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Combined use of live-attenuated and inactivated influenza vaccines to enhance heterosubtypic protection

Li-Meng Yan[a], Olive T.W. Li[a], Chek M. Poh[a], Ranawaka A.P.M. Perera[a], Sophie A. Valkenburg[a,b], Malik Peiris[a], Leo L.M. Poon[a,*]

[a] Centre of Influenza Research, School of Public Health, The University of Hong Kong, Hong Kong SAR, China
[b] HKU-Pasteur Research Pole, School of Public Health, The University of Hong Kong, Hong Kong SAR, China

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ABSTRACT

The limited protection of current commerical vaccines necessitates the investigation of novel vaccine strategies for unpredictable outbreaks. To investigate the feasibility of using vaccines derived from Group 1 influenza A virus to induce broadly cross-reactive immune responses against multiple influenza subtypes, we tested a panel of sequential 4-dose immunization regimens in mice. Mice were treated with inactivated (seasonal H1N1, pandemic H1N1 and H5N1) and vaccinia virus-based H5N1 live-attenuated vaccines in different combinations. Mice were then challenged by viruses of either Group 1 (H1N1) or Group 2 (H3N2, H7N7) influenza virus. All studied sequential 4-dose vaccinations could induce some degrees of heterosubtypic protection in mice. Amongst all these regimens, the combined use of inactivated and live-attenuated vaccines could achieve the best heterologous protection. These results highlight the synergistic effect of combining different vaccine platforms to enhance heterosubtypic protection against influenza viruses.

1. Introduction

Influenza A virus (IAV) is a medically important pathogen. Seasonal influenza strikes every year, along with the constant threat of avian influenza and occasionally pandemics. Vaccination remains one of the best control measures against seasonal influenza. Current licensed inactivated and live-attenuated influenza vaccines, however, still have their own limitations. For example, the protection induced by inactivated vaccines is entirely dependent on eliciting antibodies that can neutralize circulating seasonal influenza viruses (Petrova and Russell, 2018). The commercial live-attenuated influenza vaccine can induce both B and T cells responses for protection, but the recent recommendation against the use of live-attenuated influenza vaccine questions the realialbilty of this vaccine approach (Grohskopf et al., 2016). One should also note that both kinds of seasonal influenza vaccines only aim at developing immune responses against viruses that are antigenically highly similar to the selected vaccine strains. Major antigenic changes of ciriculating human influenza viruses, either because of drift or shift, can allow the newly emerging antigenic variants escape from vaccine-induced immuntiy.

To date, 16 hemagglutinin (HA) subtypes and 9 neuraminidase (NA) subtypes of influenza A virus have been identified in birds (Fouchier et al., 2005). These influenza viruses can be phylogenetically classified into two distinct HA groups: Group 1 HA contains H1, H2, H5, H6, H8, H9, H11, H12, H13 and H16, whereas Group 2 HA contains H3, H4, H7, H10, H14 and H15 (Throsby et al., 2008). Sporadic zoonotic infections caused by some of these avian influenza viruses can be detected in humans (e.g. H5, H6, H7 and H9) (Yoon et al., 2014). Although highly pathogenic H5 and H7 avian influenza viruses are of huge pandemic concerns, it is currently impossible to predict the subtype that will be responsible for the next pandemic. The unpredictable nature of influenza A virus can be highighted by the H1N1 pandemic in 2009. International health organizations, such as World Health Organization, therefore urge for new developments that can induce broadly reactive immune responses against influenza casued by different subtypes (Hampson et al., 2017).

The influenza virus surface glycoprotein HA can be broadly divided into 2 parts, namely, the HA1 head domain and HA2 stalk domain. The stalk domain is responsible for membrane fusion in virus entry and it is structurally and genetically well conserved among all HA subtypes. Broadly neutralizing antibodies (bnAbs) targeting conserved domains in the stalk region have been described (Ekiert and Wilson, 2012). Unlike the classical neutralizing antibodies, which normally bind to the receptor binding domains to prevent virus attachment to host cells,

* Correspondence to: School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong SAR, China.
E-mail address: llmpoon@hku.hk (L.L.M. Poon).

