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Short communication

Improved protection against avian influenza H5N1 virus by a single vaccination with virus-like particles in skin using microneedles

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ABSTRACT

To develop a more effective vaccination method against H5N1 virus, we investigated the immunogenicity and protective efficacy after skin vaccination using microneedles coated with influenza virus-like particles containing hemagglutinin derived from A/Vietnam/1203/04 H5N1 virus (H5 VLPs). A single microneedle vaccination of mice with H5 VLPs induced increased levels of antibodies and provided complete protection against lethal challenge without apparent disease symptoms. In contrast, intramuscular injection with the same vaccine dose showed low levels of antibodies and provided only partial protection accompanied by severe body weight loss. Post-challenge analysis suggested that improved protection was associated with lower lung viral titers and enhanced generation of recall antibody secreting cells by microneedle vaccination. Thus, this study provides evidence that skin delivery of H5 VLP vaccines using microneedles designed for self-administration induces improved protection compared to conventional intramuscular immunization.

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Avian H5N1 influenza viruses cause sporadic zoonotic infections of humans with high fatality rates of 60% (Sims et al., 2005; Webster et al., 2005). Furthermore, the pandemic potential of these viruses poses a serious threat to public health. The influenza pandemic caused by the 2009 H1N1 virus provided an opportunity to examine the performance of current vaccination. The available evidence indicates that the second wave of infection spread through the US population in the early Fall of 2009, before the vaccine became available to the majority of targeted high-risk population groups (Litchfield, 2009; Loeb et al., 2010). This experience indicated that development of new and faster methods of vaccine manufacturing and immunization should be a priority.

The skin has been suggested as an attractive site for immunization due to the presence of potent antigen-presenting cells such as Langerhans and dermal dendritic cells (Glenn and Kenney, 2006; Hammond et al., 2001). To improve protective efficacy while reducing the antigen mass by targeting influenza antigens to the skin, intradermal (ID) immunization has been evaluated in clinical trials (Auewarakul et al., 2007; Belshe et al., 2004; Kenney et al., 2004; Khanlou et al., 2006; Van Damme et al., 2009). However, the conventional ID injection procedure requires highly trained medical personnel and is not well tolerated by vaccinees due to pain and discomfort at the site of injection (Auewarakul et al., 2007; Belshe et al., 2004; Kenney et al., 2004). Recent studies have demonstrated a promising alternative method that delivers inactivated whole-virion vaccines to the skin using microneedles, penetrating the outer layer of the skin (Kim et al., 2010, 2009; Quan et al., 2009; Zhu et al., 2009). This simple design could permit self-administration of vaccine by patients, possibly enabling vaccination campaigns to rapidly reach large populations (Prausnitz et al., 2009).

Conventional inactivated vaccines are produced from virus propagated in eggs. A new vaccine platform, virus-like particles (VLPs) produced in cell culture, has been shown to confer protection against highly pathogenic avian-origin influenza viruses in animal models, and can be manufactured without handling pathogenic live viruses (Bright et al., 2008; Haynes et al., 2009; Kang et al., 2009). In the present study, we investigated the immunogenicity and protective efficacy after a single vaccination using microneedles coated with dried H5 VLPs, in comparison with conventional intramuscular injection.

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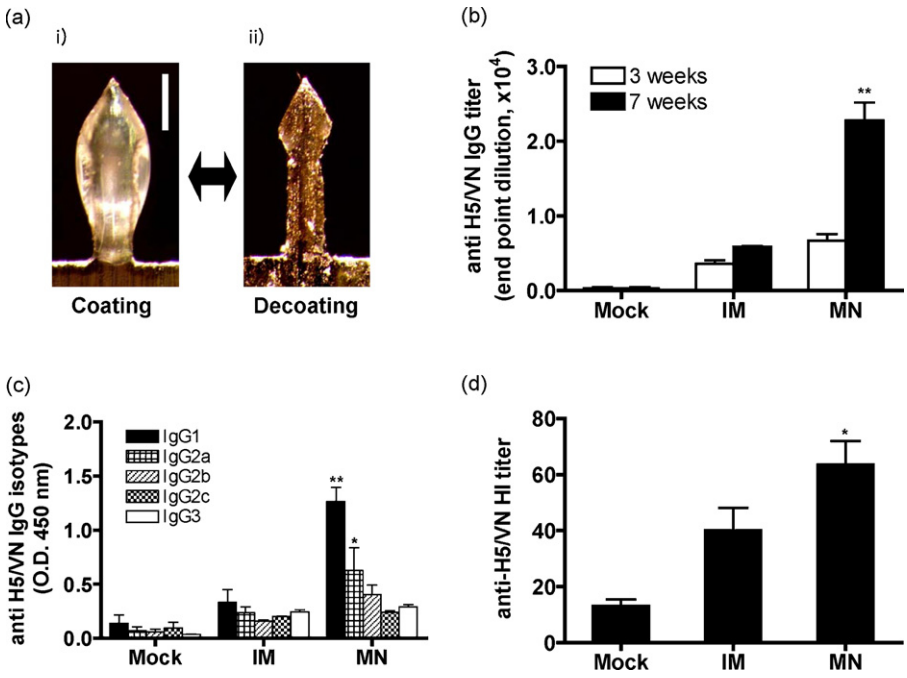

