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Improvement of immune responses to influenza vaccine (H5N1) by sulfated yeast beta-glucan

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ABSTRACT

In this study, the adjuvant activity of sulfated glucan from *saccharomyces cerevisiae* ($_S$GSC) was investigated. sGSC had molecular weight of 12.9 kDa and degrees of sulfation of 0.16. The effects of $_S$GSC on the splenic lymphocyte cells of mice were measured in vitro. The results showed that $_S$GSC could significantly promote lymphocyte proliferation singly or synergistically with Con A and LPS, and stimulate the cells to secrete IL-2 and INF-γ. The adjuvant activity of $_S$GSC was researched in BALB/c mice inoculated with inactivated H5N1vaccine in vivo. The results showed that $_S$GSC could significantly enhance lymphocyte proliferation, effectively increase the percentage of $CD4^+$ T cells, decrease the percentage of $CD8^+$ T cells, and elevate the $CD4^+/CD8^+$ ratio; enhance the HI antibody titre, and promote the production of IL-2, INF-γ, IL-4 and IL-6 at medium level. These results indicated that sulfated glucan showed an excellent adjuvant effect on H5N1vaccine in a mouse model. Therefore, $_S$GSC could be used as an effective immune adjuvant for an inactivated H5N1vaccine.

1. Introduction

Influenza is a highly infectious and potentially lethal human respiratory pathogen [1]. Influenza A viruses are dangerous clinical and veterinary pathogens. Viral infection can lead to seasonal pandemics as they cause highly contagious acute respiratory disease in humans, birds and many other species [2]. Highly pathogenic H5N1 subtype influenza virus can be transmitted directly from poultry to human and cause acute respiratory infections. Pandemic influenza virus H5N1 posed a worldwide threat to the public health because of rapid spread and high pathogenicity [3,4]. Over the past two decades, H5N1 highly pathogenic avian influenza has become a significant threat to the health of commercial poultry, wildlife and humans [5]. H5N1 outbreaks occurred frequently in the domestic fowl and migratory birds in Asia, Europe, and Africa in the recent years [6].

The H5N1 virus broke through the species barrier and infected humans, resulting in high mortality [7]. Currently, the most effective preventive measure against the influenza virus is vaccination. Vaccination has been an important strategy for the prophylaxis of H5N1 influenza infection in Chinese domestic poultry since 2004. A strong, primary immune response is important for protection against influenza viruses, since changes in the antigenic epitopes of the viruses occur rapidly. Annual vaccination is the main public health strategy for reducing the influenza burden.

Immunization is the best way to prevent seasonal epidemics and pandemics of influenza [8]. Vaccination is the most effective strategy to control the spread of a pandemic influenza virus. The unadjuvanted inactivated vaccines currently available are partially effective in preventing influenza. Inactivated H5N1 vaccines were consistently poorly immunogenic when administered to animals or human volunteers without adjuvant. Strategies to enhance influenza vaccine immunogenicity include use of higher antigen dose vaccines or inclusion of an appropriate adjuvant. Benefits of adjuvants include enhanced immunogenicity, antigen-sparing and greater duration of protection [9]. However, adjuvants can increase vaccine reactogenicity and may adversely impact on vaccine safety and hence both risks and benefits need to be carefully considered when adding adjuvants to vaccines [10]. As a natural product, polysaccharide can not only activate the immune cells, enhance the antibody titre, and promote the secretion of cytokines but can also exert important roles in cancer therapy and virus prevention [11]. Of the various biological activities of polysaccharides, its immunomodulatory efficacy is the most significant [12]. In addition, polysaccharide does not generate serious side effects when it enters the body and modulates the immune

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function. Polysaccharide is the perfect immunopotentiator for clinical use. Previous studies indicated that polysaccharides adjuvant could enhance influenza vaccine protection. Advax™ adjuvant was derived from inulin, a natural plant-derived polysaccharide that when crystallized in the delta polymorphic form, becomes immunologically active. Advax™ adjuvant increased neutralizing antibody and memory B-cell responses to influenza [13]. Chitosan, a derivative from the natural amino polysaccharide chitin, has been proved to be an effective adjuvant for inactivated influenza virus vaccine [14]. Sophy β-glucan synthesized by *Aureobasidium pullulans* (*A.pullulans*) strain AFO-202, which showed an excellent adjuvant effect on H5N1 and H5N2 vaccinations in a mouse model [15].

β-Glucan from *saccharomyces cerevisiae* is an important bioactive compound for animal and human health, but its low solubility has led to many problems. Sulfation modification could improve its solubility and change its bioactivities. Sulfated yeast glucan could stimulate animal cells proliferation, promote cytokine secretion, and enhance antibody titer of vaccine [17–19]. Previous our study was focus on the relationship between attenuated live vaccine and sulfated glucan. In this paper, we discuss the enhancing effect of sulfated glucan on the immune response to inactivated H5N1 vaccine in mice. The experiments were conducted to examine the enhanced effect of sulfated glucan on immunomodulatory activity related to the influenza virus vaccines in BALB/c mice.

