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Research Paper

Cross-reactivity of immunoglobulin A secreted on the nasal mucosa in mice nasally inoculated with inactivated H1N1 influenza A viruses in the presence of D-octaarginine-linked polymers

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ABSTRACT

We evaluated cross-reactivity of immunoglobulin A (IgA) secreted on the nasal mucosa in mice that were nasally inoculated 4 times with a mixture of inactivated H1N1 influenza A viruses and poly(*N*-vinylacetamide-co-acrylic acid) (PNVA-co-AA) bearing D-octaarginine at 7-day intervals. Three viral strains (A/Puerto Rico/8/34, A/New Caledonia/20/99 IVR116, and A/Solomon Islands/03/2006) and D-octaarginine-linked polymers with different molecular weights were used as antigens and their carriers, respectively. Secretion of intranasal IgA was barely observed when the inactivated virus alone was administered. The polymer induced the production of intranasal IgA specific to the inoculated viruses, irrespective of the viral strain and molecular weight of the polymer. The respective antibodies cross-reacted to recombinant hemagglutinin proteins of not only the viral strain used for immunization but also other H1N1 strains, including A/Puerto Rico/8/34 strain whose hemagglutinin proteins are diverse from those of other strains. Mice with high reactivity of IgA to the inoculated viruses tended to acquire clear cross-reactivity to other viral strains. Notably, IgA induced by inactivated H1N1 A/New Caledonia/20/99 IVR116 strain with the strongest immunogenicity between 3 antigens in the presence of the polymer cross-reacted to recombinant hemagglutinin proteins of the A/Brisbane/10/2007 and A/Viet Nam/1194/2004 strains, which are categorized into H3N2 and H5N1, respectively. Our polymer is a potential candidate for an efficient antigen carrier that induces mucosal IgA having cross-reactivity to antigenically drifted variants, irrespective of the subtype of viral strains.

1. Introduction

Influenza viruses are a major cause of respiratory diseases in humans. Pandemics of influenza A viruses are responsible for substantial mortality and morbidity, particularly in high-risk groups, which include infants, the elderly, and persons with chronic underlying medical conditions [1]. The modern society, which is characterized by borderless and swift movement of humans and materials, always faces with the pandemic risk of influenza viruses, as experienced pandemics caused by a new variant of H1N1 influenza A viruses in 2009.

Vaccination is the most effective tool for the prophylaxis of such infectious diseases. There are commonly 3 types of vaccines for influenza viruses which are injected subcutaneously or intramuscularly: vaccines containing hemagglutinin (HA) components (split virus vaccines), vaccines composed of formalin-inactivated viral particles (whole virus vaccines), and live attenuated (cold-adapted) viruses, while only HA vaccines are clinically available in Japan. Such parenteral vaccines are effective in prevention of severe influenza virus infection [2,3], and their effect is mediated by the induction of immunoglobulin G (IgG) in the blood. This type of

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vaccines is highly effective when they contain viral strains identical to epidemic ones. However, they are less protective against antigenically drifted variants within a subtype of influenza viruses and do not protect against viruses categorized into different subtypes [4,5]. Additionally, the systemic immunization rarely protects the mucosa of respiratory tract from influenza virus infection [6–8].

A goal of vaccination for influenza viruses is to protect humans from viral infection and maintain the magnitude of seasonal epidemics at the controllable level that pandemics can be completely circumvented. However, since parenteral vaccines are not satisfied with the requirement, vaccination on the basis of a novel strategy has been desired. Mucosal vaccination is one of the most effective strategies of adaptive immunization for protecting the hosts against infectious pathogens that invade epithelial cells [9–13]. This strategy provides significant production of antigen-specific IgG in the serum and immunoglobulin A (IgA) secreted on the mucosa. The secreted IgA, which plays an important role in preventing natural infection of influenza viruses, predominantly mediates mucosal immunity. Not only does the mucosal IgA prevent bodies from being infected with pathogens in their invasion sites [6–8,14], but the antibody is expected to exhibit cross-protection against antigenically drifted variants [5]. Such vaccination would be a powerful tool to protect humans from viral infection because the prediction of epidemic strains is not always correct. FluMist was first launched in the United State in 2003 as nasally-administered vaccines containing live attenuated (cold-adapted) influenza viral strains (H1N1 and H3N2 influenza A viruses and influenza B viruses) [15,16]. The vaccines elicit mucosal IgA and cell-mediated immunities and inoculated subjects can be protected from infection with antigenically-matched and/or distant viral strains. However, FluMist is indicated only for the active immunization of healthy subjects aged 2–49 years, indicating that high-risk groups are still jeopardized by the influenza illnesses.

