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Modulation of the severity of highly pathogenic H5N1 influenza in chickens previously inoculated with Israeli H9N2 influenza viruses

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ABSTRACT

The continued evolution of H9N2 and H5N1 viruses and their spread and re-emergence across Eurasia raise concern that prior H9N2 virus infection may limit the detection of subsequent H5N1 infection in gallinaceous poultry by attenuating the severity of disease. We show that H9N2 viruses isolated from Israeli turkeys during 2000–2004 were antigenically and genetically distinguishable. These three H9N2 viruses caused no overt signs of disease in chickens. The 2004 isolate replicated and spread most efficiently, and chickens previously inoculated with this H9N2 virus showed 90%–100% survival after inoculation 1 to 35 days later with lethal H5N1 virus. Chickens that survived did not show signs of disease but did shed lethal H5N1 virus from the cloaca. The modulation of survivability was time-dependent; the effect was maximal 5 days after H9N2 inoculation. These findings suggest that co-circulation of H9N2 viruses can contribute to the spread of lethal H5N1 viruses.

Introduction

Avian influenza viruses of the H9N2 subtype have been implicated in the genesis of highly pathogenic (HP) H5N1 strains and in the outcome of HP H5N1-associated disease (Webster et al., 2006). The G1 genotype of the H9N2 virus that circulated in Hong Kong's live poultry markets in 1997 (A/Quail/HongKong/G1/97) contained six internal genes of the H5N1 virus (A/HongKong/157/97) isolated from humans at that time (Guan et al., 1999, 2000; Lin et al., 2000). The surprisingly low mortality rate of gallinaceous poultry in the Hong Kong markets during that period is explained in part by cross-protective cell-mediated immunity induced by the G1 genotype of the H9N2 virus (Seo and Webster, 2001).

H9N2 viruses of gallinaceous poultry spread from Asia to most Eurasian countries during the 1990s (Aamir et al., 2007; Alexander, 2007). These viruses have circulated widely and cause only low-grade disease in gallinaceous poultry. An unanswered question is whether prior infection with H9N2 viruses reduces the lethality and therefore the detectability of HP H5N1 infection in poultry in the field, thereby facilitating virus spread.

In Israel, H9N2 influenza viruses were first detected in poultry in May 2000, when they caused signs of mild respiratory disease and a drop in egg production, mainly in turkeys. After the initial outbreak, H9N2 viruses were not detected again until December 2001, and they continued to circulate in poultry until April 2003, causing disease outbreaks in chickens, geese, ostriches, and turkeys. A non-homologous inactivated vaccine was widely used to control H9N2 virus spread. H9N2 viruses were introduced a third time in February 2003 and were isolated sporadically through 2006, primarily from chickens.

In March 2006, HP H5N1 virus was detected in Israel. All flocks affected by H5N1 had been vaccinated against H9N2 infection, but evidence of co-infection with H9N2 viruses was observed in the Gaza strip region. As many as one million birds were culled to stop the spread of the H5N1 outbreak (Perk et al., 2007).

The spread of HP H5N1 to poultry in Israel, where H9N2 influenza viruses continued to circulate, allowed us to assess whether the current H9N2 viruses can moderate the lethality of co-circulating HP H5N1 viruses and thus decrease the likelihood of their detection. We antigenically and molecularly characterized three H9N2 viruses isolated from domestic turkeys in Israel and determined experimentally whether these viruses influence the pathogenicity of HP H5N1. We found that some H9N2 influenza viruses modulate the lethality of H5N1 challenge in a time-dependent fashion.

Results

Sequence and phylogenetic analysis of H9N2 influenza viruses

Complete sequencing of the HA and NA genes of the three Israeli H9N2 influenza isolates confirmed that they were of the H9 subtype

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and were closely inter-related (Fig. 1). There was 96% identity in amino acid sequences between the HA gene of 2004 virus and the 2000 and 2002 viruses, whereas homology between the 2000 and 2002 viruses was close to 99%. The neuraminidase (NA) gene showed 98.5% similarity in the 2000 and 2002 viruses and 96.3% similarity between those two isolates and the 2004 virus. The internal genes differed by an average of 4% (96%–98% similarity of amino acid sequence). Greater divergence in nucleotide sequences (89%–92% similarity) was