2. Materials and methods

2.1. Reagents

RPMI-1640 was purchased from Gibco (USA). T-cell mitogen Concanavalin A (Con A), lipopolysaccharide (LPS), bovine serum albumin, and cellulose sacks were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Filter membrane was purchased from Millipore Co. (Billerica, Massachusetts, USA).3-(4,5-Dimethylthiazol-2-yl)-2,5-dipheny-ltetrazoliumbromide (MTT) was purchased from Amresco Co. Yeast β-1,3-D-glucan standard was purchased from Putus Macromolecular Sci. & Tech. Ltd. (Wuhan, China). Dimethyl sulfoxide (DMSO), phenol, trichloroacetic acid, NaOH, acetic acid, urea, and H_2SO_4 were purchased from Yixin Institute of Chemical Engineering in Jiangsu. Anti-CD3-PerCP, anti-CD4-PE, and anti-CD8-FITC antibodies were purchased from BD eBiosciences (San Diego, CA, USA).IL-2, IFN-γ, IL-4 and IL-6 kits were purchased from R&B Co. (USA). All chemicals used in the experiments were analytical grade.

2.2. Preparation of sulfated glucan

Based on the results of our previous experiment, sGSC was prepared using the mixed solution of Urea (72 g) DMSO (100 mL) and GSC (4 g) (glucan from *saccharomyces cerevisiae*). The solution was transferred into a separate flask and mixed with H_2SO_4 (10 mL) and DMSO (100 mL). Then the solution was heated to 120 °C in an oil bath with stirring for 6 h. The method for determining carbohydrate contents, molecular weight and degrees of sulfation of sGSC according to our previous report [19].

2.3. Vaccine and virus

H5N1 vaccine was bought from Harbin veterinary research institute (Heilongjiang, China). Inactivated H5N1 virus was purchased from Shenzhen lvshiyuan biotechnology co., ltd (Shenzhen, China).

2.4. Splenic lymphocyte proliferation assay in vitro

The method for preparing splenic lymphocytes was performed according to a previous report [18]. Briefly, spleen cells from mice were collected, placed in Hank's solution, minced and passed through a steel mesh to obtain a cell suspension. After centrifugation, the cells were washed, resuspended in RPMI-1640 and adjusted to 5.0×10^6 mL^{-1}. Then, 100 μL of the splenic lymphocytes were incubated into a 96-well culture plate, and Con A, LPS (20 and 10 μg mL^{-1}), or medium was then added to a volume of 50 μL. Then, in $_S$GSC groups at series of concentrations were added. Cell control group was only added RPMI-1640 media. 100 μL per well, four wells each concentration. The plates were incubated at 37 °C in a humid atmosphere of 5% CO_2. After incubation for 44 h, 20 μL of MTT (5 μg mL^{-1}) was added into each well, and continued to incubate for 4 h. The plates were centrifuged at 1000*g* for 10 min. The supernatant was removed and 100 μL of DMSO was added into each well. The absorbance was measured by microliter enzyme-linked immunosorbent assayreader (Model DG-3022, East China Vacuum Tube Manufacturer) at a wave length of 570 nm (A_{570} value) as the index of lymphocytes proliferation.

2.5. In vivo experimental design

2.5.1. Animal and experimental design

50 Male BALB/c mice (8 weeks old) were purchased from Shanghai Slac Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China). The mice were provided with water and mouse chow ad libitum and were housed in a rodent facility. All procedures involving animals and their care were approved by the Ethics Committee of the Chinese Academy of Agricultural Sciences. All mice were allowed 7 days to adapt to their environment before the experiment. The mice were randomly divided into five groups (consisting of 10 mice each). The mice, except blank control (BC) group, were vaccinated with H5N1vaccine in 0.2 mL doses by subcutaneous injection at the neck, repeated vaccination at 14 days of age. At the same time of the vaccination, the mice in three $_S$GSC groups were administered via gavage in 0.2 mL solutions, respectively, with the $_S$GSC at low (1.56 mg/kg/d), medium (6.25 mg/kg/d) and high (25 mg/kg/d) doses, in vaccination control (VC) and BC group, with equal volume of physiological saline, once a day for three successive days. On D_{35} after the first vaccination, splenocytes were harvested to determine the percentage of $CD4^+$ T cells, $CD8^+$ T cells and the lymphocyte proliferation. The serum samples of mice were collected for determining HI antibody titre and contents of cytokines by ELISA.

2.5.2. Lymphocyte proliferation assay

The mice splenic lymphocytes were prepared according to the previous report [19]. The cells were resuspended to a final density of 3×10^6 mL^{-1} in RPMI 1640 medium supplemented with 10% bovine serum albumin. Then, 1 mL of the spleen cell solution was seeded into a 24-well plate containing 75 μL of Con A, and Con A was replaced with 75 μL of deionized water as the control. The plates were cultured at 37 °C in 5% CO_2 atmosphere for 3 d. After incubation for 68 h, 0.7 mL of solution was removed, and 0.7 mL of RPMI-1640 and 50 μL of MTT (5 mg/mL) were added to each well and incubated for another 4 h. Then, 1 mL of acid isopropyl alcohol was added into each well and evenly mixed. The content of each well of the 24-well plate was added into three wells of a 96-well plate. The absorbance at 570 nm was obtained on a microplate reader (Thermo Multiskan MK3, USA).

2.5.3. Detection of $CD4^+$ and $CD8^+$ t lymphocytes

The mouse spleens cells were homogenized, sieved, and then centrifuged. The cells were counted and adjusted to 8×10^6 mL^{-1}.