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Mutation from arginine to lysine at the position 189 of hemagglutinin contributes to the antigenic drift in H3N2 swine influenza viruses

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ABSTRACT

Two distinct antigenic clusters were previously identified among the H3N2 swine influenza A viruses (IAVs) and were designated H3N2SIV-alpha and H3N2SIV-beta (Feng et al., 2013. Journal of Virology 87 (13), 7655–7667). A consistent mutation was observed at the position 189 of hemagglutinin (R189K) between H3N2SIV-alpha and H3N2SIV-beta fair isolates. To evaluate the contribution of R189K mutation to the antigenic drift from H3N2SIV-alpha to H3N2SIV-beta, four reassortant viruses with 189R or 189K were generated. The antigenic cartography demonstrated that the R189K mutation in the hemagglutinin of H3N2 IAV contributed to the antigenic drift, separating these viruses into H3N2SIV-alpha to H3N2SIV-beta. This R189K mutation was also found to contribute to the cross-reaction with several ferret sera raised against historical human IAVs with hemagglutinin carrying 189K. This study suggests that the R189K mutation plays a vital role in the antigenicity of swine and human H3N2 IAVs and identification of this antigenic determinant will help us rapidly identify antigenic variants in influenza surveillance.

Introduction

Influenza A viruses (IAVs) belong to the family *Orthomyxoviridae*. Among the 17 HA subtypes of IAVs identified, H3 is one of the most widely circulating subtypes in nature. H3 IAVs have been recovered from humans, pigs, horses, dogs, birds, and seals. H3 IAVs caused the 1968 pandemic (by H3N2 IAV), contemporary seasonal epidemics (H3N2) in humans, epidemic or endemic diseases in pigs (H3N2) (Zhou et al., 1999, 2000), horses (H3N8) (Thomson et al., 1977), and dogs (H3N2 and H3N8) (Crawford et al., 2005; Li et al., 2010; Song et al., 2008; Yoon et al., 2005).

In the North American swine population, the current predominant H3N2 IAV was associated with a "spillover" of human seasonal H3N2 IAVs to pigs in 1990′s (Vincent et al., 2008; Zhou et al., 1999). Phylogenetic analyses of HA genes of H3N2 SIVs in North America demonstrated that there have been at least four genetic groups (Cluster I–IV) (Olsen et al., 2006), and H3N2 IAVs of Cluster IV has predominated in US swine populations since 2005 (Hause et al., 2010). Neutralization assay using swine antisera demonstrated that these four genetic clusters are also antigenically distinct, varying from a 2 to 8-fold change in hemagglutination inhibition (HI) titers, although cross reaction exists among these clusters to a degree (Hause et al., 2010).

In 2011, a novel IAV, so called H3N2 variant (H3N2v), was identified in agricultural fairs. This virus caused more than 325 confirmed human influenza cases in 14 states (CDC, 2012a, 2012b; Lindstrom et al., 2012). Genetically, the hemagglutinin gene of H3N2v-like IAV belongs to Cluster IV of H3N2 SIVs. Recently, antigenic profile of four human H3N2v isolates, 12 commercial swine farm isolates, and 68 isolates recovered from pigs at 2009–2011 Ohio county fairs were characterized in our laboratory (Feng et al., 2013). These 84 isolates were clearly divided into two antigenic clusters, H3N2SIV-alpha and H3N2SIV-beta. The human H3N2v isolates were grouped with H3N2 SIV-beta while the swine isolates were divided between two antigenic clusters. Sequence analysis of these isolates showed a number of variations at antibody binding sites among these H3N2 isolates, but only the

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mutation arginine (R) to lysine (K) at the position 189 of hemagglutinin was consistent between H3N2SIV-alpha and H3N2SIV-beta. Also, our previous study showed that the viruses in the antigenic cluster H3N2SIV-beta cross-reacted with ferret antisera produced against several seasonal human influenza viruses (Feng et al., 2013). Interestingly, these human seasonal viruses also carried 189K in HA.

