International Journal of Pharmaceutics 365 (2009) 61–68

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Poly(I:C) coated PLGA microparticles induce dendritic cell maturation

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ARTICLE INFO

Article history:
Received 5 June 2008
Accepted 14 August 2008
Available online 4 September 2008

Keywords:
Poly(I:C)
PLGA microparticles
Toll-like receptor
Dendritic cells
Tumor vaccination
Adjuvant

ABSTRACT

Microparticles from poly(D,L-lactic-co-glycolic acid) [PLGA] are of steadily rising interest for the delivery of antigens to immune cells and the induction of a long-lasting immune response for vaccination or immunological tumor therapy. However, if the desired vaccine contains only weak antigens and fails to activate the antigen presenting cells (APC), the opposite effect, i.e., the induction of immunotolerance may be observed. Therefore, it was the aim of this study to show the ability of protein loaded PLGA microparticles to additionally carry a specific, surface-coated maturation signal to human dendritic cells (DC), i.e., the most potent APC. Polyinosine-polycytidylic acid [poly(I:C)], a ligand of Toll-like receptor (TLR) 3, was efficiently bound either in a single layer or a multilayer attempt to the surface of diethylaminoethyl dextran modified PLGA microparticles. These particles were effectively phagocytized by DC *ex vivo* and induced a maturation similar to that achieved with a cytokine cocktail or higher concentrations of soluble poly(I:C). In conclusion, the concept of surface coating of biodegradable microparticles with selected TLR ligands might successfully be used in DC-based cell therapies for cancer or in vaccination trials to induce DC maturation and specifically amplify the immunological response to encapsulated antigens.

1. Introduction

Within the last decade, biodegradable microparticles composed of poly(D,L-lactic-co-glycolic acid) [PLGA] have been studied not only for the encapsulation of conventional drugs, but also for proteins such as vaccine antigens (Jain, 2000; Jiang et al., 2005). Antigen loaded vehicles are discussed as a tool to continuously deliver their payload to antigen presenting cells (APC) and to control the pathway of the subsequent immune response. Dendritic cells (DC), being the most potent APC, are a promising target to induce immunity by such antigen loaded vehicles.

For an efficient antigen presentation, the DC have to be activated from the immature state (iDC) with high capacity of phagocytosis to the mature state (mDC), in which the cells efficiently interact with T cells (Guermonprez et al., 2002; Lutz and Schuler, 2002; Gilboa, 2007). These mDC are characterized by the increased expression of HLA-DR, of costimulatory molecules such as CD80 and CD86, and of the maturation marker CD83 (Zhou and Tedder, 1996). However, besides the presence of the antigen, additional maturation signals may be required to ensure a full DC maturation and a sufficient immune response (Matzinger, 1994; Reis e Sousa, 2006). This may particularly be the case if weak antigens or low antigen concentrations are used, with DC potentially remaining in a semi-mature state and inducing immunotolerance against the presented antigen (Mahnke et al., 2002; Rutella et al., 2006).

A variety of viral or microbial substances can serve as DC maturation signals. Most of these pathogen-associated molecular patterns (PAMP) bind to Toll-like receptors (TLR), triggering DC maturation (Reis e Sousa, 2004). Thus, TLR play a crucial role in the linkage of innate and adaptive immunity (Pasare and Medzhitov, 2005) and their agonists are promising candidates to induce full DC maturation and immune stimulation (Kaisho and Akira, 2003).

PLGA microparticles fulfill a number of requirements for antigen delivery to dendritic cells, since particles smaller than 10 μm are efficiently phagocytized by DC, enhancing the uptake of encapsulated antigens in comparison to soluble proteins (Waeckerle-Men and Groettrup, 2005). They can be efficiently loaded with antigens and release their payload in a prolonged manner, leading to an increased time of antigen presentation at the cell surface (Audran et al., 2003, Zimmermann et al., 2003). In contrast, soluble antigens

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doi:10.1016/j.ijpharm.2008.08.039

may be characterized by a short half-life of antigen presentation by DC (Ludewig et al., 2001). Importantly, by using encapsulated antigen the immune response can be directed towards the Th1 pathway—as desired for the immunological tumor therapy (Zimmermann et al., 2003; Waeckerle-Men et al., 2006). Recently it has been shown that PLGA microparticles themselves do not induce DC maturation (Wischke et al., 2006; Fischer et al., 2007), which is preferable since DC activation by the microparticle matrix itself would interfere with the aim to study the effect of the encapsulated antigen. However, if the encapsulated antigen fails to maturate the DC, the microparticles should be modified with maturation signals, e.g., at their surface for direct ligand–receptor-interaction.

Polyinosine-polycytidylic acid [poly(I:C)] is a synthetic analogue of viral double-stranded RNA (dsRNA) and an agonist of TLR3, a receptor selectively expressed in myeloid DC (Muzio et al., 2000; Kadowaki et al., 2001). It was shown that soluble poly(I:C) can induce a stable maturation of human DC (Verdijk et al., 1999).

