Preparation and study of modified polyethyleneimine cationic adjuvant

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Abstract. A polyethylenimine adjuvant modified by mannose, which may be used in the study of vaccine adjuvant, was prepared in this paper. Its structure was characterized by infrared spectroscopy and its micromorphology was observed by transmission electron microscopy. A series of mannose-modified polyvinylimine were prepared according to the molar ratio of primary amine groups between mannose and polyvinylimine, and their storage stability was observed at 4℃. In addition, the cytotoxicity of modified and unmodified polyethylenimine was also tested, and the results showed that the modified polyethylenimine was less toxic than unmodified polyethylenimine.

Keywords: Adjuvant, mannose, polyethyleneimine, cytotoxicity

1. Introduction

Adjuvant itself has no antigenicity and is a non-specific immune-enhancing substance. When it is injected into the body in advance or at the same time with the antigen, it can increase the immune response to the antigen or change the type of immune response[1]. Vaccination is an important means to effectively prevent and control the spread of diseases[2, 3], but traditional vaccines such as inactivated vaccines and attenuated vaccines generally have problems of short maintenance time and low safety[4]. Subunit vaccines are safer than traditional vaccines, but their immunogenicity is weak and they often need to be combined with adjuvants[5, 6]. Therefore, the development and application of adjuvants have attracted the attention of many researchers, who are committed to developing new vaccine adjuvants with broad spectrum and high efficiency to enhance the effect of new vaccines. There are three possible mechanisms of action of adjuvants: 1) Change the physical properties of antigens and promote the slow release of antigens; 2) Promote the ability of mononuclear phagocytes to present antigens; 3) Stimulate the proliferation and differentiation of lymphocytes and enhance immune response[7, 8]. Conventional adjuvants include aluminum salt adjuvant[9], oil emulsion adjuvant[10], polysaccharide adjuvant[11] and so on. The new adjuvants include polymer adjuvant[12], liposome adjuvant[13], nano adjuvant[14, 15], cytokine adjuvant[16] and so on. With the development of new vaccines, safe and effective vaccine adjuvants. Polymer materials have gradually attracted the attention of researchers because of their good biocompatibility, and more and more polymer materials such as polyacrylates, polylactic acid and polypolysaccharides have been used to prepare polymer adjuvants. What's more, some studies have shown that the nano adjuvant prepared by polymer has a more significant adjuvant effect[14, 17].

Polyethyleneimine is a cationic polymer, which is an effective delivery vector for nucleic acid transfection reagents in vitro and non-viral vaccines in vivo. In recent years, studies have shown that PEI can enhance the immune effect of a variety of vaccines, especially mucosal immunization vaccines, and significantly enhance the antiviral ability of vaccines, so it has attracted high attention from many scholars. Mannose is a yeast polysaccharide that has mannose receptors on macrophages and dendritic cells. Mannose reacts with polyethyleneimine to form a Schiff base structure. It is worth noting that in the immune physiological process, with the interaction between Th cells and APC, Schiff base structure will be formed. And most specific immune responses depend on interactions between lymphocytes and antigen-presenting cell (APC). In addition, studies have shown that exogenous Schiff base structure can enhance the immune response of T cell[18]. Hence, according to the above advantages, mannose-modified polyethyleneimine (Man-PEI), a novel vaccine adjuvant which can reduce cytotoxicity and promote endocytosis was prepared in this paper.

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2. Experiment

2.1 Materials

Polyethyleneimine (PEI, M_w=25 kDa) was obtained from BASF Co., Ltd. Mannose, Stearyl chloride and triethylamine were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Ovalbumin (OVA, chicken egg white) was purchased from Sigma-Aldrich Co., Ltd. BALB/c female mice was obtained from Hebei Medical University.

