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Heterosubtypic protection conferred by combined vaccination with M2e peptide and split influenza vaccine

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ABSTRACT

There is urgent need to develop influenza vaccines with broad-spectrum protection against the potential influenza pandemic. The extracellular domain of influenza M2 protein (M2e) is considered as an appropriate target to induce heterosubtypic protection. We investigated the immunity and protection induced by combined vaccination with synthetic M2e peptide and traditional split influenza vaccine. The combined vaccination was able to induce similar strain-specific hemagglutinin inhibition (HI) antibodies as vaccination of split virus alone. However, aluminum-adjuvant but not oil-in-water-emulsion adjuvant combined vaccination was able to induce high titers of anti-M2e antibodies and provoke M2e-specific T lymphocyte response. Furthermore, we found that the addition of M2e peptide greatly enhanced the cross-protective efficacy of split virus in aluminum adjuvant but slightly weakened the efficacy of vaccination in oil-in-water-emulsion adjuvant. Moreover, aluminum-adjuvant combined vaccination conferred complete cross-protection against heterosubtypic influenza virus. According to the results, we suggest that the M2e peptide should be added into split influenza vaccine in the preparation for the potential influenza pandemic.

1. Introduction

Antigenic variation of influenza virus poses great challenge on influenza vaccine development. The widely used influenza vaccines at present, mainly including inactivated influenza vaccines, are only effective against a few relative epidemic strains. New circulating strains, generated by frequent antigenic changes of the major glycoproteins hemagglutinin (HA) and neuraminidase (NA), make current vaccines ineffective [1]. As a result, annual revaccination is necessary to cope with antigenic drift of epidemic strains [2]. Based on a global surveillance, the WHO predicts the dominantly circulating strains in the next epidemic season and updates the vaccine formula every year [3]. When it comes to the seasonal human influenza virus caused by H1N1 or H3N2 virus, the prediction is often reliable and the selected vaccines are usually effective. However, the new circulating H5N1 avian influenza virus engenders a different situation. As a highly pathogenic virus, the H5N1 avian influenza virus would cause a great pandemic and might result in several millions deaths globally when it would acquire an efficient human-to-human transmissibility [4]. Nevertheless, it is quite difficult to predict when and which strain would acquire transmissibility between humans, to cause a worldwide pandemic. Although several inactivated H5N1 influenza vaccines have been developed, it is still unclear whether these vaccines available nowadays are effective against the potential influenza pandemic. So it is of great importance to develop influenza vaccines with broad-spectrum protection to prepare for the potential pandemic [5].

The conservative extracellular domain of influenza M2 protein (M2e) is considered as an appropriate target for the development of influenza vaccine with broad-spectrum protection. To date, the efficacy of several M2e-based vaccines have been tested in animal models, including baculovirus-expressed M2 proteins [6], M2e-HBc conjugate [7], synthetic M2e peptide conjugate [8], and so on. Vaccination with M2e could protect animals against different subtypes of influenza viruses, including avian H5N1 influenza virus [9]. The protection was mediated by M2e-specific antibodies via antibody dependent cytotoxicity [10]. However, these M2e-based vaccines are not prophylactic vaccines. A major reason is that M2e-specific antibodies are able to inhibit viral replication whereas fail to prevent viral infection. Consequently vaccination with M2e-based vaccines greatly reduced mortality but not morbidity in animal test. Therefore, M2e-based vaccines are not capable to replace traditional inactivated influenza vaccines in epidemic season.

This dilemma can be resolved by combined vaccination with inactivated influenza vaccine and M2e-based vaccine. Inactivated influenza vaccine would confer efficient protection against predicted seasonal epidemic strains. Once escaped strains or new subtype strains circulate, the activated M2e-specific immune

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response would inhibit virus spread and reduce mortality, to a more or less extent. In this paper, we investigated whether the combined vaccination was able to provide cross-protection against heterosubtypic influenza virus in mice model. The combined vaccines were composed of an inactivated split influenza vaccine and a synthetic 24-residue influenza M2e peptide, with aluminum or oil-in-water-emulsion adjuvant. The 24-residue M2e peptide was highly immunogenic and was able to induce protection against influenza virus challenge without carrier protein, as indicated in our previous report [11]. Our results in this report indicated that the combined vaccination was able to provide effective protection against heterosubtypic influenza virus, suggesting the suitability of adding M2e peptide into current widely-used split influenza vaccine in order to broaden the protection.

2. Materials and methods

2.1. Peptide and Viruses

The M2e peptide, N-KSLLTEVETPIRNEWGCRCNDSSD, was synthesized at SBS Genetech Co. (Beijing, China). The purity of the peptide was above 90%, analyzed by HPLC and Mass spectrum. Influenza virus A/Wuhan/359/95 (H3N2) was used as vaccine strain, and mouse-adapted influenza virus A/Porto Rico/8/34 (PR8, H1N1) was used as challenge strain. The viruses were propagated in allantoic fluids of 10-day-old embryonated eggs at 37 °C for 3 days. Virus titers were measured as described [12].