The aim of this study was the controlled activation of human monocyte-derived DC (MoDC) by ligand modified microparticles. The TLR3 ligand poly(I:C) was allowed to bind to the surface of cationic, diethylaminoethyl dextran (DEAE-dextran) modified PLGA microparticles via electrostatic interactions. Additionally, the microparticles were loaded with a fluorescent model protein. The scope of this work was to show in principle that a vehicle consisting of (i) an immunologically inert particle matrix, (ii) an easily detectable model protein, and (iii) a TLR ligand, can specifically induce DC maturation after microparticle phagocytosis.

2. Materials and methods

2.1. Materials

The biodegradable polymer poly(D,L-lactic-co-glycolic acid) [PLGA] used in this study was Resomer® RG 502H from Boehringer Ingelheim (Ingelheim, Germany). Bovine serum albumin bearing fluorescein isothiocyanate groups [FITC-BSA] (Sigma, Taufkirchen, Germany) served as a model protein. Diethylaminoethyl dextran (DEAE-dextran) with a molecular weight of 2×10^6 Da was purchased from Pharmacia/Pfizer (Uppsala, Sweden), polyvinyl alcohol (PVA) was Mowiol® 4-88 from Kuraray Specialities (Frankfurt, Germany), poly(I:C) was from InvivoGen (San Diego, CA, USA), and all other chemicals were of analytical grade.

For cell culture, tissue culture dishes were purchased from Greiner Bio-One GmbH (Frickenhausen, Germany), serum-free cell culture media (CELLGro DC) and IL-4 from CellGenix GmbH (Freiburg, Germany). TNF-α, IL-1β, and IL-6 were obtained from R&D Systems (Minneapolis, MN, USA). GM-CSF (Leukine®) was purchased from Berlex Laboratories, Inc. (Monteville, NJ, USA) and PGE_2 (Minprostin®) from Pharmacia/Pfizer (Erlangen, Germany).

The phycoerythrin (PE) or phycoerythrin-cyanin 5.1 conjugate (Pc5) labeled antibodies against the surface markers HLA-DR, CD1a, CD209, CD80, CD83, and CD86 as used for flow cytometry were from Beckman Coulter (Krefeld, Germany), the anti-TLR3 monoclonal antibody (mouse anti-human CD283, clone TLR3.7) and the PE-labeled secondary antibody (rabbit F(ab′)2 anti-mouse IgG-RPE) were from AbD Serotec (Oxford, UK).

2.2. Preparation of microparticles

DEAE-dextran modified microparticles were prepared under aseptic conditions by a previously described solvent evaporation procedure (Wischke and Borchert, 2006; Wischke et al., 2006). Briefly, a solution of 1% (w/v) FITC-BSA in Tris buffer pH 7.0 was emulsified in a solution of 5% (w/w) Resomer® RG 502H in methylene chloride using a rotor-stator homogenizer. Following this, a secondary emulsification was performed in a solution of 0.5% (w/v) DEAE-dextran and 0.25% (w/v) PVA in water by a static micromixer (IMM, Mainz, Germany). The resulting w/o/w emulsion was stirred for 4 h at room temperature for solvent evaporation. The generated microparticles were collected by centrifugation (10 min, $5200 \times g$), washed three times with distilled water, and lyophilized. The particle size distribution was determined by laser diffraction with an LS 230 (Beckman Coulter, Krefeld, Germany).

2.3. Coating of microparticles with poly(I:C)

Cationic DEAE-dextran modified PLGA microparticles were coated with the negatively charged poly(I:C) using two different concepts: a single layer and a multilayer approach.

For the single layer method the lyophilized microparticles were suspended (15 mg/ml) in aqueous poly(I:C) solutions of 50 or 250 μg/ml at pH 4.5 and incubated at 4 °C for 3 h on a rotating mixer (RM5, Sondheim, Germany). Microparticle-free poly(I:C) solution served as a control to exclude unspecific loss of poly(I:C), e.g., by adsorption to the tubes, whereas cationic microparticles suspended in water of pH 4.5 without poly(I:C) were used to detect potential alterations of the zeta potential during the coating procedure.

Following the incubation, an aliquot of the suspension was retained to measure the particle surface charge at pH 4.5 (Zeta-Sizer Nano ZS, Malvern Instruments GmbH, Herrenberg, Germany). The coated microparticles were collected by centrifugation (10 min, $5200 \times g$, Centrifuge 5415 C, Eppendorf, Hamburg, Germany) in order to remove unattached poly(I:C) from the samples. The amount of surface bound poly(I:C) was calculated by the decrease of the poly(I:C) concentration in the supernatant as determined from the absorption difference between 260 and 320 nm (UV-2101 PC, Shimadzu Scientific Instruments, Columbia, MD, USA). The pellet was then suspended in water of pH 4.5 for short-term storage until added to the medium employed in cell studies (see below).

For the multilayer concept, lyophilized cationic microparticles were suspended in 250 μg/ml poly(I:C) and incubated at 4 °C for 1.5 h. After centrifugation to remove unbound poly(I:C), the microparticles were incubated in 250 μg/ml DEAE-dextran for 1 h. Then the microparticles were centrifuged, followed by another treatment with 250 μg/ml poly(I:C) for 1 h. Possible loss of poly(I:C) during the coating with DEAE-dextran could be excluded by analyzing the supernatant for both remaining DEAE-dextran and potentially detached poly(I:C).

