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Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model

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Abstract

Current vaccine development includes optimization of antigen delivery to antigen presenting cells, such as dendritic cells (DC). Particulate systems have attracted increasing attention in the development of vaccine delivery systems. In the present study, we investigated DC uptake of model fluorescent polystyrene particles with a broad size range and variable surface properties. Localization of particles was investigated using confocal laser scanning microscopy and uptake was quantified by flow cytometry. Immature DC were generated from mononuclear cells isolated from human blood. The polystyrene particles interacted with the DC throughout the tested diameter range of 0.04–15 μm in a time- and concentration-dependent manner. The optimal particle diameter for fast and efficient acquisition by a substantial percentage of the DC was 0.5 μm and below. The surface of 1 and 0.1 μm polystyrene particles was covalently modified with different polyaminoacids/proteins, yielding particles with varying surface charge. Uptake of 1 μm particles was greatly enhanced when particles displayed a positive surface charge. In general, the present findings establish that particle diameters of 0.5 μm and below were optimal for DC uptake; however uptake of larger particles could be greatly enhanced by rendering the particle surface positive. Whether increased particle uptake is correlated with increased immune responses, remains to be established.
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Microspheres possess pharmaceutical and immunological advantages as vaccine delivery systems for subunit vaccines based on peptides, protein or DNA (Raychaudhuri and Rock, 1998). Particulate systems are naturally targeted to antigen presenting cells (APC) since their dimensions are comparable to those of microorganisms, and particles can through phagocytosis deliver antigens to APC 1000–10,000-fold more efficiently than soluble antigen (Kovacsovics-Bankowski et al., 1993; Vidard et al., 1996). Presentation of processed, particulate antigens by major histocompatibility complex (MHC) class I and class II can

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activate $CD4^+$ and $CD8^+$ T-cell responses. Particulate antigens might therefore mediate the induction of both humoral and cellular immunity.

Dendritic cells (DC) represent the main APC lineage able to induce primary immune responses and immunological memory (Banchereau and Steinman, 1998). Antigen acquisition by DC is mediated by several uptake mechanisms such as receptor-mediated endocytosis, macropinocytosis and phagocytosis, depending of the nature of the antigen (Reis e Sousa et al., 1993; Sallusto et al., 1995; Steinman and Swanson, 1995). Interactions between particulate formulations and cells in general depend on particle characteristics such as size and surface properties, including surface charge and hydrophobicity. In the present study we addressed the importance of particle size, charge and concentration for efficient interactions between particles and human DC.

DC were generated from human peripheral blood monocytes and characterized by flow cytometry, according to previous reports (Foged et al., 2004; Romani et al., 1996; Sallusto and Lanzavecchia, 1994). The study was approved by the local ethics committee (KF01-020/00). The 0.1, 0.5, 1.0 and 4.5 μm Fluoresbrite™ carboxylated yellow-green microspheres were purchased from Polysciences Europe GmbH, Eppelheim, Germany. 0.04, 10 and 15 μm yellow-green fluorescent (505/515) carboxylate-modified FluoSpheres® were obtained from Molecular Probes, Europe BV, Leiden, The Netherlands. Protamine sulphate (PS) (grade III), poly-L-lysine (PLL) M_W 5000–10,000, wheat germ agglutinin (WGA) from *Triticum vulgaris* and poly-D-L-alanine (PA) M_W 1000–5000 were purchased from Sigma Aldrich A/S, Vallensbaek Strand, Denmark. Tetanus toxoid (TT) was from Statens Serum Institut, Copenhagen, Denmark (Batch XXVII, 669 lf/mg, 2.23 mg/ml). The 0.1 and 1.0 μm carboxylated polystyrene particles were coated with PLL, PS, PA, WGA and TT by covalent coupling of the molecules (400 μg) to the free carboxyl groups on the particle surface using the Carbodiimide Coupling Kit for Large Microspheres according to manufacturers instructions (Polysciences Europe GmbH, Eppelheim, Germany). For the 0.1 μm particles, a special Carbodiimide Kit with Hollow Fiber Filtering System was applied to concentrate the particle suspension (Polysciences Europe GmbH, Eppelheim, Germany). Amounts of bound peptide/protein was measured indirectly by determination of non-bound material in the supernatants by the bicinchoninic acid protein assay kit according to manufacturers instructions (Sigma–Aldrich, Denmark A/S). The average size of unmodified particles was measured in 10 mM NaCl by dynamic light scattering with a Zetasizer 4 from Malvern Instruments, Sweden. The zeta potential (surface charge) was determined in Milli-Q water using a Zeta Master (Malvern Instruments Nordic AB, Uppsala, Sweden). The relative concentration of surface-modified particles was adjusted by comparison of fluorescence intensity with non-conjugated particles of known concentration using a Luminescence Spectrometer (LS50B, Perkin-Elmer, excitation wavelength 458 nm, emission wavelength 540 nm, and emission filter 515 nm).

For particulate uptake studies, DC were harvested, counted and viability was determined by trypan blue staining. The cells were seeded in 24-well culture plates at a density of 2×10^5 cells/well, and particles were added in a small volume with a micropipette after vigorous vortexing for 1 min. The tissue culture plates were swirled gently to distribute particles evenly in the wells and incubated at 37 °C. Cells were then harvested by addition of ice-cold PBS and by pipetting. Staining for surface MHC class II (human leukocyte antigen (HLA) DR) was done with phycoerythrin conjugated mouse anti-HLA-DR (or control IgG2a) as reported previously (Foged et al., 2004). Cell samples were fixed in 1% paraformaldehyde prior to acquisition and analysis by a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) using the CellQuest software (Becton Dickinson). Dead cells were gated out based on their light scattering properties. For confocal laser scanning microscopy (CLSM) DC incubated with particles were fixed for 15 min in 1% paraformaldehyde on ice. Staining was performed with a monoclonal mouse anti human CD1a antibody (Immunotech, Beckman Coulter, Marseille, France) followed by a rhodamine-conjugated donkey anti-mouse IgG (AffiniPure, Jackson Immunoresearch Laboratories Inc., West Grace, PA, USA) in PBS + 4% BSA. The cell preparations were washed with PBS and mounted using the SlowFade™ Antifade Kit (Molecular Probes Europe BV, The Netherlands). Fluorescence microscopy was performed with a Zeiss LSM510 confocal laser scanning microscope (Carl Zeiss, Jena, GmBH, Germany) equipped with an argon laser (458

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