We have addressed a unique strategy of penetration enhancement using cell-penetrating peptide-linked polymers [17,18]. Cell-penetrating peptides are cationic oligopeptides that are internalized into cells via macropinocytosis [19–21]. The cell-penetrating peptide-linked polymers have been designed with the expectation that they enable poorly membrane-permeable molecules physically mixed with them to effectively penetrate the cell membrane without their concomitant cellular uptake. Poly(*N*-vinylacetamide-co-acrylic acid) (PNVA-co-AA) bearing D-octaarginine, which is a typical cell-penetrating peptide, was first prepared to validate our strategy and its potential as a safe penetration enhancer (Fig. 1) [17,18]. Mice experiments revealed that the polymers significantly enhanced peptide penetration through the nasal membrane without mucosal irritation. *In vitro* cell penetration of poorly membrane-permeable molecules was enhanced without cytotoxicity in the presence of the polymers.

Most antigens are poorly immunogenic when solely applied to the mucosa. Therefore, antigen carriers with adjuvant activities are prerequisite for significant induction of mucosal immunity [22–31]. We next evaluated a potential of D-octaarginine-linked PNVA-co-AA as a carrier for mucosal vaccination using ovalbumin (OVA) as an antigen [32]. When mice were nasally inoculated 4 times with a mixture of OVA and the polymer at weekly intervals, OVA-specific IgG and secreted IgA were produced in sera and nasal wash fluids, respectively. A similar immunization profile with both systemic and mucosal humoral immunities was observed when OVA was substituted with influenza virus HA vaccines.

In the present study, we demonstrate that intranasal inoculation with inactivated H1N1 influenza A viruses accompanied by D-octaarginine-linked PNVA-co-AA elicited secretion of mucosal IgA cross-reactive to not only antigenically drifted variants within a subtype of the inoculated viruses but also viruses categorized into different subtypes. There are a couple of important properties required for mucosal vaccines: IgA induction and its cross-reactivity. A series of our studies strongly support the potential use of our cell-penetrating peptide-linked polymer as a promising antigen carrier for mucosal vaccination.

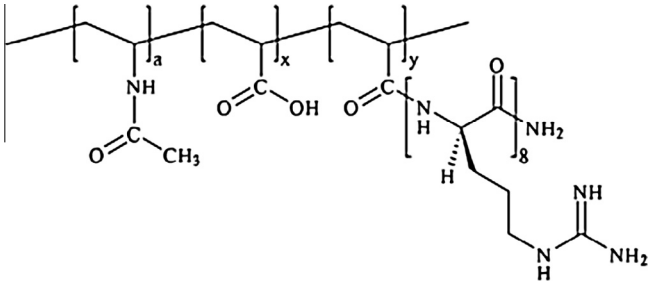

Fig. 1. Chemical structure of D-octaarginine-linked PNVA-co-AA.

2. Materials and methods

2.1. Materials

Sodium salts of PNVA-co-AA were obtained from Showa Denko Co. (Tokyo, Japan). PNVA-co-AA (NVA units/AA units = 70/30) with a weight-average molecular weight (M_w) of 1600 kDa and 350 kDa, which are assigned as GE-160 and GE-160-105, respectively, was used in this study. Octaarginine (D-configuration) with amidated terminal carboxyl groups was purchased from Kokusan Chemical Co. Ltd. (Tokyo, Japan). Other chemicals were commercial products of analytical or reagent grade and were used without further purification.

Trivalent influenza virus HA vaccines used clinically in Japan (SEIKEN in the 2011–2012 season, viral strains: A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008) were obtained from Denka Seiken Co. Ltd. (Tokyo, Japan). Three strains of inactivated H1N1 influenza A viruses (whole particles inactivated by β-propiolactone) were used in this study. The A/Puerto Rico/8/34 strain was purchased from Advanced Biotechnologies, Inc. (Columbia, MD). Other strains (A/New Caledonia/20/99 IVR116 and A/Solomon Islands/03/2006) were obtained from Prospec-Tany TechnoGene Ltd. (Ness-Ziona, Israel). Recombinant HA (rHA) proteins of five H1N1 strains (A/Puerto Rico/8/34, A/New Caledonia/20/99, A/Solomon Islands/03/2006, A/Brisbane/59/2007, and A/California/07/2009), one H3N2 strain (A/Brisbane/10/2007), and one H5N1 strain (A/Viet Nam/1194/2004) were used in this study. The rHA proteins of the A/Puerto Rico/8/34 strain and the A/Viet Nam/1194/2004 strain were purchased from Sino Biological, Inc. (Beijing, China). Other rHA proteins were obtained from eEnzyme LLC (Montgomery Village, MD).

Bovine serum albumin (BSA) and polyoxyethylene (20) sorbitan monolaurate (Tween 20) were obtained from Wako Pure Chemical Industries Co. Ltd. (Osaka, Japan). Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgA and IgG antibodies were purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL). Biotin-conjugated goat anti-mouse IgA and HRP conjugated-streptavidin (ELISA grade) were purchased from Life technologies (Carlsbad, CA). TMB 1-Component Microwell Peroxidase Substrate, SureBlue Reserve, and TMB Stop Solution were obtained from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Dulbecco's phosphate-buffered saline, modified (PBS without calcium chloride and magnesium chloride) was purchased from Sigma–Aldrich (St. Louis, MO). Plates (Immuno Modules, framed, 96 wells per frame, MaxiSorp surface, Nunc