2.4. Cell studies

The general procedure of the DC cell culture has been described previously in detail (Wischke et al., 2006). Briefly, immature monocyte derived DC (iMoDC) were generated by cultivating human peripheral blood monocytes in the presence of GM-CSF (800 U/ml) and IL-4 (500 U/ml) for 5 days.

Microparticles incubated with either 50 or 250 μg/ml poly(I:C), microparticles coated with two layers of poly(I:C) (multilayer MP), or uncoated microparticles were added to these iMoDC (50 μg microparticles/1×10^6 cells) for two additional days in the continued presence of GM-CSF and IL-4. As a negative control, iMoDC were cultured in the presence of GM-CSF and IL-4 without any additional substance (untreated DC). As positive controls served MoDC maturated either with a cytokine cocktail containing TNF-α, IL-1β (each 10 ng/ml), IL-6 (100 U/ml), and PGE_2 (1 μg/ml) (Jonuleit et al., 1997) or with 10 μg/ml soluble poly(I:C).

In order to determine the maturation of DC, cells were stained with PE- or Pc5-labeled antibodies against the surface markers

HLA-DR, CD209 (DC SIGN), CD83 (HB15), CD80 (B7.1), and CD86 (B7.2) for 30 min at 4 °C in phosphate buffered saline (PBS) containing 0.5% BSA, 0.1% sodium azide, and 10% rabbit serum to block unspecific binding. The cells were washed twice before being analyzed by flow cytometry on a FACS-Calibur using ProCellQuest software (BD-Bioscience, Heidelberg, Germany). Intact cells were gated based on their forward and side scatter characteristics, the exclusion of propidium iodide was used to gate living cells.

For intracellular staining of TLR3, cells were fixed with 4% paraformaldehyde in PBS for 5 min at room temperature and permeabilized with 0.6% octyl-β-D-glucopyranoside for 2 min, washed, and then incubated with 1 μg anti-TLR3 monoclonal antibody for 20 min. After washing, the cells were incubated with a PE-labeled secondary antibody, washed again, and analyzed by flow cytometry.

3. Results

3.1. Characteristics of the microparticles

The DEAE-dextran modified microparticles employed in this study, specifically their preparation procedure and their characteristics, have been described in detail previously (Wischke et al., 2006). Briefly, the DEAE-dextran modified microparticles prepared with the micromixer exhibit a smooth surface, particle sizes of about 3 μm (Fig. 1a), and a distinct positive charge at pH 4.5 (Fig. 1c).

Incubating these cationic microparticles with anionic poly(I:C) resulted in an efficient binding of the dsRNA to the particle surface as determined by measuring the concentration of residual poly(I:C) in the supernatant. Increasing the poly(I:C) concentration in the suspension medium from 50 to 250 μg/ml resulted in a larger amount of surface bound poly(I:C) (Fig. 1b). Typically, 90–100% of the soluble poly(I:C) was bound to the particles in the 50 μg/ml samples while lower binding efficiencies were observed for 250 μg/ml poly(I:C). The largest amount of microparticle bound poly(I:C) was obtained using the multilayer concept, where the cationic DEAE-dextran modified microparticles were first coated with poly(I:C), then with another layer of DEAE-dextran, and finally with a second layer of poly(I:C). As depicted in Fig. 1c, a reversion of the zeta potential to negative values was observed after poly(I:C) binding to the particles. During the coating procedure a loss of poly(I:C), e.g., by adhesion to the wall of tubes, or a detachment of poly(I:C) from the microparticle surface during the incubation with DEAE-dextran (multilayer particles) was not observed, indicating that the binding to the microparticle surface was stable and the determined values of surface bound poly(I:C) were accurate.

However, as will be discussed below, some aggregation of the particles was observed after the last coating step, particularly for the multilayer samples. This did not affect the suitability of the particles for cellular uptake.