2.2 Synthesis of Man-PEI

Add 10 g of PEI and 80 g of purified water to a three necked flask equipped with mechanical stirring and thermometer, and the system was heated to 60℃ for stirring and dissolution. Add a amount of mannose into 10 g purified water and stir to dissolve. Then the mannose solution was slowly added to the flask, and the water bath was heated to 85℃ and stirred for 1 h. After the reaction, the reaction solution was collected and dialysis bags with interception molecular weight of 3.5 kDa were used for dialysis. The water in the dialysis system was changed every 12 h and perform dialysis. After dialysis, freeze dry it using a freeze-vacuum drying oven to obtain a yellowish viscous substance, which is polyethyleneimine modified by mannose (Man-PEI).

The molar ratio of primary amine groups in mannose and PEI reaction was 1:25, 1:20, 1:15, 1:10 and 1:5, respectively. They are named Man-PEI_x, which are Man-PEI_a, Man-PEI_b, Man-PEI_c, Man-PEI_d, and Man-PEI_e, respectively.

2.3 Synthesis of Man-PEI/OVA composite particles

A certain amount of OVA was added to purified water to prepare a solution of 5 mg/mL. In addition, a series of Man-PEI prepared by a series of different mannose-primary amine molar ratios prepared in 2.1 was prepared into a solution of 0.2 mg/mL with purified water (pH value adjusted to 7.4). A quantitative OVA solution was added to the beaker, the speed of the magnetic stirrer was adjusted to 600 r/min, and then the same volume of Man-PEI solution was slowly dropped into it, stirring for 20 min, and standing for 10 min after the completion of stirring, Man-PEI/OVA composite particles were obtained.

2.4 Structure of Man-PEI

The structure of Man-PEI was characterized by Fourier transform infrared spectrometer (FT-IR, IRL280301), and the scanning wavenumber range from 650 to 4000 cm^{-1}.

2.5 Microstructure of Man-PEI/OVA composite particles

The microscopic morphology of PEI/OVA and Man-PEI_d/OVA composite particles was observed by transmission electron microscope (JEM-2100 Plus, Japan Electronics), the accelerating voltage of the transmission electron microscope is 200 kV.

2.6 Stability of Man-PEI/OVA composite particles

The particle sizes stability (average particle size and distribution, polydispersity index) of PEI/OVA and Man-PEI_x/OVA composite particles was measured on a PSS Z3000 (Particle Sizing Systems LLC, USA) to characterize the storage effect of the particles. Sample was prepared by individually diluting 10 μL of the PEI/OVA or Man-PEI_x/OVA composite particles in 990 μL of distilled water. The samples were placed at 4℃, and conduct particle size testing at 12 h, 24 h, 72 h, and 168 h, respectively. The detection temperature was set at 25℃, the temperature of the detection chamber was balanced for 60 s before detection, and each sample was measured 3 times.

2.7 Cytotoxicity experiment of peritoneal macrophages

Mouse peritoneal macrophages were isolated according to the method in 2.3, and the cell concentration was adjusted to 1×10^5 cells per well. Macrophage suspension was added to 96-cell plate at 100 μL/well for 6 h to make macrophages stick to the wall. After sticking to the wall, supernate was discarded and fresh RPMI 1640 was added to complete culture medium. PEI and Man-PEI_d were added to the cell plate at 100 μL/well, where the drug concentration is 1 μg/mL and 2 μg/mL, repeat five wells for each drug, for 18 h of cultivation, as well as control well and blank well. CCK-8 method was used to detect the cytotoxicity of each group of drugs on macrophages, and the absorbance at 450 nm was measured with Multiskan Go, then calculate the cell viability according to the formula below.

$$\text{Cell viability}=\left(\frac{A_s-A_b}{A_c-A_b}\right)\times100\%$$

A_s—Absorbance of experimental well (including cell, medium, CCK-8).

A_c—Absorbance of control well (including cells, culture medium, CCK-8 solution, no drugs).

A_b—Absorbance of blank well (including culture medium and CCK-8 solution, no cells and drugs).

