subtype have not been reported.

Modified vaccinia virus Ankara (MVA) is a promising viral vector platform for the development of influenza vaccines (*5*). We previously conducted a randomized double-blind phase 1/2a trial in young healthy persons to evaluate an MVA-based H5 vaccine (registered in the Netherlands' trial register under NTR3401). Preclinical testing was conducted before this trial (*6,7*). Thirty-nine study participants received MVA-H5-serumfree Munich-Rotterdam (sfMR), which encoded hemagglutinin of influenza virus A/Vietnam/1194/2004 (H5N1), and 40 received vector control. Persons received 1 or 2 doses (with an interval of 4 weeks) of 10^7 or 10^8 PFU. Twenty-seven of the MVA-H5-sfMR–vaccinated persons received a booster vaccination 1 year later (again 10^7 or 10^8 PFU). The MVA-based vaccine was well tolerated and induced antibodies to both the homologous (A/Vietnam/1194/2004, clade 1) and a heterologous (A/Indonesia/5/2005, clade 2.1) H5N1 virus (*8*).

Although the newly emerged HPAI (H5N8) virus thus far has been detected only in birds, zoonotic transmission to humans exposed to large numbers of infected birds might occur (e.g., during culling operations). Therefore, shortly after the H5N8 outbreak in poultry in the Netherlands, we determined whether MVA-H5-sfMR–induced antibodies cross-react with the new H5N8 strain. Post-infection A/Vietnam/1194/2004 (clade 1) ferret serum (infected with a low pathogenic reverse genetics virus produced with hemagglutinin and

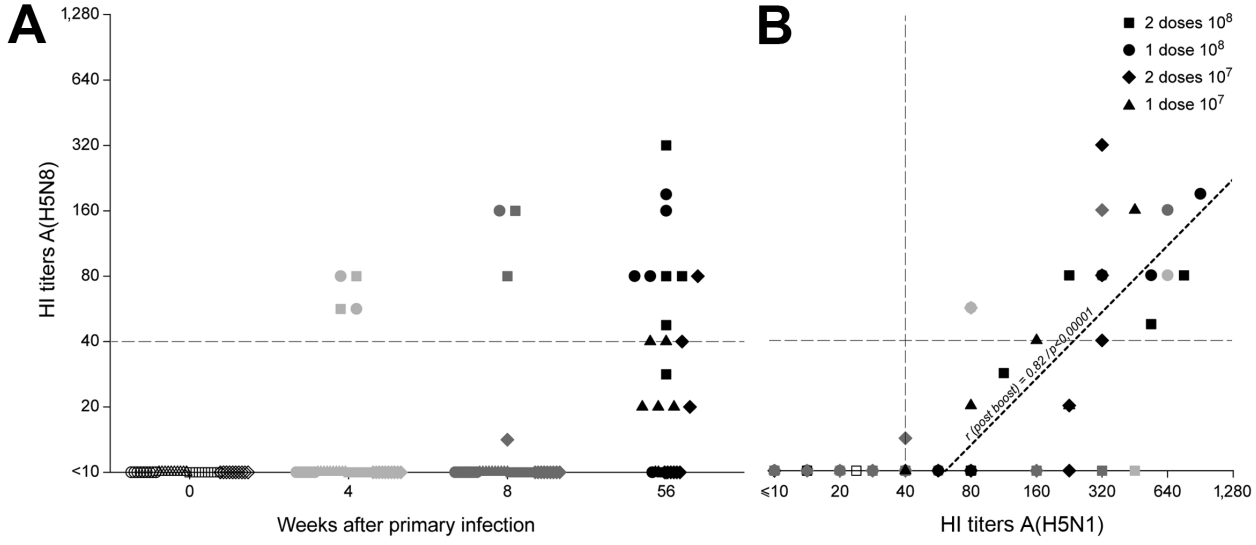

Figure. Results of hemagglutination-inhibition (HI) testing of modified vaccinia virus Ankara influenza vaccine cross-reactivity. Each symbol represents a person in the clinical trial; symbol shapes indicate different vaccination regimens. A) Timeline for development of HI titers against influenza virus A(H5N8) (A/chicken/Netherlands/EMC-3/2014). B) Correlation between HI titers against H5N8 and A/Vietnam/1194/2004 (H5N1) viruses. Linear regression for samples after booster vaccination is shown (r = 0.82, p<0.0001).