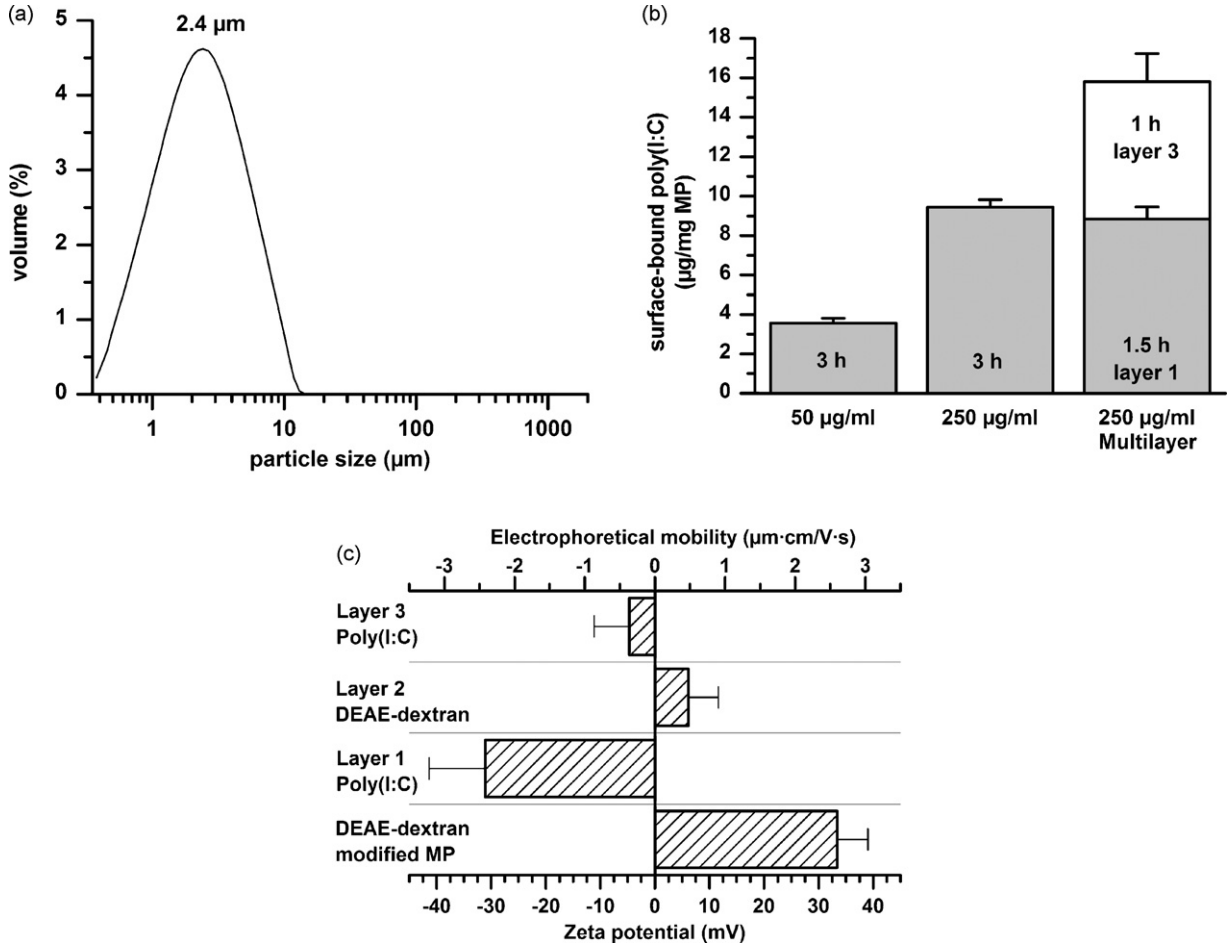

Fig. 1. Microparticle (MP) characteristics. (a) Particle size distribution of cationic DEAE-dextran modified microparticles as determined by laser diffraction (representative for numerous batches). (b) Extent of poly(I:C) binding to cationic DEAE-dextran modified MP depending on the coating procedure (coating time is stated in the column; $n = 3$, median, range). (c) Electrophoretical mobility and calculated zeta potential (for details, see Wischke et al., 2006) of multilayer particles ($n = 5$, mean, S.D.).

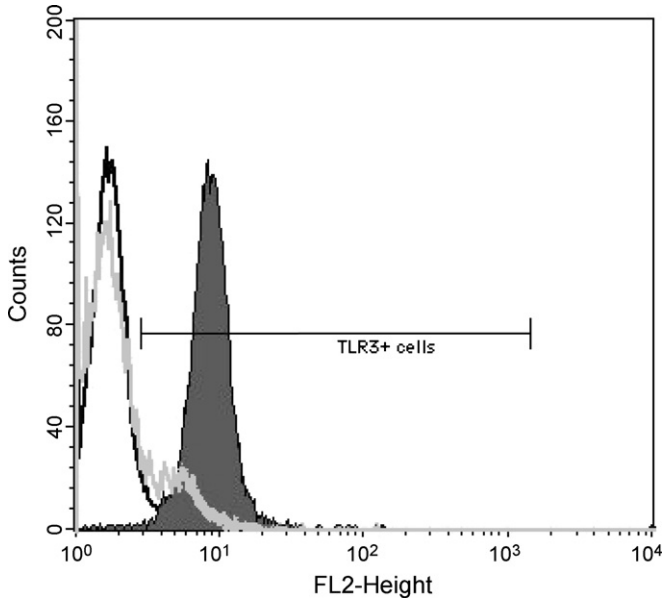

Fig. 2. Intracellular and surface expression of TLR3 in iMoDC. The open black line represents the isotype control, the open light gray line TLR3 at the cell surface, and the filled dark gray line the intracellular expression of TLR3.

3.2. TLR3 expression pattern of monocyte-derived DC

It has been reported that TLR3, a receptor for poly(I:C), is exclusively expressed by dendritic cells and predominantly resides inside the cells (Muzio et al., 2000; Matsumoto et al., 2003). However, TLR3 is commonly not expressed in monocytes but should be upregulated during their differentiation to human MoDC (Visintin et al., 2001). Because TLR3 was the target of the described poly(I:C) functionalized microparticles, the authors had to show on the protein level that it was present to a normal extent inside the used iMoDC.

