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Nanoencapsulated budesonide in self-stratified polyurethane-polyurea nanoparticles is highly effective in inducing human tolerogenic dendritic cells

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ABSTRACT

The design of innovative strategies to selectively target cells, such antigen-presenting cells and dendritic cells, *in vivo* to induce immune tolerance is gaining interest and relevance for the treatment of immune-mediated diseases.

A novel loaded-nanosystem strategy to generate tolerogenic dendritic cells (tol-DCs) was evaluated. Hence budesonide (BDS) was encapsulated in multiwalled polyurethane-polyurea nanoparticles (PUUa NPs-BDS) based on self-stratified polymers by hydrophobic interactions at the oil-water interface. DCs treated with encapsulated BDS presented a prominent downregulation of costimulatory molecules (CD80, CD83 and MHCII) and upregulation of inhibitory receptors. Moreover, DCs treated with these PUUa NPs-BDS also secreted large amounts of IL-10, a crucial anti-inflammatory cytokine to induce tolerance, and inhibited T lymphocyte activation in a specific manner compared to those cells generated with free BDS. These results demonstrate that PUUa NPs-BDS are a highly specific and efficient system through which to induce DCs with a tolerogenic profile. Given the capacity of PUUa NPs-BDS, this delivery system has a clear advantage for translation to *in vivo* studies.

1. Introduction

In the last decade, nanoimmunotherapy has emerged as a novel strategy to bolster the capacity of the immune system to counteract different diseases ranging from cancer to immune-mediated diseases (Tacken et al., 2007; De Fuente et al., 2014; Cheung and Mooney, 2015). Several studies have demonstrated the therapeutic potential of manipulating and unleashing our own immune system to tackle cancer or infectious diseases (Kirkwood et al., 2012). Dendritic cells (DCs) are key regulators of the immune response. They are a heterogeneous subset of immune cells recognized as highly potent antigen-presenting cells (APCs) that link innate and adaptive immune responses. DCs are crucial in inducing immunogenic and tolerogenic responses to pathogens or harmless antigens, respectively (Shao et al., 2014; Gharagozloo et al., 2015). DCs are highly specialized in capture and processing

Abbreviations: APCs, antigen-presenting cells; Bayhydur 3100, hydrophilic aliphatic polyisocyanate based on hexamethylene diisocyanate (HDI); BDS, budesonide; DCs, dendritic cells; DiI, 1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate+; IL-, interleukin; MLR, mixed leucocyte reaction; PBMCs, peripheral blood mononuclear cells; PBLs, peripheral blood lymphocytes; PUUa NPs, polyurethane-polyurea nanoparticles; PUUa NPs-BDS, polyurethane-polyurea nanoparticles loaded with Budesonide; PUUa NPs-DiI, polyurethane polyuria nanoparticles loaded with DiI; YMER N-120, linear difunctional polyethylene glycol monomethyl ether; Tol-DCs, tolerogenic dendritic cells; TNFα, tumor necrosis factor α.

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antigens in order to convert proteins into small peptides. These peptides are then presented to T-cells by major histocompatibility complexes (MHC) to initiate immune responses (Banchereau and Steinman, 1998; Steinman and Banchereau, 2007). Once immature DCs have recognized pathogens through specialized receptors, they mature to acquire the capacity to stimulate T-cells. The activation and polarization of T-cells is induced through costimulatory molecules, such as CD80, CD83 and CD86, which are upregulated on the mature DC (mDC) membrane, as well as through the secretion of pro- or anti-inflammatory cytokines such as interleukin IL-6, IL-12p70, IL-23 TNF-α or IL-10. The cytokine secretion pattern strongly defines the resulting polarization of T-cells, thereby determining the type of immune response, namely effector Th1, Th2 or Th17 immunity (IL-12p70, IL-23 and TNF-α) or tolerance induction through regulatory T-cells or Tr1 (IL-10) (Collin et al., 2013).

Due to their physiological properties and the availability of clinical grade reagents, immunogenic DCs have been safely and successfully used in clinical trials aiming to generate an efficient immune response against tumors and infectious diseases (Butterfield, 2013; Anguille et al., 2014; Banchereau et al., 2000). Furthermore, as DCs play a key role in maintaining immune tolerance, the generation of tolerogenic DCs (tol-DCs) has great potential in immunotherapy approaches in several immune-mediated diseases such as diabetes, rheumatoid arthritis, multiple sclerosis, and Crohn's disease (Pulendran et al., 2010; Steinman et al., 2003; Cabezón and Benítez-Ribas, 2013; Hu and Wan, 2011; Benham et al., 2015).

Several protocols, including the generation of DCs in the presence of corticosteroids, such as BDS, have been described to produce tol-DCs *in vitro* (Hackstein and Thomson, 2004; Van Kooten et al., 2009). These cells present a semi-mature phenotype, a pronounced shift towards anti-inflammatory versus inflammatory cytokine production and a low capacity to stimulate T-cells. The increased secretion of IL-10 by tol-DCs is considered critical to induce tolerance (Zheng et al., 2013; Cabezón et al., 2012; Kalantari et al., 2011; García-González et al., 2013).

