ELSEVIER

Institut Pasteur

Microbes and Infection xx (2017) 1–7

www.elsevier.com/locate/micinf

Self-assembly polymerization enhances the immunogenicity of influenza M2e peptide

Peng Zou[1], Yuan Li[1], Jinghe Huang**, Fan Wu*

Shanghai Public Health Clinical Center, Shanghai 201508, China

Received 30 July 2017; accepted 5 September 2017
Available online ■ ■ ■

Abstract

The extracellular domain of Influenza M2 protein (M2e) was considered as a promising target for universal influenza vaccine development. Several M2e-based influenza vaccines have been developed and many of them used a mutant M2e peptide, in which the two conserved cysteine residues were substituted by serine residues. In this paper, we compared the antigenicity and immunogenicity of wild type and cysteine-mutant M2e peptides. We found that the cysteine substitution slightly affected the antigenicity of M2e epitope, but greatly reduced the immunogenicity of M2e peptide. The cysteine substitution also disabled the M2e peptide from inducing protection against influenza virus challenge in mice. Further analysis revealed that the immunogenicity of M2e peptide was enhanced by the self-assembly of the peptide through inter-peptide disulfide bonds. These results provide new information to improve the design of M2e-based vaccines against potential influenza pandemics.
© 2017 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Influenza; M2e; Vaccine

1. Introduction

The prevalence of avian influenza viruses (AIV) in birds, poultry and environment poses an extensive threat to the public health and economic recovery worldwide [1–5]. Sporadic cases of the newly emerging pathogenic AIV infections in mammalian hosts, especially in humans, present great challenge to the current influenza vaccine production and vaccination strategies [6–13]. Current influenza vaccine, including inactivated and live attenuated vaccines, are targeted on influenza hemagglutinin (HA) protein and induce protective neutralizing antibodies [14–16]. The vaccine efficacy is dependent on the antigenic similarity between circulating influenza virus and vaccine strains. The influenza vaccine strains need to be updated regularly to match the circulating strains. Currently, the influenza vaccine strains are selected based on the global surveillance and prediction of epidemic and pandemic by new emerging influenza strains. However, the process from seed strain selection to influenza vaccine production is time-consuming. There may be not enough vaccine available to protect public against influenza pandemic when a new pathogenic avian influenza virus acquires ability of human to human transmission [17]. One potential solution is to develop universal influenza vaccine which can provide cross protection against different influenza strains and subtypes [18–22]. Different from the traditional HA-based influenza vaccines, universal influenza vaccines were developed targeted on the influenza conserved proteins or epitopes. One of the promising target is influenza matrix 2 (M2) protein [23–28]. M2 protein is the third protein on virus membrane which is rare in influenza viral particles but abundantly expressed on virus-infected cells. Different forms of influenza vaccines targeted on the extracellular domain of M2 protein (M2e) were developed in the past decade and they

* Corresponding author.
** Corresponding author.
E-mail addresses: Jinghe02@foxmail.com (J. Huang), wufan@shphc.org.cn (F. Wu).
[1] These authors contributed equally.

https://doi.org/10.1016/j.micinf.2017.09.004
1286-4579/© 2017 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

UNCORRECTED PROOF

could induce cross-subtype protection against different subtypes of influenza viruses [29−33], suggesting that M2e-based vaccine can be used as supplementation of traditional influenza vaccine for precaution of potential influenza pandemics.

The M2e domain consists of 24 amino acids and is conserved in different subtypes of influenza viruses [34]. It contains two cysteine residues at positions 17 and 19. The two cysteine residues are identical in most subtypes of influenza viruses. They form disulfide bonds to stabilize the M2 tetramer but the biological function was not clearly understood [35]. The two cysteine residues were intentionally substituted by serine residues to prevent disulfide bond formation and aggregation in many M2e-based vaccines [36−39]. The substitution of serine for cysteine in M2e peptide does not affect the peptide binding to protective M2e-specific monoclonal antibody. The mutant M2e can also induce protective antibodies when coupled to carrier proteins. However, whether the substitution of serine for cysteine affects M2e-specific antigenicity and immunogenicity has not been completely evaluated. In this paper, we compared the antigenicity and immunogenicity of wild type and cysteine-mutant M2e peptide and investigated whether the difference of cysteine substitution affects the efficacy of M2e-based influenza vaccines.

