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Broadly protective anti-hemagglutinin stalk antibodies induced by live attenuated influenza vaccine expressing chimeric hemagglutinin

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ABSTRACT

The development of influenza vaccines that can provide broad protection against all drifted seasonal virus variants, zoonotic infections and emerging pandemic strains, has been a priority for two decades. Here we propose a strategy of inducing broadly-reactive anti-stalk antibody by sequential immunizations with live attenuated influenza vaccines (LAIVs) expressing chimeric HAs (cHAs). These vaccines are designed to contain identical hemagglutinin stalk domains from H1N1 virus but antigenically unrelated globular head domains from avian influenza virus subtypes H5, H8 and H9. Mouse experiments demonstrated enhanced cross-protection of cHA-containing LAIVs compared to the relevant vaccine viruses expressing natural HAs, and this enhanced protection was driven by stalk-HA-reactive IgG antibodies. The establishment of fully functional cross-protective immunity after two doses of cHA LAIV vaccination in naïve animals suggests that a similar effect might be expected after a single cHA LAIV dose in primed individuals, or after two to three doses in naïve children.

1. Introduction

Influenza is an acute respiratory disease caused by influenza virus. Annual influenza epidemics result in significant morbidity and up to 500,000 mortalities worldwide (WHO, 2016). The majority of human influenza cases are caused by influenza A viruses which is also the etiological agent of avian influenza which can cause devastating outbreaks on poultry farms and in wild bird populations. The surface glycoproteins of influenza A viruses show high diversity with 18 subtypes of hemagglutinin (HA) and 11 subtypes of neuraminidase (NA) currently recognized. Aquatic birds serve as a natural reservoir for almost all influenza A virus subtypes, however only a small subset of them have historically caused sustained infection in humans, namely H1N1, H2N2 and H3N2. In addition, zoonotic infections with H5NX, H7NX, H9N2 and H10N8 avian influenza viruses are sporadically detected, but these viruses are not yet easily transmissible from human to human (Zhang et al., 2015; RahimiRad et al., 2016; Richard and Fouchier, 2016; Harris et al., 2017; Imai et al., 2017). Periodically, a new influenza A subtype cross the interspecies barrier, acquires the ability to efficiently spread from human to human and causes an influenza pandemic with devastating consequences.

It is widely accepted that vaccination can significantly reduce the burden of influenza, both during pandemics and in interpandemic periods. Most influenza virus vaccines induce strain-specific immunity and lack protection against drifted influenza virus variants. Because of the extremely high variability of the antigenic properties of influenza viruses, the global scientific community is paying close attention to the development of universal influenza virus vaccines that induce long-lasting and cross-reactive immune responses to the most conserved regions of the viral proteins (Hughes et al., 2012; Krammer, 2015; Paules et al., 2017). Such vaccines are designed to provide universal protection against any influenza A viruses, eliminating the need for annual vaccination and, as a consequence, significantly reducing the costs of the immunization campaigns. Significant progress was made in recent years in the development of a universal influenza virus vaccine, and the strategy of inducing HA stalk-specific antibodies is one of the most promising approaches for the design of such vaccine (Hai et al., 2012; Krammer et al., 2012b, 2013; Pica et al., 2012; Krammer and Palese, 2013; Margine et al., 2013a; Nachbagauer et al., 2015; Liu et al., 2017). The HA stalk domains contain epitopes that are highly conserved within influenza A phylogenetic groups 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17, H18) and 2 (H3, H4, H7, H10, H14, H15), but this region is immunosubdominant as compared to the highly variable head domain. Therefore, several strategies were proposed to