As can be seen from Fig. 2, TLR3 was predominantly detected intracellularly and only few iMoDC showed surface expression of TLR3 as determined by flow cytometry. Therefore the iMoDC were suitable for further experiments using poly(I:C) coated microparticles targeting TLR3.

3.3. Induction of DC maturation by phagocytosis of poly(I:C) coated microparticles

Microparticle phagocytosis and maturation of MoDC were determined by flow cytometry (Fig. 3). The expression of maturation related surface markers was analyzed for samples with non-coated and poly(I:C) coated microparticles as well as for untreated iDC or mDC, maturated either by high concentrations of soluble poly(I:C) or by a cytokine cocktail (Jonuleit et al., 1997). The monocyte differentiation into DC was proven by the expression of the DC specific surface marker CD209 (DC-SIGN) (Teunis et al., 2000). Due to the encapsulation of FITC-BSA into the microparticles, the whole particles were fluorescent and their phagocytosis could be detected by a strong increase of the FITC fluorescence of the cells (Wischke et al., 2006).

The phagocytosis of the non-coated microparticles did not induce a strong upregulation of the DC surface markers (Fig. 3). When poly(I:C) coated microparticles were incubated with the cells, an increased expression of CD80, CD86, and CD83 was observed. Higher microparticle loadings with poly(I:C) (see Fig. 1b) resulted in a rising expression of these markers (Table 1). The strongest effect was observed for multilayer particles. The results from these samples are depicted in Figs. 3 and 4 for comparison with mMoDC. As the authors will discuss below, a full maturation was induced by poly(I:C) coated microparticles.

4. Discussion

For a cationic surface modification of PLGA particles, different methods are described in the literature including the usage of cationic PLGA derivatives (Barrera et al., 1993; Hrkach et al., 1995; Caponetti et al., 1999; Dailey et al., 2003), physical mixtures of PLGA with a cationic polymer (Zeng and Hornsby, 1999; Manuel et al., 2001), and surface active cationic substances. The latter, including *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (Singh et al., 2000, 2006), polylysine (Cui and Schwendeman, 2001), polyethyleneimine (Shakweh et al., 2005), and chitosan (Kumar et al., 2004), are coated to the surface of either the nascent microparticles during the particle formation or after the complete hardening of the microparticles. Recently, the surface modification of PLGA with DEAE-dextran was described (Wischke et al., 2006). Due to the higher pK_a value of DEAE-dextran compared to, e.g., chitosan, these particles exhibited a stronger interaction with negatively charged phagocytic cells and were taken up more effectively in the cited study.

Besides the approach to obtain a stronger interaction with anionic cell surfaces, cationic microparticles are also evaluated for their ability to bind and deliver plasmid DNA to non-phagocytic cells (Oster et al., 2005). In the present study the authors describe the use of cationic DEAE-dextran modified microparticles as a vehicle for the delivery of a synthetic analogue of viral dsRNA to human dendritic cells, where the ligand is expected to interact with intracellular TLR3 after particle phagocytosis. The scope was to show in principle that particle surface coating with poly(I:C) can be used to induce DC maturation.

4.1. Characteristics of the microparticles

The extent of microparticle phagocytosis depends on the characteristics of the microparticles, mainly on their size and surface properties including their charge (Thiele et al., 2001; Wischke, 2006). As shown in Fig. 1a, the prepared microparticles have a suitable size for the uptake by phagocytic cells, i.e., smaller than 5–10 μm in diameter. When such cationic DEAE-dextran modified microparticles were incubated with poly(I:C), the dsRNA was effectively bound to the particle surface (Fig. 1b). Some particle aggregation was observed during the multilayer coating, perhaps resulting from bridging of particles by the polyionic polymers (DEAE-dextran, poly(I:C)). However, this did not inhibit the cellular uptake of the particles (Fig. 3).

In order to quantify the extent of poly(I:C) binding, the amount of the unbound TLR3 ligand was measured in the supernatant after the incubation with the microparticles. The highest loading was observed with the multilayer concept (Fig. 1b). For these samples a coating time of only 1.5 h was employed, since longer incubation resulted only in a minor increase in surface bound poly(I:C) (compared to the 250 μg/ml single layer sample). This indicates that the equilibrium of surface bound poly(I:C) adjusted within the first hour of incubation.

The binding was also shown by a change of the zeta potential of the cationic microparticles to negative values after incubation with poly(I:C). In the case of the multilayer samples, the coating with another layer of DEAE-dextran resulted in a positive surface charge, which switched back to a negative value after the second treatment with poly(I:C) (Fig. 1c). It is obvious that after coating with layer 2 (DEAE-dextran) the charge did not reach the starting