neuraminidase gene segments of A/Vietnam/1194/2004 and the remaining 6 gene segments of A/Puerto Rico/8/34) was tested by hemagglutination-inhibition (HI) for cross-reactivity with viruses belonging to clade 0 (A/Hong Kong/156/1997), 2.1 (A/Indonesia/5/2005), 2.2 (A/Turkey/turkey/1/2005), and 2.3 (A/Anhui/1/2005) and the emerging H5N8 strain A/chicken/Netherlands/EMC-3/2014. A/Vietnam/1194/2004-specific serum (homologous titer 80) displayed low cross-reactivity with the clade 0, 2.2, and 2.3 viruses and completely failed to react with H5N8 strain A/chicken/Netherlands/EMC-3/2014. Inversely, A/chicken/Netherlands/EMC-3/2014-specific ferret serum (homologous titer 160) completely failed to cross-react with A/Vietnam/1194/2004. This finding demonstrates an antigenic distance between these viruses. Furthermore, the World Health Organization Collaborating Centers have only found limited cross-reactivity of a panel of H5 vaccine candidates with subtype H5N8 (*9*).

The clinical trial serum samples were pretreated with receptor-destroying enzyme and horse erythrocytes and tested by HI assay for their reactivity with A/chicken/Netherlands/EMC-3/2014 according to standard procedures (*10*). HI antibodies were induced after MVA-H5-sfMR vaccination that displayed considerable reactivity with the antigenically distinct H5N8 strain A/chicken/Netherlands/EMC-3/2014 (Figure, panel A). The titers of cross-reactive antibodies correlated with those to the homologous strain A/Vietnam/1194/2004 (r = 0.82, $p<0.0001$; Figure, panel B).

As shown previously (*8*), the magnitude of the antibody response was dose-dependent. Also, the highest cross-reactive response to the H5N8 strain was observed after vaccination with 10^8 PFU (Figure, panel A) of MVA-H5. None of the study participants had prevaccination HI antibody titers ≥1:40 against A/Vietnam/1194/2004 or A/chicken/Netherlands/EMC-3/2014. Although most of the study participants had detectable HI antibody titers against the homologous virus 4 and 8 weeks after vaccination (*8*), antibodies against the H5N8 virus were barely detectable at these time points. HI antibody titers against the homologous virus increased in persons who received a booster vaccine at 52 weeks after primary vaccination. A large proportion (9 [82%] of 11 study participants; geometric mean titer 63) of participants who received a vaccine dose of 10^8 PFU (equally divided among groups that received 1 or 2 previous doses) also had detectable cross-clade titers against the H5N8 virus A/chicken/Netherlands/EMC-3/2014. Furthermore, virus neutralizing antibodies against H5N8 virus were detected in 10 of 27 persons and correlated with antibody titers measured by HI assay.

We showed that an MVA-based H5 (A/Vietnam/1194/2004) vaccine can elicit cross-clade antibodies against the newly emerging HPAI (H5N8) virus that is genetically and antigenically distinct from the clade 1 H5N1 virus A/Vietnam/1194/2004. The cross-reactive antibody response observed after the 1-year booster vaccination suggests that the use of MVA-H5-sfMR is an effective emergency vaccination strategy in case tailor-made vaccines are not yet available in an outbreak situation. Thus, such a strategy might also be effective against the newly emerging influenza A(H5N8) viruses, in case these viruses would cause human infections.

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Klebsiella pneumoniae Co-Producing NDM-5 and OXA-181 Carbapenemases, South Korea

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To the Editor: Carbapenemase-producing *Enterobacteriaceae* are being reported worldwide. Travel, medical tourism, and cross-border transfer of patients might play a role in the spread of these bacteria (*1,2*). *Klebsiella pneumoniae* co-producing New Delhi metallo-β-lactamase 5 (NDM-5) and oxacillinase 181 (OXA-181) carbapenemases was detected in South Korea in 2014.