2. Materials and experiments

2.1. Peptides

Three peptides were synthesized in solid phase method by Synpeptide Co. (Shanghai, China). M2e peptide, N′-KSLLTEVETPIRNEWGCRCNDSSD-C′, was a universal M2e peptide with an additional "K" in the N-terminus for coupling with BSA [23]. M2SC peptide, N′-KSLLTEVETPIRNEWGSRCNDSSD-C′, was an M2e mutant peptide with substitution of Cys^{17} by serine. M2SS peptide, N′-KSLLTEVETPIRNEWGSRSNDSSD-C′, was an M2e mutant peptide with substitution of both Cys^{17} and Cys^{19} by Ser. All the peptides were analyzed by HPLC and the purity was above 90%. The peptides were dissolved in sterile water at concentration of 10 mg/ml and stored at −20 °C. The peptides were chemically linked to carrier protein BSA (Bovine Serum Albumin, Sigma) via glutaraldehyde.

2.2. Immunization

Thirty-six 6−8 weeks old female BALB/c mice were divided into six groups and each mouse was intraperitoneally immunized with 10 μg peptide or peptide−BSA conjugates. The immunogens were diluted in phosphorated buffer solution (PBS) and 1:1 mixed with aluminum adjuvant. Booster immunization was given with the same immunogen in each group two weeks after the first immunization. Sera were collected at day 7 post the second immunization and normal sera were collected before immunization as negative controls.

2.3. ELISA

Peptide-specific antibodies were analyzed by enzyme-linked immunosorbent assay (ELISA) as described [31]. Briefly, 96-well microtitre plates (Costar) were coated with 50 μl PBS-diluted peptide solution (10 μg/ml) at 37 °C for two hours. Unspecific binding was blocked with PBS diluted 0.25% gelatin solution at room temperature for two hours. 50 μl serum samples, which were serially diluted with PBS solution, were added into each well and incubated at 37 °C for one hour. After extensive washes with PBS diluted 0.05% tween-20 solution, the bound antibodies were detected by addition of 50 μl horseradish peroxidase-linked anti-mouse antibodies (DAKO) and substrate o-phenylenediamine dihydrochloride peroxide solution (OPD, Sigma) sequentially. The absorbance at 450 nm (A450) was read on multi-plate reader after 15 min.

2.4. ELISPOT

The number of peptide-specific IL-4-secreting cells in mouse was determined using commercial ELISPOT assay kits (U-Cytech, Netherlands) as described [31]. Briefly, the 96-well ELISPOT plates were coated with anti-mouse IL-4 monoclonal antibodies and blocked with PBS containing 1% BSA. Mice were sacrificed at day 9 post the second immunization and spleen lymphocytes were isolated. The suspension of spleen lymphocytes were transferred to ELISPOT plates and 1 μg/ml peptide was added as a stimulator. An irrelevant peptide 2F5, N′-CELDKWAGELDKWA, was added as a negative control. After incubation for 40 h, the visible spots of IL-4-secreting cells were developed and counted with microscope system as described [31].

2.5. Influenza virus challenge

Twenty-four 6−8 weeks old female BALB/c mice were divided into four groups and intraperitoneally immunized with 10 μg peptides of M2e, M2SC and M2SS respectively. The peptides were diluted in PBS and 1:1 mixed with aluminum adjuvant. Mice were immunized with PBS mixed with aluminum adjuvant as negative controls. Booster immunization was given with the same immunogen two weeks after the first immunization. Seven days post the final immunization, mice were challenged intranasally with 5 MLD50 (dose lethal to 50% of mice) of mouse-adapted influenza virus PR8. The bodyweight and death of mice were monitored every day. Survival curves were calculated by Kaplan−Meier methods with Graphpad Prism software. The survival percentages in each group were compared by Log-rank test and the loss of bodyweight were compared by multiple t tests.

2.6. SDS-PAGE

The cross-link of M2e wild type and mutant peptides were analyzed by SDS-PAGE. 10 μg peptides were dissolved in PBS and denatured by SDS with or without 2%

UNCORRECTED PROOF

Please cite this article in press as: Zou P, et al., Self-assembly polymerization enhances the immunogenicity of influenza M2e peptide, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.09.004

Download English Version:

https://daneshyari.com/en/article/8749131

Download Persian Version:

https://daneshyari.com/article/8749131

Daneshyari.com