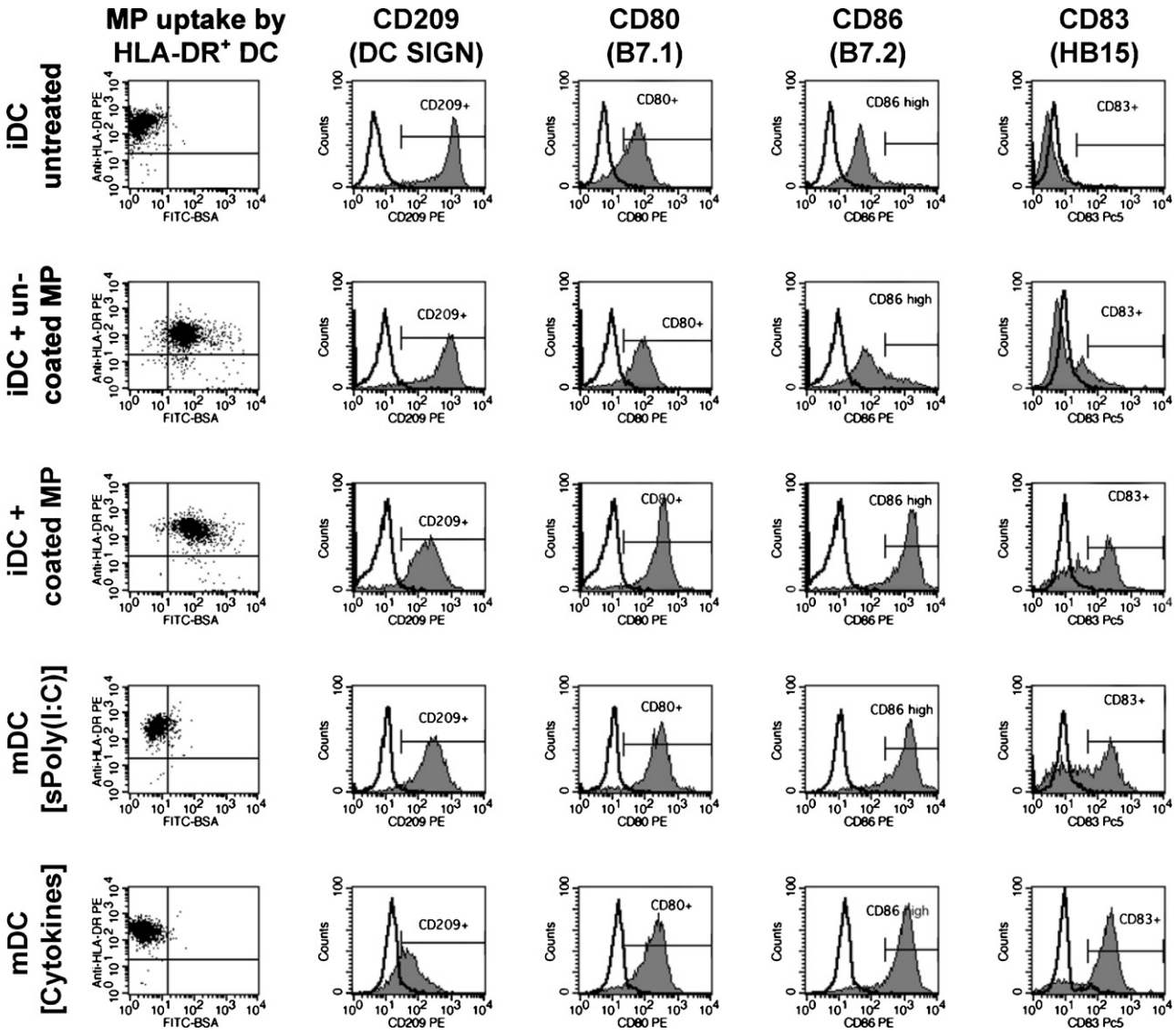

Fig. 3. Phenotype and maturation status of MoDC as analyzed by flow cytometry. Dotplots show the microparticle (MP) uptake by HLA-DR+ cells (expression of HLA-DR vs. the FITC fluorescence from FITC-BSA loaded microparticles). Histograms represent the expression of CD209, CD80, CD86, and CD83 for: (i) untreated immature DC (iDC), (ii) iDC with ingested uncoated MP, (iii) iDC with ingested poly(I:C) coated multilayer MP, (iv) mature DC (mDC) stimulated with 10 μg/ml soluble poly(I:C) (no MP), and (v) mDC matured with a cytokine cocktail (no MP). Gating of live cell populations was done by their forward and side scattering characteristics. Open lines represent staining with isotype matched control antibodies, filled gray lines display staining with the indicated specific antibody. Data shown are representative for three experiments with similar results.

value of about +35 mV. This might be attributed to a lower extent of DEAE-dextran binding to layer 1 compared to its initial binding to PLGA during the microparticle formation. In future studies higher concentrations of DEAE-dextran for layer 1 coating, the possibility of additional layers of DEAE-dextran and poly(I:C) as well as the tendency for aggregation with increasing numbers of coating steps should be addressed.

Table 1
Effect of poly(I:C) loading of microparticles (MP) on the CD86 expression

Microparticle (MP) coating procedure	DC with CD86^{high} after MP phagocytosis[1]
50 μg/ml poly(I:C)	45%
250 μg/ml poly(I:C)	80%
Multilayer samples	93%

[1] Data correspond to the experiment shown in Fig. 3.