In this study, four reassortants with 189R or 189K were generated by reverse genetics, and serological assays were conducted for these reassortants to determine if the R189K mutation drives the antigenic drift of these H3N2 SIVs. In addition, this study was conducted to determine if the R189K mutation contributes to the cross-reaction to sera against human seasonal viruses.

Results and discussion

To evaluate contribution of R189K mutation to antigenic drift of H3N2 IAVs from Ohio county fairs, four reassortant viruses were generated by reverse genetics and designated as 09SW64_189R (wt), 09SW64_189K (mt), 11SW347_189K (wt) and 11SW347_189R (mt) (Table 1). The 09SW64_189R (wt) represented an IAV in the antigenic cluster H3N2SIV-alpha whereas 11SW347_189K (wt) represented an IAV in the antigenic cluster H3N2SIV-beta. The HI results showed that the antisera against H3N2SIV-alpha isolates had a HI titer of 1280 to 2560 against 09SW64_189R (wt) whereas the antisera against H3N2SIV-beta isolates had a HI of 40–80 against 09SW64_189R (wt). In contrast, the antisera against H3N2SIV-beta isolates had a HI titer of 480–960 against 11SW347_189K (wt) whereas the antisera against H3N2SIV-alpha isolates had a HI of 10–20 against 11SW347_189K (wt). The antigenic profile of these two reassortant viruses resembled their wild type isolates (A/swine/Ohio/09SW64/2009(H3N2) and A/swine/Ohio/11SW347/2011 (H3N2), respectively. Antigenic cartography confirmed each of these two reassortant viruses was located in the corresponding antigenic cluster: 09SW64_189R (wt) belonged to H3N2SIV-alpha whereas 11SW347_189K (wt) was clustered to H3N2SIV-beta (Fig. 1).

The antisera against H3N2SIV-alpha isolates had HI titers ranging from 640 to 1280 to 09SW64_189K (mt), and these HI titers were two folds lower than those HI titers for the same set of antisera to 09SW64_189R (wt); the antisera against H3N2SIV-beta isolates had HI titers ranging from 240 to 320 to 09SW64_189K (mt), and these HI titers were at least four-fold higher than those HI titers for the same set of antisera against 09SW64_189R (wt) (Table 1). On the other hand, the antisera against H3N2SIV-alpha isolates had HI titers ranging from 20 to 80 to 11SW347_189R (mt), and these HI titers were at least two folds higher than those HI titers for the same set of antisera to 11SW347_189K (wt); the antisera against H3N2SIV-beta isolates had HI titers ranging from 120 to 320 to 11SW347_189R (mt), and these HI titers were at least three folds lower than those HI titers for the same set of antisera to 11SW347_189K (wt). These HI data demonstrate that the R189K mutation in the hemagglutinin of H3N2 IAV contributed to the antigenic drift. Antigenic cartography demonstrated that the R189K mutation could move A/swine/09SW64/2009 (H3N2) from antigenic cluster H3N2SIV-alpha forward to antigenic cluster H3N2SIV-beta whereas the K189R mutation could drive A/swine/11SW347/2011(H3N2) from antigenic cluster H3N2SIV-beta toward antigenic cluster H3N2SIV-alpha (Fig. 1).

In this study, we also produced ferret antisera against the two mutant strains 09SW64_189K and 11SW347_189R. The HI assay demonstrated the antisera against 09SW64_189K showed 2-fold higher HI titer for 11SW347_189K than 11SW347_189R, and the antisera against 11SW347_189R had 4-fold higher HI titer for 09SW64_189R than 09SW64_189K (Table 1). This data further supported our conclusion that the single R189K mutation in the HA of swine H3N2 IAV contributed to the antigenic drift, separating these viruses into H3N2SIV-alpha to H3N2SIV-beta.