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stalk-specific bnAbs prevent the conformational change of HA2 required for membrance fusion. Some stalk-specific bnAbs are also known to have inhibitory effects on other viral processes (DiLillo et al., 2014). HA2 stalk region is not as immunogenic as HA1 head domain and the level of HA2-specfiic antibodies induced by a natural infection is normally much lower than the one specific for the HA1 region. However, recent animal and human studies have suggested that the production of HA2-specific bnAbs can be stimulated by repeated heterologous infections (Li et al., 2012; Margine et al., 2013). Sequential immunizations with chimeric HA carrying different HA1, but with conserved HA2, can also achieve such stimulatory effects (Qiu et al., 2011; Nachbagauer et al., 2014).

We have recently developed several novel vaccine approaches to induce heterologous immunity. One of these vaccines is a vaccinia virus-based live-attenuated vaccine (Wyeth/IL-15/5Flu) (Poon et al., 2009; Valkenburg et al., 2014, 2016; Fan et al., 2015). Wyeth/IL-15/5Flu is a novel pentavalent vaccine, expressing HA, NA and NP proteins from H5N1/A/Vietnam/1203/2004, M1 and M2 proteins from H5N1/A/CK/Indonesia/PA/2003 virus, and human IL-15 as a molecular adjuvant (Poon et al., 2009). As this vaccine is able to trigger both MHC I and II antigen processing machineries, it is able to elicit robust $CD4^+$ and $CD8^+$ T cell responses. Our previous studies have revealed that this vaccinia virus-based H5 vaccine can induce good heterosubtype protection against both Group 1 and Group 2 HA viruses, indicating that this vaccine might be a promising candidate to serve as a universal vaccine (Poon et al., 2009; Valkenburg et al., 2014). Here, we investigate that the combined use of this live-attenuated H5N1 vaccine and inactivated H1N1 viral antigens can further enhance this heterosubtypic protective effect.

2. Materials and methods

2.1. Viral antigens and live-attenuated virus for vaccination

Inactivated seasonal H1N1 (sH1: A/Brisbane/59/07), pandemic H1N1 (pdmH1: A/California/7/2009) and highly pathogenic H5N1 (H5: A/VN/1203/04) viral stocks were purchased from National Institute for Biological Standards and Control, United Kingdom. Vaccinia virus-based live-attenuated H5N1 vaccine (Wyeth/IL-15/5Flu, hereafter called “5Flu”) used in this work has been previously described (Poon et al., 2009). Briefly, this virus can express HA, NA and NP proteins of A/Vietnam/1203/2004 (H5N1), M1 and M2 proteins of A/CK/Indonesia/PA/2003 (H5N1) and human IL-15 in infected cells.

2.2. Vaccination regimens

Use of all animals was approved by the Committee on the Use of Live Animals in Teaching and Research, The Hong Kong University. Female BALB/c mice (8 week of age) were supplied by Laboratory of Animal Unit, The University of Hong Kong. The studied vaccine scheme consisted of 2 doses of inactivated seasonal/pandemic H1N1 vaccines followed by 2 doses of inactivated H5N1 or vaccinia virus-based live-attenuated H5N1 vaccines (Fig. 1). BALB/c mice were sequentially immunized via intramuscular route 3 weeks apart (100 µl per dose, each dose containing 5 µg HA for inactivated virus or 10^7 pfu for live-attenuated H5N1 vaccine). For vaccinating mice with inactivated viral antigens, AddaVax (InvivoGen) was used as an adjuvant (1:1; Volume/Volume). PBS treatments were also used as controls. Five different vaccination regimens were studied in this work and mice were vaccinated with different immunogens as follows:

A) Group (-) (control): PBS, PBS, PBS and PBS
B) Group V (5Flu vaccine only): PBS, PBS, 5Flu and 5Flu
C) Group inH1 (Inactivated H1 vaccines only): sH1, pdmH1, PBS, PBS
D) Group inH1/V (Inactivated H1 and 5Flu vaccines): sH1, pdmH1, 5Flu and 5Flu
E) Group inH1/H5 (Inactivated H1 and H5 vaccines): sH1, pdmH1, H5 and H5.