3. Results and Discussion

3.1 Structure of Man-PEI

Man-PEI_d was selected as the sample compared with the infrared spectra of PEI, and the results were shown in Figure 1.

The anti-stretching vibration characteristic absorption peaks of -NH_2 in PEI and Man-PEI_d are 3358 cm^{-1} and 3350 cm^{-1}, respectively, and their symmetric stretching characteristic absorption peaks are 3281cm^{-1} and 3286cm^{-1}, respectively. The absorption intensity of -NH_2 characteristic peaks of Man-PEI_d decreased, indicating that some primary amine groups participated in the reaction. The stretching vibration peak of carbohydrate C-OH was found at 1112 cm^{-1}, and the nucleophilic addition reaction between the aldehyde group of mannose and the

primary amine group of PEI resulted in Schiff base with C=N structure. The characteristic absorption peak of C=N appeared at 1656 cm^{-1} of Man-PEI_d, indicating that mannose successfully modified the molecular structure of PEI.

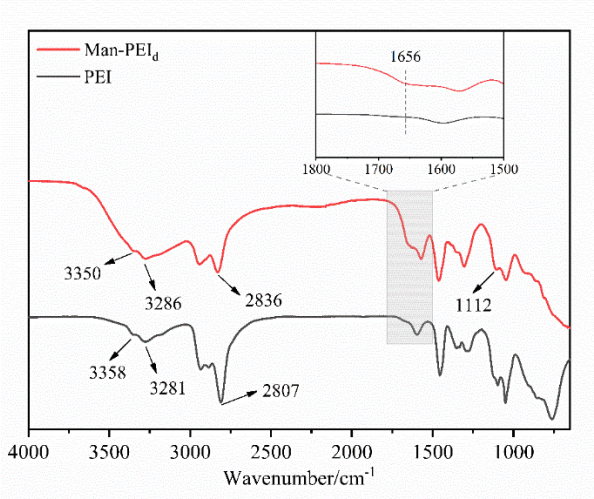

Figure 1. Infrared spectra of PEI and Man-PEId.

3.2 Microstructure of Man-PEI/OVA composite particles

As shown in Figure 2, transmission electron microscopy was used to test the microstructure of PEI/OVA and Man-PEI_d/OVA composite particles.

As can be seen in Figure 2a, the particle size of PEI/OVA composite particles is between 50 and 400 nm, and the particle size of most particles is greater than 200 nm, indicating that the particle size of PEI/OVA composite particles is uneven, and cracks can be observed on the surface of the particles, which may be caused by insufficient stability of the particles. As can be seen from Figure 2b, the particle size of Man-PEI_d/OVA composite particles is about 200 nm, the distribution is relatively uniform, and no loose cracking phenomenon occurs. Uniform and stable composite particles are conducive to effective functioning in the body.

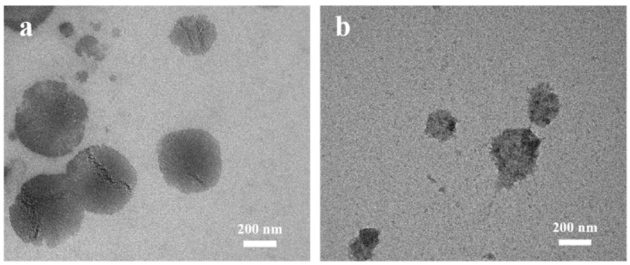

Figure 2. TEM of composite particles: (a)PEI/OVA. (b)Man-PEId/OVA.

3.3 Stability of Man-PEI/OVA composite particles

In order to observe the long-term storage stability of composite particles, Man-PEI_x/OVA composite particles were placed at 4℃ and compared with PEI/OVA composite particles. Dynamic light scattering particle size analyzer was used to investigate the particle size changes in aqueous solution, and the results were shown in Figure 3.