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increase the immunogenicity of the HA stalk domain, one of which utilizes immunization with headless HA (Impagliazzo et al., 2015; Wohlbold et al., 2015; Yassine et al., 2015). Another strategy proposes sequential immunizations with vaccines expressing chimeric HAs (cHAs); such vaccines are designed to contain identical stalk domains (e.g. from H1) but antigenically unrelated globular head domains (from avian influenza virus subtypes). Several influenza virus vaccine platforms have been tested thus far for the induction of stalk-specific antibodies using these chimeric HA constructs in mice and ferrets, such as DNA vaccinations followed by protein vaccines or protein-protein vaccines in mice (Goff et al., 2013; Krammer et al., 2013); adjuvanted inactivated influenza vaccine (IIV) in mice (Nachbagauer et al., 2016); live attenuated influenza vaccine (LAIV) followed by IIV in ferrets (Nachbagauer et al., 2017a, 2017b); vesicular stomatitis virus (VSV)-vectored cHAs followed by adenovirus-vectored cHAs in ferrets (Krammer et al., 2014a; Nachbagauer et al., 2015); and VSV vectored vaccines in mice (Ryder et al., 2015). Since LAIVs are known to induce all arms of the adaptive immune system, the use of the LAIV platform for the design of chimeric HA-based universal vaccine is a very attractive option (Isakova-Sivak and Rudenko, 2015). Most recently, a LAIV expressing a chimeric HA molecule was tested in a prime-boost strategy for the induction of stalk-specific antibody in ferrets (Nachbagauer et al., 2017a, 2017b). In this preclinical trial cH8/1 LAIV was compared to cH8/1 IIV as a prime prior to a cH5/1 IIV boost, and despite the induction of similar levels of cross-reactive antibody responses, the LAIV-IIV regimen afforded better protection of ferrets against pandemic H1N1 influenza virus than the IIV-IIV regimen. Here we tested the protective potential of sequential vaccination with cHA expressing LAIVs in the mouse model.

2. Materials and methods

2.1. Plasmids, viruses and proteins

This study utilized several LAIV reassortant viruses generated by the means of reverse genetics (RG). The six genes encoding internal and non-structural proteins were derived from the cold-adapted master donor virus (MDV) A/Leningrad/134/17/57 (H2N2) [Len/17] (Gustin et al., 2011; Isakova-Sivak et al., 2011). The chimeric HA genes containing stalk domain of A/California/7/2009 (Cal09, pH1N1) virus and head domains of A/Vietnam/1203/2004 (ΔH5N1) [polybasic cleavage site deleted] and A/mallard/Sweden/24/02 (H8N4) were chemically synthesized at Icahn School of Medicine at Mount Sinai (New York, USA), followed by its subcloning into dual-promoter RG plasmids. Additional chimeric HAs bearing the stalk domain of A/PR/8/34 (PR8, H1N1) or A/South Africa/3626/2013 (SA13, pH1N1 virus with 99% amino acid stalk domain homology with the stalk of Cal09) and globular head domains of A/Vietnam/1203/2004 (ΔH5N1) and A/quail/Hong Kong/G1/1997 (H9N2) were generated by gene engineering methods at the Department of Virology, IEM (St. Petersburg, Russia) (Table 1). To do this, the HA head domains (aa 52–277, H3 numbering) of PR8 and SA13 viruses were removed by an overlap PCR strategy, and a BsmBI restriction site was introduced at this position. Subsequently, the HA head domains of H5N1 and H9N2 viruses were subcloned using the BsmBI restriction enzyme. All the resulting cHA constructs were sequence-confirmed by Sanger sequencing (ABI 3130xl, Applied Biosystems, USA). To prepare control LAIV viruses with non-chimeric HAs, the full-length HAs of the H5N1, H8N4 and H9N2 viruses were cloned into RG plasmids from corresponding wild-type influenza viruses, with an additional removal of the HA polybasic cleavage site of the H5N1 virus. The Len/17-based LAIV candidates bearing natural or chimeric HAs were rescued by electroporation of Vero cells using the Neon transfection system (Invitrogen, USA). In addition, an A/17/quail/Hong Kong/97/84 (H9N2) LAIV control virus generated by classical reassortment in eggs was obtained from the LAIV strain repository of the Institute of Experimental Medicine (Table 1) (Desheva et al., 2015).

Table 1
Overview of the Len/17-based LAIV viruses used in this study.