2.2. Inactivated split influenza vaccine

The allantoic fluids containing H3N2 virus were inactivated by 0.1% formaldehyde at 4 °C for 24 h. Virus was then enriched by centrifugation at 75,600 × *g* for 90 min. Pellet was dissolved in phosphate-buffered saline (PBS) and split by the detergent Trixton-X100 at a final concentration of 0.5% (v/v) for 2 h at room temperature (RT). After centrifugation at 10,000 rpm for 5 min, supernatant was collected, and the detergent was removed by ultrafiltration with Amicon Ultra 100K NMWL device (Millipore, USA). The split H3N2 virus was stored at −40 °C and the concentration of total viral protein was determined by Coomassie Blue dying assay.

2.3. Immunization

Female Balb/C mice were given intraperitoneal immunization with the combined vaccine including 10 μg inactivated split influenza H3N2 virus (total protein) mixed with 10 μg M2e peptide for each mouse, in aluminum or oil-in-water-emulsion adjuvant. Mice were immunized with split virus or peptide alone as controls. The oil-in-water-emulsion adjuvant was composed of 5% (v/v) squalene, 0.5% (v/v) Span 85 and 0.5% (v/v) Tween-80 in PBS and emulsified by ultrasonication for 30 min in ice bath [13]. Antigens were dissolved in PBS and adsorbed with adjuvant for 30 min before injection. A booster was given with the same immunogen two weeks later. Sera were collected on day 7 post the second immunization and normal sera were prepared before immunization as negative controls.

2.4. Antibody determination

Influenza-specific anti-HA antibodies were measured in the hemagglutinin inhibition (HI) assay as described [12]. In brief, 100 μL serially diluted sera were incubated with 4 hemagglutinin units (HAU) of influenza virus for 20 min at RT. For each well, 50 μL 1% hen red blood cell suspension in PBS was added and incubated for 30 min at RT. In order to eliminate unspecific reactions, sera were previously inactivated in a 56 °C water bath for 30 min and treated with potassium periodate (KIO_4). HI antibody titers were calculated as reciprocal of the highest serum dilution which was able to inhibit hemagglutination.

The titers and subtypes of M2e-specific antibodies were determined by enzyme-linked immunosorbent assay (ELISA) as previously described [11].

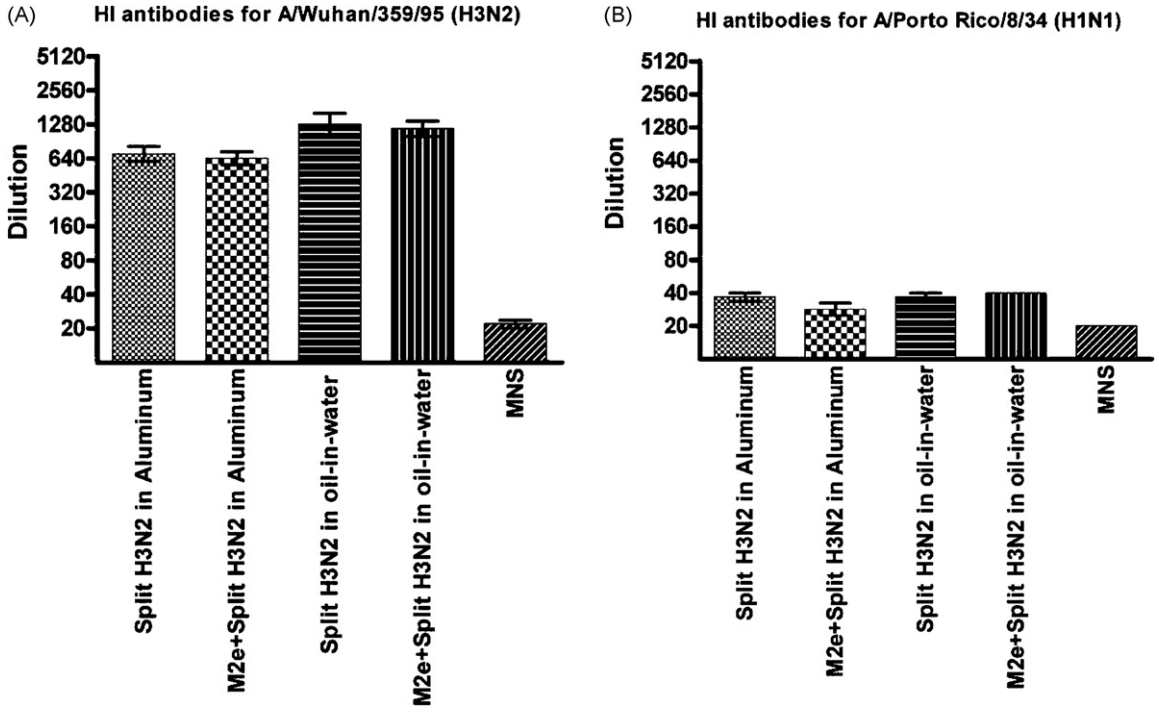

Fig. 1. HI antibodies were determined after the second immunization. The titers of HI antibodies were calculated as reciprocal of the highest serum dilution which was able to inhibit hemagglutination. HI antibodies specific for A/Wuhan/359/95 (H3N2) (A) and A/Porto Rico/8/34 (H1N1) (B) were detected respectively.