Attenuation of a human H9N2 influenza virus in mammalian host by reassortment with an avian influenza virus

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Summary. In order to develop a surrogate virus strain for production of an inactivated influenza vaccine against a human H9N2 virus, A/Hong Kong/1073/99 (HK1073: H9N2) was co-infected in embryonated chicken eggs with an apathogenic avian influenza virus, A/Duck/Czechoslovakia/56 (Dk/Cz: H4N6), for gene segment reassortment. Multiple-gene reassortants obtained were examined for replication in mammalian hosts in vitro and in vivo by infecting MDCK cells and by intranasal administration to hamsters, respectively. A 2–6 gene reassortant with both surface glycoproteins of HK1073 origin and the rest of Dk/Cz origin, HK/CZ-13, was shown to replicate poorly in the mammalian hosts both in vivo and in vitro comparing with HK1073, although this reassortant replicated as efficiently as each parental strain in embryonated eggs. No sequence difference was observed in the HA1 region between HK1073 and HK/CZ-13, indicating that the reassortant would be equivalent in its immunogenicity to the parental HK1073 strain when it is used as an inactivated vaccine. A virus strain with attenuation in mammalian hosts is preferable for production of an H9 vaccine, since it should reduce the risk of manufacturing-related infections of employees during the vaccine production. HK/CZ-13 can therefore be a surrogate strain for production of an inactivated vaccine as well as diagnostic antigens in case of a possible future pandemic caused by an HK1073-like H9 influenza virus.

Introduction

Four influenza pandemics have been recorded during the last century with novel subtypes of Influenza A virus having appeared in human population in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1). In addition to these
pandemics, 6 people died of infection with a highly pathogenic avian influenza virus of the H5N1 subtype in 1997 in Hong Kong [1]. Following to this incident, two child residents of Hong Kong were infected with avian H9N2 influenza virus in 1999 [10]. Although recent report suggested that the transmissibility of the H9 virus among humans appeared low [17], the virus can recognize 5-N-acetylneuraminic acid-α-2, 3-galactose (Neu5Aα2,3 Gal) and Neu5Aα2,6 Gal as its receptor [4, 11], suggesting that it binds to receptors of both avian and human. Although none of the incidents developed into influenza pandemic, preparedness against a next pandemic is one of the major concerns of health authorities worldwide.

One of the best measures against a pandemic influenza is immunization of naïve population with an adequate vaccine, although recent development of anti-influenza virus drugs has been remarkable. For selection of a vaccine strain against a pandemic or a potential pandemic virus at present, there would be several approaches to be taken practically. Using a pandemic strain itself for vaccine production would be a straightforward approach for this purpose and was actually taken in the cases of the pandemics in 1957 and 1968. However, there would be several problems in adopting such an approach at the case of a next pandemic, although it assures the exact match of antigenicity between circulating virus and vaccine strain. One of the important issues, that was ignored previously, would be a potential hazard to manufacture workers who may be exposed to the novel virus during vaccine production processes. It should be essential to reduce manufacturing-related risk of infections, since such incidence may result in creating an artificial epicenter of a pandemic. To avoid the exposure, handling of a potential pandemic virus would be required to be done in a biosafety level 3 (BSL-3) or higher condition, and consequently, the conventional vaccine manufacturing process could not be allowed [16].

In the present study, we propose an approach to develop a safer vaccine production strain against a human virus with a pandemic potential by the conventional reassortment technique. A human H9N2 strain (A/Hong Kong/1073/99; HK1073) was co-infected with an avirulent avian influenza virus [A/Dk/Czechoslovakia/56 (H4N6); Dk/Cz] in embryonated chicken egg to develop a 2–6 gene reassortant which contains the hemagglutinin (HA) and the neuraminidase (NA) surface glycoprotein genes from HK1073 and the other 6 genes from Dk/Cz. This reassortant was expected to be attenuated in humans. A high-yield vaccine seed strain against epidemic strains of Influenza A virus is produced by reassortment between an epidemic strain and the attenuated A/PR/8/34 (H1N1) strain [7, 8]. On the other hand, by making 2–6 gene reassortants possessing the surface antigens from a human H3N2 epidemic strain and the other 6 genes from an apathogenic avian strain, Murphy and co-workers intended to develop live attenuated vaccines against epidemic strains of influenza [9, 13]. They provided evidences that such reassortants were well attenuated in monkeys [9] as well as humans [2].

For evaluation of attenuated phenotype of the reassortant obtained in this study, replication of the viruses in hamster lungs was measured. Saito et al. demonstrated that hamsters were highly susceptible to HK1073, allowing it to