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Assessment of the enhancement of PLGA nanoparticle uptake by dendritic cells through the addition of natural receptor ligands and monoclonal antibody

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ABSTRACT

Targeting of specific receptors on antigen-presenting cells is an appealing prospect in the production of novel nanoparticulate vaccines. In particular, the targeting of vaccines to dendritic cell (DC) subsets has been shown in models to significantly improve the induction of immune responses. This paper describes the evaluation of natural ligands, mannan and chitosan, and monoclonal antibodies as targeting motifs to enhance uptake of PLGA nanoparticle carriers by bovine DCs. To assess enhancement of uptake after the addition of natural ligands a bovine monocyte derived DC (MoDC) model was used. For the assessment of monoclonal antibody targeting, the model was expanded to include afferent lymph DCs (ALDCs) in a competitive uptake assay. Mannan, proved unsuccessful at enhancing uptake or targeting by MoDCs. Chitosan coated particle uptake could be impeded by the addition of mannan suggesting uptake may be mediated through sugar receptors. Inclusion of monoclonal antibodies specific for the DEC-205 (CD205) receptor increased the number of receptor expressing DCs associated with particles as well as the number of particles taken up by individual cells. These results support the further evaluation of active targeting of nanovaccines to DCs to enhance their immunogenicity in cattle and other large mammalian species including humans.

1. Introduction

Nanoparticulate delivery of subunit vaccines offers a number of potential advantages over conventional approaches including preferential uptake by dendritic cells (DC) and prolonged release of antigen resulting in enhanced T cell stimulation [1]. Furthermore, nanoparticles offer a versatile system that allows for targeted delivery of antigen and adjuvant to the same DC [2]. It is generally acknowledged that DCs are capable of taking up particles with a varied range of physical properties. Studies have shown enhanced DC uptake of particles *in vitro* but few have addressed specific targeting and the uptake of particles by different cell types [3]. A recent study showed liposomes are taken up primarily by $CD11c^+$ DCs and macrophages in murine lymph nodes, with the latter being more efficient [4]. Similarly, it was shown that PLGA nanoparticles are more efficiently taken up by macrophages than DC [5]. Since DCs are the primary target of most vaccines, these data suggest that particulate systems may need to be further targeted to specific cell types to achieve optimal induction of immune responses.

Targeting of vaccines to DCs *via* specific receptors can be facilitated with the use of natural receptor ligands or mAbs specific for DC receptors. Targeting mannose receptors through mannosylation is the most common means of targeting DCs through natural

Abbreviations: DC, dendritic cell; MoDC, monocyte derived dendritic cell C; ALDC, afferent lymph dendritic cell; PLGA, poly(lactic-*co*-glycolic) acid; OVA, ovalbumin; MAN, mannan; mAb, monoclonal antibody.

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ligands. Indeed protein mannosylation has proved effective at both improving antigen uptake and adjuvanting immune responses [6]. Mannosylation of particles however has yielded variable results both in terms of enhancing particle uptake or DC maturation [7–9].

Targeting of particles to a cell type using specific mAbs was first evaluated in the context of cancer drug delivery [10,11]. Employing the same principle, nanoparticles targeted to DC using DC-SIGN-specific mAbs generated 10–100 fold increases in the efficiency of antigen-presentation compared to non-targeted particles [12]. mAb targeting of microparticles to DEC-205 resulted in a two-fold enhancement of immune responses [13]. A second study targeting nanoparticles using DEC-205 specific mAb reported only a 15% increase in uptake over particles coated with an irrelevant isotype matched mAb when targeting immature DCs but a 70% increase when targeting mature DCs [14]. Furthermore, the properties of different particle types such as their size can affect which DC subset becomes associated with the particle and thus the type of immune response evoked [15,16]. Dendritic cell targeting of biomaterials has been comprehensively reviewed in [17].

Cattle represent an important veterinary species susceptible to a number of infectious diseases for which there is a need to develop new or improved vaccines to support control programmes. Bovine DC subsets have been characterized suggesting that they represent orthologous populations to those described in humans [18,19]. As such, cattle provide a large mammal model species for the evaluation of DC-targeted nanovaccines, which may be useful in cattle, other animals and humans. As a first step to using the bovine system for the development of novel nanoparticle vaccine systems, this study describes the synthesis of targeted PLGA nanoparticles using both natural ligands and mAb and an evaluation of their effects on uptake by bovine DC *in vitro*.

2. Materials & methods

2.1. Isolation of bovine monocytes

All animal work was approved by the APHA Ethics Committee and conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood collected from adult male Holstein-Friesian cattle [20]. Monocytes were purified from PBMC by magnetic activated cell sorting (MACS), using anti-human CD14-microbeads as described by the manufacturer (Miltenyi Biotec).

