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Enhancement of the safety of live influenza vaccine by attenuating mutations from cold-adapted hemagglutinin

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ABSTRACT

In our previous study, X-31ca-based H5N1 LAIVs, in particular, became more virulent in mice than the X-31ca MDV, possibly by the introduction of the surface antigens of highly pathogenic H5N1 influenza virus, implying that additional attenuation is needed in this cases to increase the safety level of the vaccine. In this report we suggest an approach to further increase the safety of LAIV through additional cold-adapted mutations in the hemagglutinin. The cold-adaptation of X-31 virus resulted in four amino acid mutations in the HA. We generated a panel of 7:1 reassortant viruses each carrying the hemagglutinins with individual single amino acid mutations. We examined their phenotypes and found a major attenuating mutation, N81K. This attenuation marker conferred additional temperature-sensitive and attenuation phenotype to the LAIV. Our data indicate that the cold-adapted mutation in the HA confers additional attenuation to the LAIV strain, without compromising its productivity and immune response.

Introduction

Vaccination remains the most important and effective strategy to provide protection against influenza infection and has been known to alleviate severe symptoms during annual epidemics and occasional pandemics (Nichol, 2003). To date, the licensed influenza vaccines are the two types: an inactivated vaccine (IV) or a live attenuated influenza vaccine (LAIV). IVs are widely used with variable levels of effectiveness for preventing influenza infection, but less effective against mismatched influenza viruses (Carrat and Flahault, 2007). LAIVs induce a robust mucosal and cell-mediated immune response with a broader cross-protection than IVs (Belshe, 2004; Beyer et al., 2002; Glezen, 2004; Jang and Seong, 2013). Currently, two different type of influenza A cold-adapted master donor viruses (MDVs), A/Ann Arbor/6/60ca and A/Leningrad/134/17/57ca strains, are used for the generation of LAIVs for humans. It has been well documented that during the cold-adaptation process, various mutations are accumulated in the internal genes (Cox et al., 1988; Herlocher et al., 1996; Isakova-Sivak et al., 2011; Murphy and Coelingh, 2002) as well as the surface genes (Herlocher et al., 1993; Klimov et al., 1995) that confer cold-adapted (*ca*), temperature-sensitive (*ts*), and attenuated (*att*) phenotypes to the virus, and their specific contributions to the attenuation has been examined through genetic reassortment analyses under non-attenuated backbones (Jin et al., 2003, 2004; Snyder et al., 1985, 1988; Subbarao et al., 1992).

Virtually all cold-adapted LAIVs carry six internal genes derived from the already attenuated MDV and two surface glycoprotein genes from the wild-type virus. Thus, the attenuation characteristics of LAIVs are specified by the six internal genes of the MDV and it is possible that the replacement of the surface proteins of the cold-adapted MDV with those of wild type virus may acquire virulence, compromising the safety of the LAIVs. Previously, the X-31 (H3N2) was developed as a high-yield reassortant virus carrying the HA and NA genes derived from A/Aichi/2/68 (H3N2) and the six internal genes from PR8 (H1N1) (Baez et al., 1980). With a view to provide an alternative influenza A type MDV, X-31ca has been developed by serial passages of the parental X-31 at low temperatures (Lee et al., 2006a). The X-31ca exhibited desired levels of *ca*, *ts*, and *att* phenotypes and, similar to other MDV strains, various mutations were found in the six internal genes (unpublished data) and also in the surface HA gene. While the X-31ca-based LAIVs against seasonal influenza and 2009 pandemic H1N1 retained virtually all the attenuated characters of their MDV (Jang et al., 2012a, 2013a,