2.3. Virus challenge studies

Treated mice were anesthetized and challenged intranasally by influenza viruses 3 weeks after the final vaccination using protocols as described before (Poon et al., 2009). The viruses include: Human H1N1 (A/Puerto Rico/8/1934, PR8; $10MLD_{50}$), mouse-adapted H3N2 (A/Hong Kong/1/68, HK68; $10MLD_{50}$), or highly pathogenic avian H7N7 (A/Netherlands/219/2003, H7N7; $1MLD_{90}$). Morbidity and weight loss were monitored daily for 14 days, and survival curves were presented ($n \geq 10$ per group). Humane endpoint was set to be 25% of body weight loss. Tissue from representative mice from each group were harvested at various days post-infection (dpi) as specified. All experiments involving highly pathogenic H7N7 viruses were conducted in a biosafety level 3 laboratory.

2.4. Lung viral load and protein concentration assays

Lungs from challenged mice ($n = 3$ per group) were harvested for 3 and 7 days post-infection (dpi). Harvested tissues were mechanically homogenized (1 ml PBS per lung) and the virus titres were titrated using MDCK cells. The virus titres of infected lung tissues were determined by $TCID_{50}$ assays using the Reed-Muench formula.

Bronchoalveolar lavage (BAL) were harvested at 7dpi ($n = 9$ per group). The total protein concentration in supernatants of BAL was measured using Pierce BCA Protein Assay kit (Thermo Scientific).

2.5. Serological assays

Serum harvested at day 21 after the final vaccination and at 7 dpi were studied by ELISA and microneutralization (MN) assay for determining the level of influenza virus-specific antibodies. For conducting MN assays, RDE-treated, heated-inactive serum samples were 2-fold serially diluted starting from 1:10, and tested against PR8 or HK68 virus using standard protocols ($n \geq 10$ per group) (INFL and MANU, 2002).

For conducting ELISA assays ($n \geq 6$ per group), recombinant HA of sH1, pdmH1 and HK68 and NP of PR8 (Sino Biological) were used as antigens to coat ELISA plates (Nunc MaciSorp) at 4 °C overnight. Heat-treated serum samples were 2-fold serially diluted (PBS, 0.1% BSA, 0.05% Tween-20) starting from 1:80. Anti-mouse IgG1 and IgG2α HRP conjugates (Invitrogen) were used as the secondary antibodies as appropriate. ELISA tests were conducted according to the manufacturer's guidelines (DuoSet reagent kits, R&D Systems). Absorbance at 450 nm was detected using a standard microplate reader.

2.6. Intracellular cytokine staining

$CD4^+$ and $CD8^+$ T cell recall responses were determined by intracellular cytokine staining (ICS) assays, as described previously (Horton et al., 2007). A panel of immunodominant HA- and NP- derived epitopes were selected to measure influenza virus-specific $CD4^+$ (MHC class II peptides: H1N1/H5N1 $NP_{55–69}$:RLIQNSITIERMVLS, H1N1 $HA_{140–154}$:AKSFYKNLIWLVKKE, H5N1 $HA_{140–154}$:KSSFFRNVVWLIKKN) and $CD8^+$ (MHC class I peptides: $NP_{147–155}$:TYQRTRALV, H1N1/H5N1 $HA_{518–526}$:IYSTVASSL) T cell responses (Valkenburg et al., 2014; Richards et al., 2015). The production of type 1 (IFN-γ, TNF-α, IL-2) and type 2 (IL-4) cytokines of these T cells was detected by flow cytometry analyses. Briefly, splenocytes ($n = 5–6$ per group) and cells from BAL ($n = 9$ per group) were harvested from infected mice at 7 dpi. After hemolysis with Red blood cell lysis buffer (eBioscience), isolated lymphocytes were co-stimulated by the above virus-specific peptides (1uM), anti-CD28 and anti-CD49d (BD biosciences, 300 ng/ml), IL-2 (Roche, 25U/ml) for 2 h, followed by an incubation of Golgi plug