2.2. Generation of monocyte derived dendritic cell (MoDC)

Monocytes were resuspended in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin (cRPMI) and plated at 2×10^6 cells/well in 24 well Ultra-Low Attachment Tissue Culture Plates (Costar). Differentiation to MoDCs was induced by the addition of recombinant bovine GM-CSF and IL-4 according to the manufacturer's recommended dilution (1:20) (Bovine Dendritic Cell Growth Kit, AbD Serotec, Oxford, UK). Cells were incubated for 6 days at 37 °C in a 5% CO_2 humidified atmosphere.

2.3. Bovine afferent lymph dendritic cells

Cells were collected from afferent lymph following pseudo-afferent lymphatic cannulation [21] and cryopreserved in 10% (v/v) DMSO in FBS. Cells were thawed rapidly in a 37 °C water bath and washed in pre-warmed cRPMI before use.

2.4. PLGA nanoparticle synthesis

Acid terminated PLGA (Resomer RG502 H, 50:50, M_w 7–17 kDa, Sigma, Poole, UK) was dissolved in ethyl acetate (Sigma) at a concentration of 25 mg/ml to form the oil phase (o). The model antigen ovalbumin (OVA) conjugated Alexa Fluor 488 or Alexa Fluor 647 (Life Technologies) diluted to 5 mg/ml in DPBS formed the first water phase (w_1). 1% (w/v) poly vinyl alcohol (PVA; 98% hydrolysed M_w 13–23 kDa, Sigma) in distilled water was used as a stabilizer in the second water phase (w_2). To form the first emulsion (w_1/o), w_1 was rapidly added to the oil phase at a ratio of 1:10 and sonicated for 30 s at an intensity of 14 μm using a Soniprep 150 sonicator (MSE, London, UK). To form the second emulsion ($w_1/o/w_2$), w_1/o was added to w_2 at a ratio of 1:5 and sonicated for 2 min at an intensity of 18 μm. The final emulsion was added to a larger vessel and the solvent was evaporated overnight with stirring (250 rpm) at 37 °C. The particles were collected by ultracentrifugation at 40,000 × *g* for 20 min and washed twice with distilled water to remove excess PVA.

2.5. Particle sizing and assessment of surface charge

The hydrodynamic diameter of the particles was calculated using dynamic light scattering (DLS). Particles were sonicated, dispersed in distilled degassed water and analysed on a Zetasizer 3000 (Malvern Instruments, Malvern, UK) in a disposable cuvette at 25 °C with a scatter angle of 90°. Eleven readings were made in triplicate. Zeta potential measurements (20 readings/sample in triplicate) were made using a disposable zeta cell (Malvern Instruments).

2.6. Scanning electron microscopy

Particles were air dried onto poly-L-lysine coated coverslips (BD Biosciences, Oxford, UK). Coverslip were applied to double sided carbon (Agar Scientific, Stansted, UK) attached to a 13 mm aluminium stub (Agar Scientific) and sputter coated with gold to a thickness of 15 nm using an Emitech K550X Sputter Coater (Quorum Technologies, Ashford, UK). Particles were visualized using a Zeiss Evo LS10 scanning electron microscope (Zeiss, Welwyn Garden City, UK) at an accelerating voltage of 10–20 kV.

2.7. Conjugation of mannan and ovalbumin to PLGA nanoparticles

Ligands were conjugated to the surface of PLGA nanoparticles post-synthesis using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), a carbodiimide cross-linker that activates carboxyl groups for spontaneous reaction with primary amines as schematically illustrated in Fig. 2A. Particles were suspended at 10 mg/ml based on the starting amount of PLGA in 0.1 M 2-(*N*-morpholino)ethanesulfonic acid buffer (MES) (Sigma) adjusted to pH 5.2 with 0.1 M hydrochloric acid (HCl). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and sulpho-*N*-hydroxysuccinimide (Sulpho-NHS) (Sigma) were added at a final concentration of 0.1 M, and then mannan (MAN) and OVA (Sigma) were added at a final concentration of 5 mg/ml, unless otherwise indicated. The reaction was carried out overnight with stirring (250 rpm) at room temperature. Particles were collected by centrifugation at 10,000 × *g* for 2 min and washed twice with Tris buffered saline (TBS). To neutralize reactive groups not utilized in the process and isourea intermediates, particles were resuspended in TBS for 1 h. The coating of particles was confirmed by staining with concanavalin A-FITC (ConA-FITC; Sigma) or anti-OVA mAb (clone OVA-14, Sigma) followed by APC-conjugated anti-mouse IgG1 secondary antibody (clone X56; BD Biosciences). The particles were washed twice with DPBS and fluorescent staining assessed

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