4.2. Induction of DC maturation by phagocytosis of poly(I:C) coated microparticles

The cellular uptake of the FITC-BSA loaded microparticles was confirmed by the shift in the fluorescence intensity of the phagocytically active iMoDC (Fig. 3). Recently, this shift was proven to correlate with the intracellular localization of the microparticles (Wischke et al., 2006). Moreover, the phagocytosis of microparticles without strong antigens encapsulated did not induce a maturation of iMoDC with both cationic DEAE-dextran modified and anionic non-modified microparticles. This result was confirmed for microparticles without (Waeckerle-Men et al., 2004) or with different surface coatings (Fischer et al., 2007). However, there are also studies showing an increased expression of some but not all maturation-related DC surface markers after phagocytosis of blank PLGA microparticles (Yoshida and Babensee, 2004, 2006a,b; Babensee, 2008). This indicates that differences in the cell

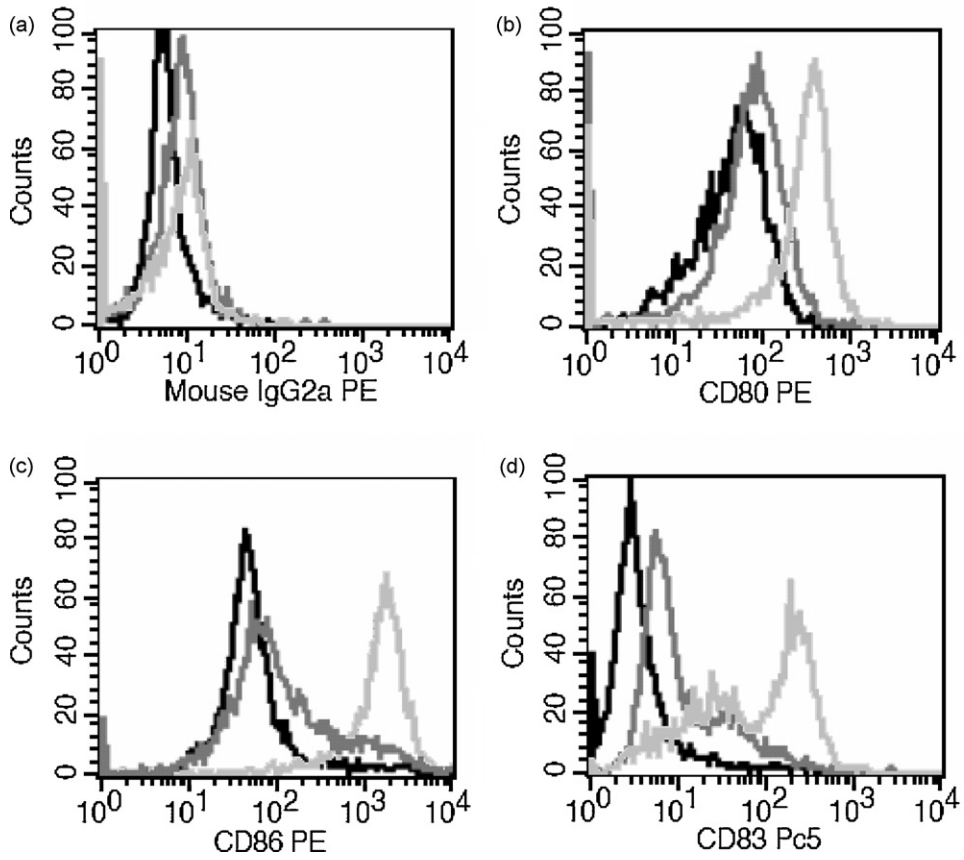

Fig. 4. Upregulation of costimulatory molecules and maturation markers after ingestion of poly(I:C) coated microparticles (MP). Overlays of selected histograms from Fig. 3 show the isotype control (a) and the expression of CD80 (b), CD86 (c), and CD83 (d). Black lines represent the untreated iDC, dark gray lines the iDC incubated with non-coated MP, and light gray lines the DC after incubation with poly(I:C) coated multilayer MP.

source, the cell culture procedure, or some unknown differences in the microparticle characteristics could influence the result of the respective study.

The differentiation of immature dendritic cells to fully mature DC results in the upregulation of costimulatory molecules, e.g., CD86 and the maturation marker CD83. In order to obtain DC maturation *ex vivo*, a cytokine cocktail (Jonuleit et al., 1997) is usually employed in cell culture systems. However, maturation can also be obtained by high concentrations of soluble poly(I:C) (Verdijk et al., 1999). This might be considered to be surprising, because TLR3 is present inside DC rather than on the cell surface (see Fig. 2). However, besides the ability to phagocytize particles, dendritic cells are also characterized by other mechanisms of endocytosis such as macropinocytosis (Sallusto et al., 1995). Thus they can internalize soluble or nanometer-sized pathogens like viruses from their environment at a low rate. This is the reason why high concentrations of soluble extracellular poly(I:C) activate DC through intracellular TLR3 that transduces the signal inside the cells (Matsumoto et al., 2003). However, the favored way to activate dendritic cells for vaccination or tumor therapy would be a vehicle that (i) contains the respective antigen, (ii) activates DC after complete phagocytosis by a specific interaction of particle-bound ligands with their targets, and (iii) allows the DC to bias the T-helper cell response into the antigen adapted and desired Th1/Th2 pathway. In this study, one part of this puzzle was analyzed, i.e., the suitability of PLGA microparticles to deliver a surface coated maturation signal.

