European Journal of Pharmaceutics and Biopharmaceutics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Influence of particle size, an elongated particle geometry, and adjuvants on dendritic cell activation

Roman Mathaes [a], Gerhard Winter [a], Teruna J. Siahaan [b], Ahmed Besheer [a,1], Julia Engert [a,*,1]

[a] Department of Pharmacy, Pharmaceutical Technology & Biopharmaceutics, Ludwig-Maximilians-University Munich, Butenandtstr. 5, D-81377 Munich, Germany
[b] Department of Pharmaceutical Chemistry, The University of Kansas, Simons Research Laboratory, 2095 Constant Avenue, Lawrence, KS 66047, United States

ARTICLE INFO

Article history:
Received 26 February 2015
Revised 16 June 2015
Accepted in revised form 18 June 2015
Available online xxxx

Keywords:
Non-spherical particles
Flow cytometry
Nano-rods
Adjuvants
Vaccination
Dendritic cells

ABSTRACT

Modern subunit vaccines have many benefits compared to live vaccines such as convenient and competitive large scale production, better reproducibility and safety. However, the poor immunogenicity of subunit vaccines usually requires the addition of potent adjuvants or drug delivery vehicles. Accordingly, researchers are investigating different adjuvants and particulate vaccine delivery vehicles to boost the immunogenicity of subunit vaccines. Despite the rapidly growing knowledge in this field, a comparison of different adjuvants is sparsely found. Until today, little is known about efficient combinations of the different adjuvants and particulate vaccine delivery vehicles.

In this study we compared three adjuvants with respect to their immune stimulatory potential and combined them with different particulate vaccine delivery vehicles. For this reason, we investigated two types of polyI:C and a CL264 base analogue and combined these adjuvants with differently sized and shaped particulate vaccine delivery vehicles. A high molecular weight polyI:C combined with a spherical nano-sized particulate vaccine delivery vehicle promoted the strongest dendritic cells activation.

1. Introduction

Vaccination is a potent and cost-effective tool to treat and prevent fatal diseases [1]. The morbidity of life threatening disease such as smallpox, polio, hepatitis B, or measles has been significantly reduced [2]. Therefore, vaccination contributed to the drop of children-mortality from 20 million cases worldwide in 1960 to less than 10 million in 2005 [2].

In addition, the development of subunit vaccines facilitates the industrial large scale production, which reduces the production costs, and therefore increases the accessibility of vaccines to poor regions. Living pathogens can cause serious side effects or death in immune-deficient patients, as they can revert to virulent wild-type strains [3]. Subunit vaccines consist of purified oligopeptides, recombinant proteins, or viral subunits, which cannot lead to disease and thus have a lower toxicological profile [4]. Despite these benefits, subunit vaccines are rather poorly immunogenic and require the addition of a potent adjuvant [5].

Adjuvants and particulate vaccine delivery vehicles have been applied to boost the immunogenic potency of subunit vaccines [6]. Specifically, adjuvants enhance antigen presentation and co-stimulation. A common mechanism is the activation of pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) in antigen-presenting-cells (APCs) [7]. For example, TLR 3 recognizes double-stranded (ds) RNA and activates the TRIF-dependent pathway, which leads to the induction of type I interferon [8,9].

One example of adjuvants acting via TLR 3 is polyinosine–polycytidylic acids (polyI:C), which are synthetic dsRNAs available in different molecular weights [9]. Similarly, TLR 7/8, which is highly homologous to TLR9, recognizes several synthetic imidazoquinolines and induces several inflammatory cytokines like interferon alpha [8,10]. The natural ligands of the TLR 7/8 family remain unclear. The adenine analog CL264 is a resiquimod analogue recognized by TLR 7/8 and is functionalized by a carboxy group for chemical conjugation [11–13].

Upon administration of TLR agonists, like the above mentioned vaccine adjuvants, antigen presenting cells (APCs) such as dendritic cells (DCs) are activated, which leads to DC maturation. The maturation process can be described by several factors. For example, a loss in phagocytic capacity, an increased expression of major histocompatibility complex (MHC) molecules, the production of cytokines, and the up-regulation of co-stimulatory molecules including CD80, CD83, and CD86. The co-stimulatory molecules play an

* Corresponding author. Tel.: +49 89 2180 77025; fax: +49 89 2180 77020.
E-mail address: julia.engert@cup.uni-muenchen.de (J. Engert).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.ejpb.2015.06.015
0939-6411/

important role in the immune response and indicate DC activation [14].

Besides adjuvants, particulate vaccine delivery vehicles are used to efficiently protect and deliver antigens or TLR ligands to APCs. TLR 3 and TLR 7/8 are intracellular receptors, which require a cellular delivery of TLR ligands. For this reason, numerous particulate vaccine delivery vehicles such as liposomes [15], microparticles [16], nanoparticles [17], immune stimulating complexes (ISCOMs) [18–20], and virus-like particles (VLPs) [21] have been used as vaccine delivery vehicles in order to mimic pathogens. These particulate vaccine delivery vehicles mimic pathogens in size [22], charge [23], surface chemistry [24], and shape [25,26].

Particle shape has recently been identified to influence particle uptake into different immune cells. Champion and Mitragotri showed that an elongated particle shape reduces the incidence of particle phagocytosis into macrophages [27]. In addition, different pathogens such as *Escherichia coli* or *Aeromonas hydrophila* display an elongated shape [28]. It is still unclear whether a non-spherical particle shape could influence the activation of immune cells.