Fig. 1. Immunogenicity of influenza H5 VLP vaccination in the skin using microneedles. (a) A microneedle coated with H5 VLPs before and after dissolution in PBS. A microneedle is shown as observed by bright field microscopy after coating with influenza H5 VLPs for skin vaccination (i) and after dissolution of the coating from the microneedle in PBS (ii). Bar = 250 μm. (b) H5 HA-specific IgG titers at weeks 3 and 7 after a single vaccination. Titers of antibodies specific to inactivated H5 A/Vietnam/1203/04 virus are expressed as the highest dilution of sera having a value of optical density at 450 nm (OD450) greater than the mean plus 2 standard deviations of similarly diluted naïve sera as described previously (Quan et al., 2007). (c) H5 HA-specific isotype antibodies as presented in OD450 values of 100× diluted sera at week 7. BALB/c mice (n = 11 per group) were immunized once with H5 VLPs using microneedles (MN), intramuscular injection (IM), or uncoated placebo microneedles (Mock). Bars indicate means ± S.E.M. (d) Hemagglutination inhibition (HI) titers determined at week 7 after vaccination. Asterisk indicates significance between MN and IM groups (**p < 0.01, *p < 0.05, Student's 2-tailed t-test).

H5 VLPs derived from influenza A/Vietnam/1203/04 (A/VN/04) virus were produced in insect cells using recombinant baculovirus expression as previously described (Kang et al., 2009). Stainless steel microneedles were fabricated as arrays of 5 needles (Kim et al., 2010). The 700 μm length of microneedles used in this study is suitable for effective delivery of vaccine into mouse skin with a thickness of 500–600 μm (Azzi et al., 2005), because the whole microneedle is not fully inserted into the skin due to skin deformation during insertion. For vaccination in the skin, microneedles were coated on their surfaces with H5 VLPs in coating solution (1% carboxymethylcellulose (CMC) sodium salt as viscosity enhancer, 0.5% (w/v) Lutrol F-68 NF as surfactant, and 15% trehalose as stabilizer) and then air dried (Kim et al., 2010). A change in thickness of the microneedle was observed by bright field microscopy after coating with H5 VLPs and dissolution of coated H5 VLPs into PBS buffer (Fig. 1a). The amount of H5 VLPs coated onto each 5-microneedle array was 2.0 ± 0.15 μg total proteins (approximately 0.2 μg HA) as determined after elution into PBS using a protein assay kit (Quan et al., 2009).

Groups of mice (BALB/c, 6–8 weeks old, n = 11 per group) were immunized using either (i) microneedles without antigen (mock), (ii) microneedles coated with 2 μg of H5 VLPs (MN), or (iii) 2 μg of H5 VLPs in PBS buffer solution dissolved from coated microneedles given by intramuscular injection (IM). At weeks 3 and 7 after a single dose vaccination, antibody responses in sera were determined by quantitative ELISA using recombinant H5 HA protein as a coating antigen (Fig. 1b). Interestingly, at week 7 after a single immunization, 5-fold higher levels of H5 HA specific antibodies were observed in the microneedle vaccination group compared to the intramuscular control, which is significantly higher than those at week 3 (Fig. 1b).

The pattern of antibody isotypes may provide informative insight into the T helper type 1 or 2 immune responses (Hocart et al., 1989). Therefore, we determined antibody isotypes after a single microneedle or intramuscular vaccination. IgG1 isotype antibody was induced at significantly higher levels than IgG2a antibody after microneedle delivery (p = 0.031, Fig. 1c). This IgG1 isotype-dominant pattern following H5 VLP microneedle immunization is similar to that observed with microneedle vaccination using whole inactivated A/Aichi/68 virus (H3N2) (Koutsonanos et al., 2009), but different from results obtained from 0.4 μg low vaccine dose of the A/PR/8/34 (H1N1) virus or VLPs (Quan et al., 2010, 2009), which predominantly induced IgG2a isotype antibody after microneedle vaccination of BALB/c mice. Interestingly, the IgG1 isotype antibody was previously shown to have higher neutralizing and similar HI titers, respectively, compared to the IgG2a isotype in immune sera of BALB/c mice (Hocart et al., 1989). Although it is not clear what factor(s) influences IgG1/IgG2a ratios, there are several potential parameters affecting the antibody isotype pattern, which include mouse strains and types of vaccines (Hocart et al., 1989), stability and integrity of vaccine antigens (Quan et al., 2010, 2009), routes and doses of vaccines (Bright et al., 2008), and intrinsic immunogenicity properties of vaccines such as H5 VLPs of A/Vietnam/1203/04 observed in this study.

Next, we determined hemagglutination inhibition (HI) antibody responses using 1% horse red blood erythrocyte (Fig. 1d). HI titers over 60 were detected in the microneedle vaccination group, which is 4-fold higher than the titers in the mock control and 1.5-fold higher than the IM group (p = 0.03). As high doses of H5 vaccine were reported to be required in humans for moderate immunogenicity, this observation suggests that a MN H5 vaccine could bring significant improvement compared to other non-adjuvanted vaccines tested thus far in clinical trials. The low antibody response reported in a study by Patel et al. (2010) is likely related to the small doses of antigen injected intradermally. In other clinical trials indicating low immunogenicity of H5 vaccines, prime-boost immu-

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