To date, DC-based therapies involve the isolation and *ex vivo* generation of DCs (Jauregui-Amezaga et al., 2015; Suwandi et al., 2016). These approaches require the preparation of individualized autologous cells and thus call for costly culture protocols in certified GMP facilities (Naranjo-Gómez et al., 2011) and standardization of the procedures among laboratories when Phase II or III are planned. An alternative approach to *ex vivo* cell generation is the *in vivo* targeting of specific immune cells in order to manipulate and modulate their function. Several approaches to deliver immunogenic or regulatory agents have been explored, including nanopolymeric systems of PLGA (poly(lactic-*co*-glycolic acid)) and PLLA (poly-L-lactide), and liposomes (Park et al., 2013). Nanoparticle systems improve DC-targeted delivery of tumor antigens, amplify immune activation via the use of immunostimulatory materials, and increase the efficacy of adoptive cell therapies (Amoozgar and Goldberg, 2015; Cho et al., 2011; Fang et al., 2014). Interestingly, the possibility to target DCs *in vivo* paves the way for immunotherapeutic approaches to treat human diseases by modifying immune responses without the need to culture cells. Recent studies have shown that nanoencapsulated corticosteroids in controlled release polymeric systems boost the therapeutic efficiency of the drug, as BDS becomes more water soluble (increased bioavailability) and is released only under the required conditions, thus reducing systemic side effects (Siddique et al., 2015; Leonard et al., 2012; Ali et al., 2014; Beloqui et al., 2013). However, many of these delivery systems show insufficient stability under *in vivo* conditions and limited encapsulation capacity as the drug is prematurely released (Zou et al., 2013). We have recently demonstrated that common approaches based on drug encapsulation with monowalled nanostructures are not stable upon interaction with amphiphilic and hydrophobic cell membrane molecules (*e.g.* phospholipids, cholesterol) (Rocas et al., 2015). Thus, the cargo is non-specifically released from the nanoparticle core, poorly internalized by target cells, and less bioactive (Chen et al., 2008). Hence, we envisaged that a disulfide-rich nanopolymeric system based on hydrophobically stratified polymers creating robust multiwalled nanostructures would be an interesting approach to improve encapsulation stability and maintain in-target redox biodegradation and drug release. However, the effect of these multiwalled nanostructures on human primary DCs remains unknown.

Here we evaluated the performance of our previously described redox-sensitive self-stratified multiwalled nanoparticles (Rocas et al., 2015, 2014) to quantitatively encapsulate various amounts of budesonide (BDS) and analyzed the effects of these particles on primary human monocyte-derived DCs (Fig. 1). In addition, we studied the generation of tol-DCs, comparing encapsulated versus soluble BDS. For this purpose, polyurethane-polyurea nanoparticles (PUUa NPs) carrying BDS were incubated with human monocyte-derived DCs and the toxicity and internalization of these particles were evaluated over time. To validate PUUa NPs as an appropriate carrier for immunosuppressive drugs, we assessed costimulatory molecule expression, cytokine production and the capacity to activate T-cells.

2. Material and methods

2.1. Materials

YMER N-120 was provided by Perstorp (Perstorp, Sweden) and N-Coco-1,3-propylenediamine (Genamin TAP 100D) by Clariant (Barcelona, Spain). The capric/caprylic triglyceride mixture (Crodamol GTCC) was obtained from Croda (Barcelona, Spain), and

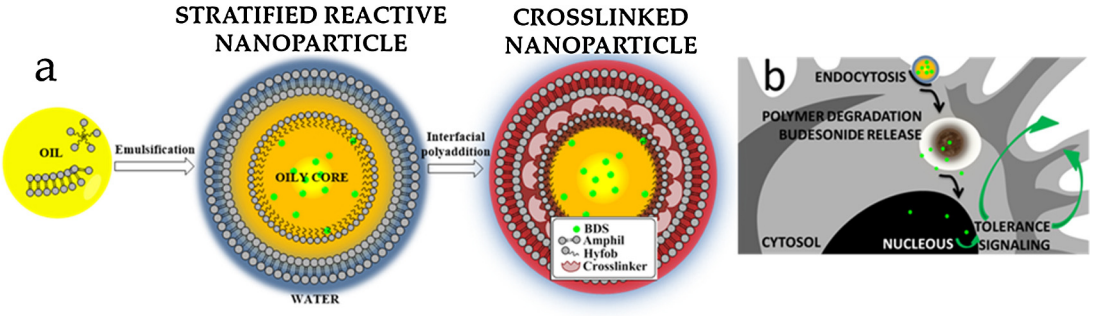

Fig. 1. Nanoparticle synthetic strategy and cell internalization. (a) Emulsification of Hyfob and Amphil leads to reactive nanostructures that are further crosslinked in the o/w interface. (b) DC internalization, drug release, and tolerance signaling scheme.