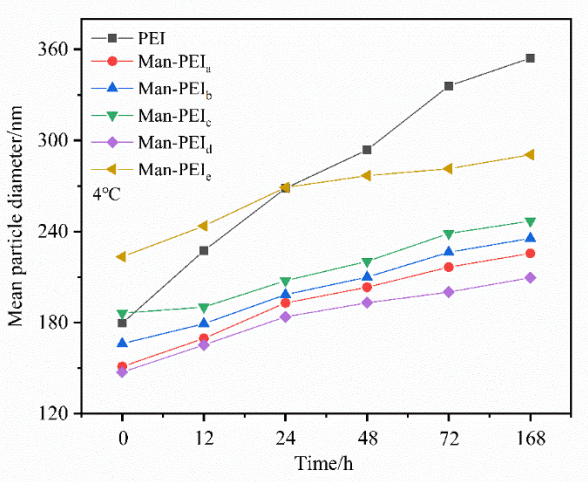

Figure 3. The particle size of Man-PEIx/OVA varies with time at 4℃.

It can be seen from Figure 3 that under the ambient temperature of 4℃, the particle size of PEI/OVA changes significantly when the placement time is 0h, 12h, 24h, 48h, 72h and 168h respectively, and the particle size is doubled after 168h. The stability of mannose-modified polyethyleneimine and OVA composite particles is far less than that of mannose-modified polyethyleneimine and OVA composite particles, among which Man-PEI_d/OVA composite particles have the smallest particle size. The stability of polyethyleneimine modified by mannose was improved at low temperature. In addition, the subsequent experiments were all used on the same day combined with the same day to ensure its consistency.

3.4 Cytotoxicity experiment of peritoneal macrophages

In order to study the safety of modified polyvinylimide, the toxicity of PEI and Man-PEI_d to macrophages was detected by CCK-8 method, and the survival rate of cells was calculated, as shown in Figure 4.

As can be seen from Figure 4, the cell survival rate at 1 μg/mL was lower than that at 2 μg /mL. In addition, the toxicity of polyvinylimide modified with mannose to macrophages was significantly reduced, which may be due to the high molecular weight of polyethyleneimine and the high positive charge density. Appropriate cation density will promote endocytosis, while excessive cation density will destroy the structure of the cell membrane. After the modification of polyethyleneimine, the positive charge density is reduced, so the cell survival rate is high, which further indicates that the cell survival rate is related to the cation density.

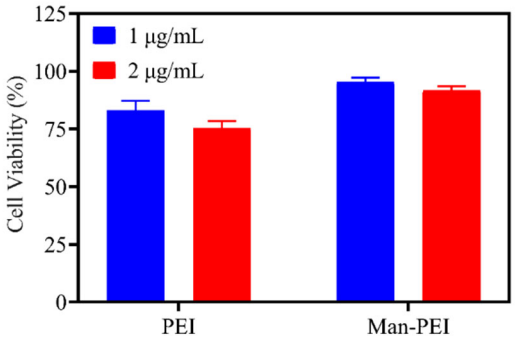

Figure 4. Cell viability of PEI and Man-PEI.

4. Conclusion

In this paper, polyvinylimine modified with mannose was successfully prepared as a new adjuvant by utilizing the targeting and immunomodulatory effects of mannose. The structure of Schiff base was formed after nucleophilic addition between the aldehyde group of mannose and the primary amine group of polyvinylimine by infrared spectroscopy. The particle size of modified polyethylenimine was found to be uniform by transmission electron microscopy, about 200 nm. The storage stability evaluation of composite particles shows that Man-PEI$_d$/OVA composite particles have better storage stability at 4℃ than PEI/OVA composite particles. The positive charge density of polyvinylimine modified with mannose was lower than that of unmodified polyvinylimine, and the toxicity to macrophages was reduced. Therefore, mannose modified polyvinylimine adjuvant is expected to be applied in the field of vaccine adjuvant.

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