Confirming earlier results, the phagocytosis of the non-coated microparticles did not induce a strong upregulation of maturation related surface markers in the present study. By contrast, the incubation of DC with poly(I:C) coated microparticles resulted in an increased expression of CD80, CD86, and CD83 at the cell surface (Fig. 4). The expression of CD80 and CD86, which are costimulatory molecules, was similarly high for the multilayer microparticles and both mDC controls (Fig. 3). For CD83 a similar expression was found for poly(I:C) coated microparticles and the mDC control with high concentrations of soluble poly(I:C), which was only slightly below that of the mDC obtained with the cytokine cocktail.

In order to evaluate the effect of microparticle attached poly(I:C), the difference in the poly(I:C) concentration that induced the upregulation of the surface markers should be noted. Soluble poly(I:C) was used at its optimal concentration of 10 μg/ml (as tested previously), whereas the microparticle samples were diluted prior to their use resulting in a final poly(I:C) concentration of only 800 ng per ml cell culture medium (multilayer samples). In conclusion the poly(I:C) coated microparticles were more effective in the induction of the DC maturation since lower concentrations of the TLR3 ligand could cause the same effect. This confirms the assumption that certain ligands bound to the surface of microparticles can interact more intensively with receptors, especially intracellular ones, than the same concentration of soluble ligands. Therefore they could achieve a stronger cellular response.

Besides the higher efficiency in inducing the DC maturation, the authors wish to emphasize the advantageous ability of such microparticles to do both; deliver encapsulated proteins or antigens into the cell, and stimulate the DC maturation once the particles are phagocytized (Fig. 5). The relevance of this concept is reflected in a recent review, whereby novel strategies are demanded to overcome inhomogeneous or incomplete activation of APC obtained by clas-

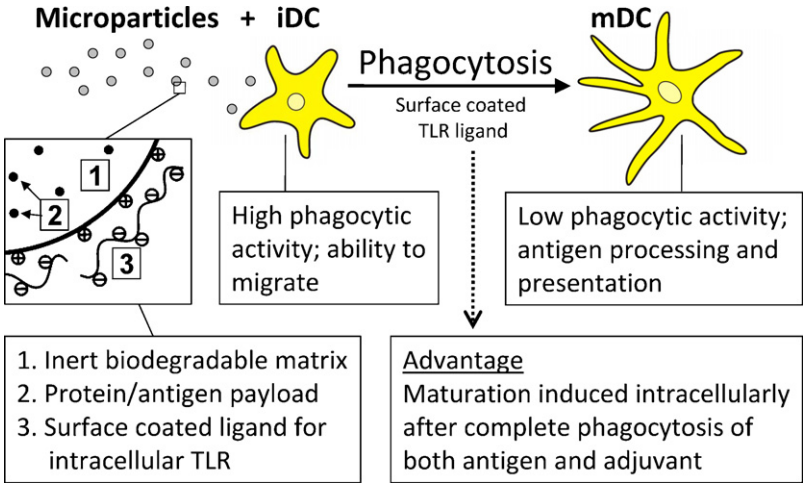

Fig. 5. Microparticle properties advantageous for the homogeneous maturation of DC populations.

sical vaccination protocols with a mixture of both soluble antigen and adjuvant (Heit et al., 2008).

Based on the findings of this work, PLGA microparticles may further be studied as a vehicle for the controlled induction of DC maturation, with a focus on the coating procedure, on different ligands for the onset of either Th1 or Th2 responses, the dose–response relationship of the ligand, and the effect of different encapsulated antigens. Additionally, a direct comparison of both present strategies, i.e., the coencapsulation of antigen and TLR ligand (Heit et al., 2008; Schlosser et al., 2008) and the surface coating as described herein will give insights of the potential of each method.

5. Conclusions

Microparticles of poly(lactic-co-glycolic acid) are suitable, non-toxic protein delivery systems to human dendritic cells (DC). Anionic substances, such as poly(I:C), can bind to the surface of cationic DEAE-dextran modified microparticles. This coating with ligands of Toll-like receptors can induce DC maturation in the presence of only weak antigens. This suggests that by using poly(I:C) coated rather than non-coated standard microparticles for antigen delivery the issue of low immunogenicity of some antigens such as certain tumor associated proteins might be overcome. Therefore such vehicles may successfully be used in DC-based cell therapies of cancer or in vaccination trials.

Acknowledgements

The authors thank Dr. J. Riggert and the personnel from the Dept. of Transfusion Medicine, University of Göttingen, for supplying the leukapheresis products and Andrea Struck, Thomas Schulz, Tania Fuhrmann-Selter, Dr. Holger Brosig, Dr. Werner Herrmann, and Sam Reinhold for their support or excellent technical assistance. Parts of this study were financially supported by the DC Gesellschaft for Dendritic-Cell-Therapy mbH, Göttingen, Germany.

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