The response of the immune system to an antigen depends on processing and presentation of the antigen by APCs. Vaccine delivery vehicles are used to enhance the uptake into APCs and thus promote efficient antigen presentation. The overall efficiency of a vaccine delivery vehicle is a complex interplay of the physical and chemical vaccine delivery vehicle properties and is further boosted in a combination with an adjuvant [29]. Consequently, the synergistic combination of adjuvants and a vaccine delivery vehicle represents a promising strategy to enhance immunogenicity of subunit vaccines [5,30].

Despite the progress in understanding the effect of adjuvants and particulate vaccine delivery vehicles, little is known about potent combination of different adjuvants and vaccine delivery vehicles, which are promising concepts to boost the immunogenicity of vaccines [31]. In particular, a comparison of different molecular weight polyI:Cs and TLR 7/8 adjuvants has not been reported. Zhou et al. compared a soluble low molecular weight and a soluble high molecular weight polyI:C. The high molecular weight polyI:C promoted a stronger immune response than the low molecular weight polyI:C *in vitro* [32]. Nevertheless, the two different polyI:C forms have not been compared in combination with different particulate vaccine delivery vehicles to further boost their immune stimulatory potential. Micro- and nanoparticles have clearly proven their ability as vaccine delivery vehicles, however the preferred size and shape are still debated [22].

In this study, we compared two different molecular weight polyI:C TLR 3 ligands, as well as the TLR 7/8 CL264 adenine base analogue. In addition, we adsorbed the above mentioned adjuvants to differently sized and shaped polystyrene particles. All adjuvant samples were then applied to the DC line JAWSII and tested for their potential to stimulate the DCs. JAWSII stimulation was followed by flow cytometry measurements of the co-stimulatory molecules CD83 and CD86.

2. Materials and methods

2.1. Micro- and nanoparticles

Fluorescently (FITC) labeled (excitation 460/emission 500) and label free 150 nm and 2 μm polystyrene particles were purchased from Phosphorex (Hopkinton, Ma, USA).

2.2. Non-spherical micro- and nanoparticle fabrication

The film stretching method established by Champion et al. [33] was used to stretch spherical 150 nm and 2 μm polystyrene particles to elliptical particles with tailored aspect ratios as mentioned earlier [34]. In brief, spherical particles were incorporated into a polyvinyl alcohol 40–88 (PVA) film (Sigma–Aldrich, Steinheim, Germany). PVA 40–88 (10% w/v) was dissolved in highly purified water at 80 °C. Then, 2% of glycerol (Sigma–Aldrich, Steinheim, Germany) and spherical polystyrene particles (0.2% w/v) were added to this solution. The mixture was dried at room temperature for 4 days in 4 × 6 cm molds until a flexible film was formed. The PVA film was cut into 2 × 3 cm pieces and fixed in an in-house built film-stretching device. The 2 cm long PVA – film including the spherical polystyrene particles was stretched to the length of 6 cm in a silicone oil bath at 120 °C at a stretching speed of 2 cm per minute. Afterward, the stretched PVA film was cooled down to room temperature and washed with 100% isopropanol (Sigma–Aldrich, Steinheim, Germany). Finally, the PVA films were first washed in 100% isopropanol and then dissolved in highly purified water to recover the non-spherical particles. The recovered particles were washed 5 times in highly purified water. Non-spherical particles stretched to three fold of their original length are referred to as 3X stretched particles. To ensure comparability, the spherical particles used in the following *in vitro* study were treated in the same manner but without stretching the PVA film.

2.3. Scanning electron microscopy (SEM)

Particle morphology was confirmed using a Joel JSM 6500F scanning electron microscope (Joel Ltd, Tokyo, Japan). Spherical and non-spherical particles were applied to a filter paper and attached to self-adhesive tape on aluminum scanning electron microscope stubs. Afterward, the samples were sputtered with carbon. SEM image collection was performed at 8000× magnification for the nanoparticles and 2000× magnification for the microparticles. The aspect ratio of the 3X stretched micro- and nanoparticles was calculated using the SEM images (20 particles measured per group). This test setup has shown good correlation to high throughput methods in previous studies [35]. The Inca software (Oxford instruments, Oxfordshire, UK) was utilized to process image acquisition data.

2.4. Light obscuration measurements

Microparticles were measured by light obscuration (LO) utilizing a SVSS-C system (PAMAS, Partikelmess- und Analysesysteme GmbH, Rutesheim, Germany). All microparticle batches were analyzed in triplicates. The measuring volume was set to 0.3 ml and the pre-run volume was set to 0.5 ml. The system was cleaned with highly purified water until the particle count was less than 25 counts/ml between each measurement.

2.5. Dynamic light scattering

Nanoparticles were analyzed using a Malvern Zetasizer Nano ZS (Malvern, Herrenberg, Germany). Each sample was measured in triplicates; each triplicate was performed with 11 sub-runs at 24 °C. 400 μl of the polystyrene particle suspensions was measured in a poly(methylmethacrylate) cuvettes (Brand, Wertheim, Germany) with a path length of 12.5 mm. Particle size was measured in water or culture media. Particle zeta potential was measured in water including 10 mM sodium chloride. The particle concentration was always 0.06 mg/ml. Size, zeta potential, and polydispersity index (PDI) were calculated using the Malvern Dispersion Technology software (version 4.20, Malvern, Herrenberg, Germany).

Please cite this article in press as: R. Mathaes et al., Influence of particle size, an elongated particle geometry, and adjuvants on dendritic cell activation, Eur. J. Pharm. Biopharm. (2015), http://dx.doi.org/10.1016/j.ejpb.2015.06.015