Virus designation	Origin of viral surface genes[a]		
	HA head domain	HA stalk domain	NA
H5N1 LAIV	H5N1	H5N1	H5N1
H9N2 LAIV	H9N2	H9N2	H9N2
$H5N1_p$ LAIV	H5N1	H5N1	pH1N1
$H8N1_p$ LAIV	H8N4	H8N4	pH1N1
$H9N1_p$ LAIV	H9N2	H9N2	pH1N1
cH5/1N1 LAIV	H5N1	PR8 (H1N1)	H5N1
cH9/1N2 LAIV	H9N2	PR8 (H1N1)	H2N2
$cH5/1N1_p$ LAIV	H5N1	pH1N1	pH1N1
$cH8/1N1_p$ LAIV	H8N4	pH1N1	pH1N1
$cH9/1N1_p$ LAIV	H9N2	pH1N1	pH1N1

$N1_p$: NA of pH1N1 virus.
[a] the remaining six genes originate from MDV A/Leningrad/134/17/57 (H2N2).

All LAIV viruses were propagated in 10-day-old embryonated chicken eggs for 2 days at 33 °C. Virus stocks were harvested, clarified by low-speed centrifugation and stored in aliquots at − 70 °C.

For the purpose of estimating vaccine efficacy in mice, the following mouse-pathogenic influenza viruses were obtained from the influenza virus repository of either Centers for Disease Control and Prevention (CDC, Atlanta, USA), Research Institute of Influenza (St.Petersburg, Russia) or the Institute of Experimental Medicine: (1) PR8 (H1N1); (2) A/Tokyo/3/67 (H2N2) [Tokyo H2N2]; (3) A/Vietnam/1303/04 CDC-RG (ΔH5N1) [VN-PR8 H5N1], a PR8-based 6:2 reassortant candidate vaccine virus; (4) A/herring gull/Sarma/51/2006 (H6N1) [Sarma H6N1]; (5) A/Hong Kong/1073/97 (H9N2) [HK H9N2] and (6) A/Mississippi/10/2013 (pH1N1). The challenge viruses were grown in eggs for 2 days at 37 °C.

Recombinant proteins including cH6/1 (containing HA head domain of A/mallard/Sweden/81/02 (H6N1) and H1 HA stalk domain of PR8), H2 (from A/Albany/1/1968), H3 (from A/Perth/16/2009), H6 (from A/mallard/Sweden/81/02) and H9 (from A/chicken/Hong Kong/G9/1997) were expressed in High Five cells as described previously (Krammer et al., 2012a; Margine et al., 2013b).

2.2. In vitro studies

Temperature sensitive and cold-adapted (*ts/ca*) phenotypes of the LAIV viruses were determined by their titration at different temperatures in eggs: 38 °C compared to 33 °C for *ts* phenotype and 26 °C compared to 33 °C for *ca* phenotype. Eggs inoculated with 10-fold virus dilutions were incubated either 48 h (for 33 °C and 38 °C) or 6 days (for 26 °C). In addition, the LAIV virus growth was analyzed in Madin-Darby canine kidney (MDCK) cells to determine the 50% tissue culture infectious dose ($TCID_{50}$). Virus titers in eggs and MDCK cells were calculated using the Reed and Muench method and expressed in $\log_{10}$ 50% egg infectious dose (EID_{50})/ml and $\log_{10}TCID_{50}$/ml, respectively. A virus was considered to be temperature sensitive (*ts* phenotype) if the infectious titers at 33 °C was at least 5.0 $\log_{10}EID_{50}$ lower than at 38 °C. A virus was considered to be cold adapted (*ca* phenotype) if the infectious titers at 26 °C was not more than 3.0 $\log_{10}EID_{50}$ lower than at 33 °C (Kiseleva et al., 2010).

2.3. Virus replication kinetics in mice

Female BALB/c mice were randomized into groups of eight, anesthetized with ether and inoculated with a volume of 50 µl of virus suspension containing 10^7 EID_{50} by the intranasal (i.n.) route. Nasal turbinates and lungs were collected at 3 and 6 days post infection (dpi) and stored frozen at − 70 °C until used for homogenization. Tissue homogenates were prepared using a small TissueLyser LT (QIAGEN, Germany) bead mill in 1 ml of sterile phosphate buffered saline (PBS)

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