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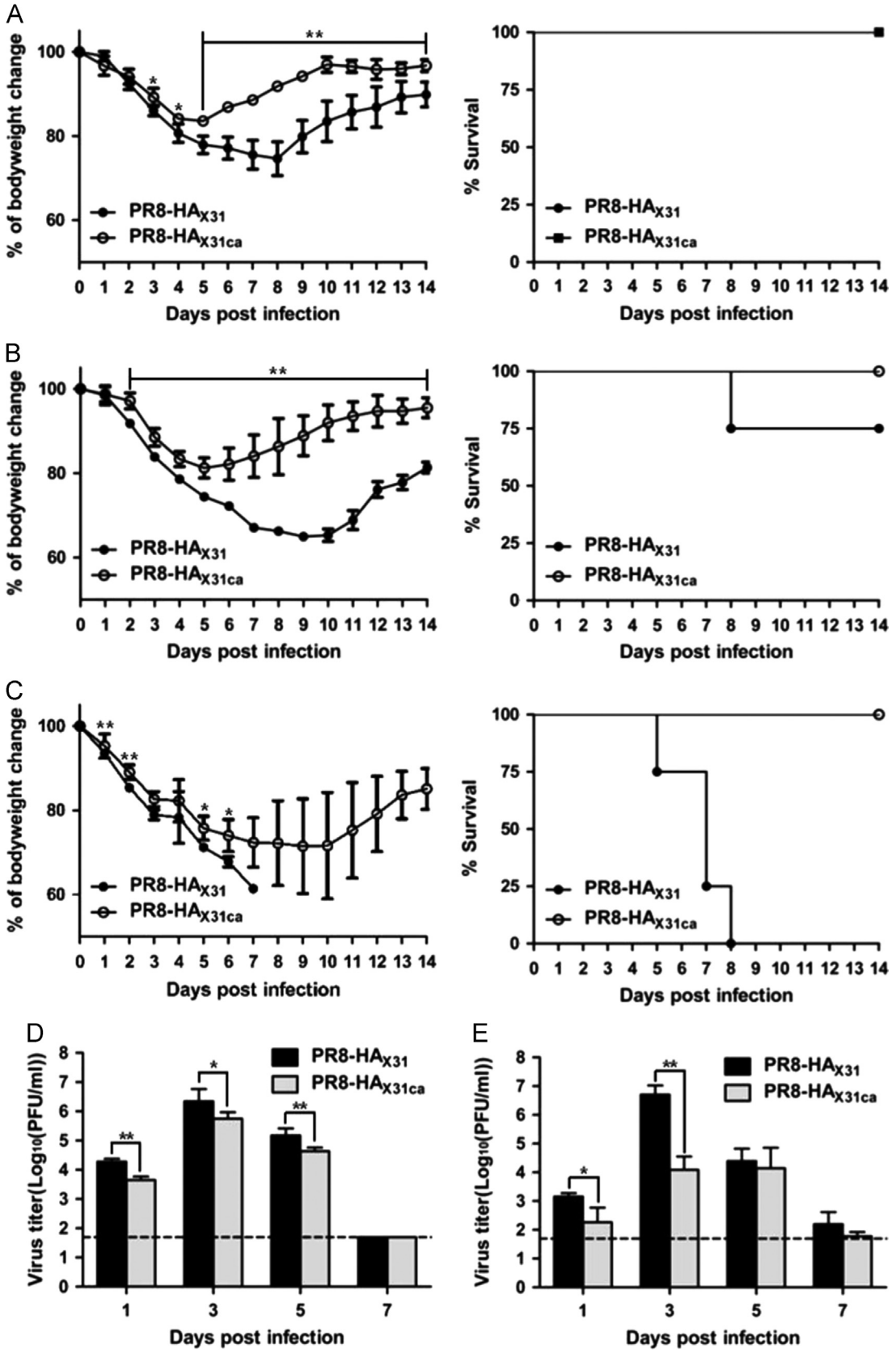

Fig. 1. Effect of cold-adapted mutations of the HA on the attenuation of PR8 virus. (A–C) Virulence of PR8-HA$_{X31}$ and PR8-HA$_{X31ca}$ virus in mice. Weight changes (left) and survival (right) of the mice groups ($n=4$) infected with 10^3 PFU (A), 10^4 PFU (B), and 10^5 PFU (C) of PR8-HA$_{X31}$ or PR8-HA$_{X31ca}$. (D and E) Viral replications in the respiratory tracts of the viruses. Mice ($n=4$) were infected with 10^4 PFU of PR8-HA$_{X31}$ or PR8-HA$_{X31ca}$, and the viral titers in the lungs (D) and nasal washes (E) were measured by viral plaque assay. Dashed line is the detection limit, 1.69.