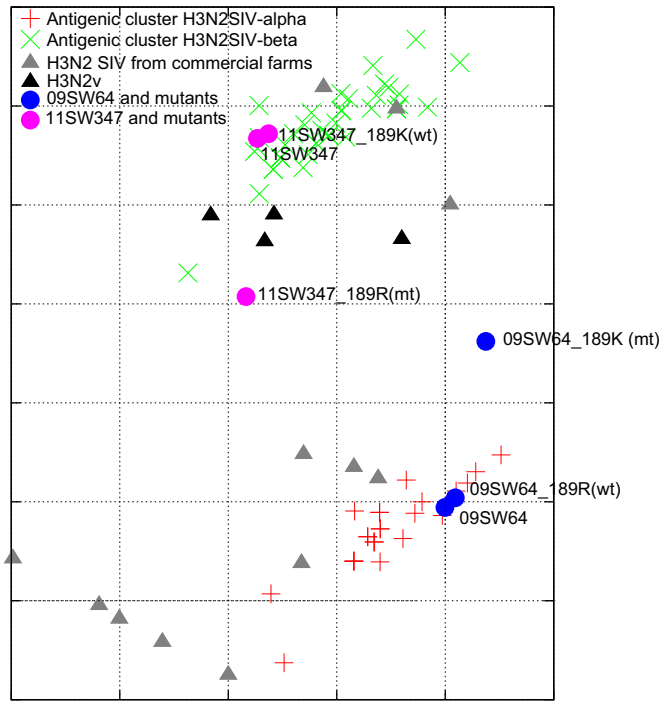

Fig. 1. Antigenic cartography was constructed using HI data for 68 SIV isolates from Ohio agricultural fairs (2009–2011),12 SIV isolates from commercial farms (2006–2012), and four human H3N2v influenza virus isolates, and four reassortant viruses (09SW64_189R, 09SW64_189K, 11SW347_189R, and 11SW347_189K). The HI data for 80 SIV isolates and four human H3N2v isolates were adapted from (Feng et al., 2013), and those for four reassortant viruses were shown in Table 1. Antigenic clusters H3N2SIV-alpha and H3N2SIV-beta and the viruses in each clusters were described in (Feng et al., 2013). 09SW64 and the corresponding reassortants (09SW64_189R and 09SW64_189K) were marked in blue whereas 11SW347 and the corresponding reassortants (11SW347_189R and 11SW347_189K) in pink. This cartography demonstrates that R189K moves 09SW64 from antigenic cluster H3N2SIV-alpha towards H3N2SIV-beta and that K189R moves 11SW347 from antigenic cluster H3N2SIV-beta towards H3N2SIV-alpha. Antigenic cartography was constructed using AntigenMap (http://sysbio.cvm.msstate.edu/AntigenMap) (Barnett et al., 2012; Cai et al., 2010).

Our results also suggested that the changes in heterologous titers were more than homologous titers. For example, the changes in 11SW347_189K and 11SW347_189R against their corresponding antibody were about two fold, whereas those in 10SW215 against 11SW347_189K and 11SW347_189R ferret antisera were four fold. This indicated that some other mutations (not in reported antibody binding sites of H3N2 IAVs(Wilson and Cox, 1990)) in HA, NA, or MP protein might affect influenza antigenicity indirectly, although R189K is the predominant mutation leading to antigenic drift in H3N2 SIVs.

Our previous study showed that the viruses in the antigenic cluster H3N2SIV-beta cross-reacted with ferret antisera produced against three seasonal human influenza viruses, including A/Caen/1/1984(H3N2), A/Ann Arbor/03/1993(H3N2), and A/Nanchang/933/1995(H3N2). Among these, the ferret antiserum against A/Caen/1/1984(H3N2) exhibited the highest HI titer, 1:160, to the viruses in the antigenic cluster H3N2SIV-beta whereas the ferret antiserum against A/Caen/1/1984(H3N2) does not have any cross reactions against the viruses in the antigenic cluster H3N2SIV-alpha (Feng et al., 2013). Sequence analysis showed that hemagglutinin of A/Caen/1/1984(H3N2) also carried 189K, like the hemagglutinin of IAV in the antigenic cluster H3N2SIV-beta. To evaluate the contribution of the R189K mutation in that cross-reaction, HI assay was performed on the four reassortant viruses using antisera produced against several historic seasonal human