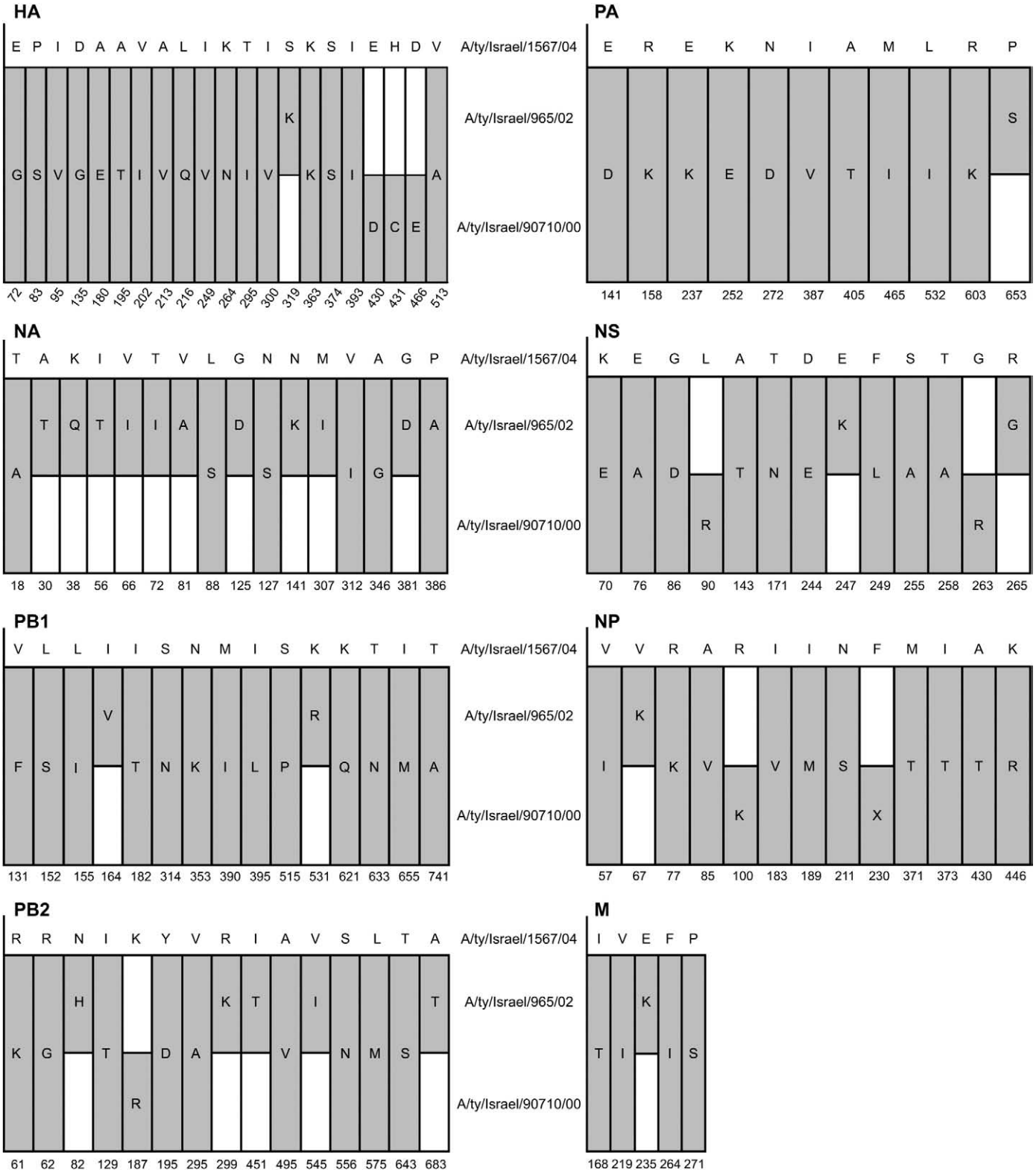

Fig. 1. Representation of amino-acid differences between the three H9N2 viruses. Upper sequence in each graph is the sequence of the reference virus, A/ty/Israel/1567/04. Capital letters indicate amino acids. Dark bars indicate changes in the two other H9N2 viruses as compared to the reference sequence; white bars indicate no change. Positions not shown are identical in the three viruses. H9 numbering was used for the HA gene.