On April 13, a 75-year-old man who had had a cerebral infarction was transferred from a tertiary care hospital in Abu Dhabi, United Arab Emirates (UAE), to Samsung Medical Center (Seoul, South Korea) for rehabilitation therapy. In Abu Dhabi, he had received broad-spectrum antimicrobial drugs for aspiration pneumonia. While at Samsung Medical Center, he experienced septic shock and acute respiratory failure due to pneumonia and was transferred to the medical intensive care unit (ICU). Carbapenem-resistant *K. pneumoniae* (strain CC1409-1) was isolated from a culture of bronchoalveolar lavage fluid. He was given meropenem and colistin for treatment of pneumonia, was discharged, and returned to the UAE.

Four months later, carbapenem-resistant *K. pneumoniae* (strain CC1410-1) was identified in the tracheal aspirate of a 74-year-old woman admitted to the surgical ICU at Samsung Medical Center for traumatic intracranial hemorrhage. She had no underlying disease or previous history of hospitalization or travel abroad. She was given colistin and piperacillin/tazobactam. Following the identification of colistin resistance, colistin was switched to tigecycline. However, her clinical condition worsened (aggravated pneumonia), and she died of refractory respiratory failure.

In vitro antimicrobial drug susceptibility tests of 2 isolates were performed by using broth microdilution. Results were interpreted following Clinical and Laboratory Standards Institute guidelines (*3*), except for those for colistin and tigecycline, for which European Committee on Antimicrobial Susceptibility Testing breakpoints were used (*4*). The first isolate was susceptible to colistin but none of the other antimicrobial agents tested (cefepime, ceftriaxone, ceftazidime, aztreonam, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole, ertapenem, imipenem, and meropenem)whereas the second isolate was susceptible only to tigecycline. Modified Hodge tests for both isolates showed positive results. Production of metallo-β-lactamase was detected by an imipenem-EDTA double-disk synergy test.

The presence of carbapenemase genes was determined by PCR and DNA sequencing (*2*). The bla_{NDM} and bla_{OXA-48} genes were detected in both isolates. The PCR product sequences were consistent with those of NDM-5 (GenBank accession no. JN104597.1) and OXA-181 (GenBank accession no. JN205800.1). Further analyses for other β-lactamases (TEM-type, SHV-type, and CTX-type) and 16S rRNA methylase aminoglycoside resistance determinants (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtF*, and *npmA*) revealed that both isolates carried bla_{TEM-1}, bla_{SHV-11}, $bla_{CTX-M-15}$, and *rmtB* genes.

Clonal relatedness was investigated by using multilocus sequence typing and pulsed-field gel electrophoresis (PFGE) (*5,6*). Multilocus sequence typing revealed that both isolates belonged to sequence type 147. PFGE showed that both isolates were the same strain (Figure).

The 2 patients were never hospitalized in the same ward and there was a substantial time lag between their hospitalizations. However, given sequence type and PFGE patterns between 2 isolates, we suspected nosocomial cross-transmission and performed infection control measures, including strict contact precautions and enhanced environmental cleaning with daily monitoring in the surgical ICU. In addition, environmental cultures and active surveillance cultures (rectal swabs and respiratory samples) on all patients in the units where these isolates were identified were performed to find asymptomatic carriers or contaminated environments as potential sources of transmission. All samples tested were negative for carbapenemase-producing *Enterobacteriaceae*. No further cases were reported in the hospital.

NDM-5 was first identified in a multidrug-resistant *Escherichia coli* sequence type 648 isolate from a patient in the United Kingdom who had a recent history of hospitalization in India (*7*). NDM-5 differs from existing enzymes due to substitutions at positions 88 (Val→Leu) and 154 (Met→Leu). OXA-181, a variant of OXA-48, was initially

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