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Tuning complement activation and pathway through controlled molecular architecture of dextran chains in nanoparticle corona

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ABSTRACT

The understanding of complement activation by nanomaterials is a key to a rational design of safe and efficient nanomedicines. This work proposed a systematic study investigating how molecular design of nanoparticle coronas made of dextran impacts on mechanisms that trigger complement activation. The nanoparticles used for this work consisted of dextran-coated poly(isobutylcyanoacrylate) (PIBCA) nanoparticles have already been thoroughly characterized. Their different capacity to trigger complement activation established on the cleavage of the protein C3 was also already described making these nanoparticles good models to investigate the relation between the molecular feature of their corona and the mechanism by which they triggered complement activation. Results of this new study show that complement activation pathways can be selected by distinct architectures formed by dextran chains composing the nanoparticle corona. Assumptions that explain the relation between complement activation mechanisms triggered by the nanoparticles and the nanoparticle corona molecular feature were proposed. These results are of interest to better understand how the design of dextran-coated nanomaterials will impact interactions with the complement system. It can open perspectives with regard to the selection of a preferential complement activation pathway or prevent the nanoparticles to activate the complement system, based on a rational choice of the corona configuration.

1. Introduction

Nanoparticles have been extensively studied as drug delivery systems for therapeutic purposes (Couvreur and Vauthier 2006; Crucho and Barros, 2015; Li et al., 2015; Shi et al., 2017). Success to improve therapeutic index of molecules carried by such systems depends on the fate of the nanoparticles in the body. The rational design of new carriers is pending to a better understanding of the relationship between nanoparticles' properties and their interactions with the biological medium surrounding the nanoparticles once they are in the body (Clogston et al., 2016Ilinskaya and Dobrovolskaia, 2016a,b; Őrfi and Szebeni, 2016; Szeto and Lavik, 2016; Xiang et al., 2013; Truel and Nienboss, 2013). In general, nanoparticles are designed to be administered intravenously. Once in the blood, they are immediately confronted with components of the innate immune system (Anchordoquy et al., 2017; Dobrovolskaia and McNeil, 2013; Moghimi and Farhangrazi, 2013; Szebeni, 2014). Without specific engineering, nanoparticles are generally recognized by the innate immune system and rapidly cleared from the bloodstream due to the uptake by the mononuclear phagocyte system (MPS) (Bazile et al., 1995; D'Addio et al., 2012; Gref et al., 1995, 1994; Peracchia et al., 1999; Suk et al., 2016). Several works have demonstrated that the grafting of a hydrophilic corona at the surface of the nanoparticles can reduce the uptake by MPS. Hydrophilic polymers, such as poly-ethylene glycol (PEG), polysaccharides (e.g. dextran, heparin, and chitosan) and polyphosphoesters were the most used materials on this purpose (Alhareth et al., 2012; Bazile et al., 1995; Bertholon et al., 2006a; D'Addio et al., 2012; Gref et al., 1995, 1994; Moghimi and Szebeni, 2003; Müller et al., 2017; Lemarchand et al., 2006; Peracchia et al., 1999; Suk et al., 2016). However, a review of the literature shows that even with a hydrophilic corona, some nanocarriers undergo a rapid clearance that is accompanied by the activation of the complement system (Alhareth et al., 2012; Gref et al., 2000; Moghimi et al., 2003; Szebeni, 2014). The effectiveness of the hydrophilic shell to reduce uptake by MPS is associated with its capability to reduce interactions of blood proteins with the nanoparticle surface. All parameters that define the molecular feature of the hydrophilic corona formed around the nanoparticles, i.e. chain length, grafting

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density, composition and structural arrangement of the chains, were found important factors influencing the *in vivo* fate of nanoparticles (Chen and Borden, 2011; D'Addio et al., 2012; Du et al., 2015; Hamad et al., 2010; Müller et al., 2017; Peracchia et al., 1997; Toda et al., 2010; Szebeni and Storm, 2015; Szeto and Lavik, 2016; Vittaz et al., 1996; Vonarbourg et al., 2006).

The complement system (CS) has an important role in innate immunity to recognize and eliminate pathogens, defective cells and foreign biomaterial devices, including nanoparticles circulating in the blood (Carroll and Sim, 2011). The CS consists of more than 30 plasma and cell-membrane proteins. It is the most important biochemical cascade that defines the type of "danger" signals sent to the rest of the immune system after it has detected an intruder, hence defining the type of response. Its activation can be triggered essentially by three distinct routes, the classical, lectin and alternative pathways (Gasque, 2004; Harboe et al., 2011; Merle et al., 2015). The classical pathway is activated primordially by the interaction of C1q with immunoglobulins. Non-immune molecules such as the C reactive protein (CRP) can also trigger the classical pathway. The lectin pathway is triggered by the recognition of carbohydrate patterns exhibited on the surface of foreign particles. Proteins involved in the recognition are the mannose-binding lectin (MBL), the H-, L- or M-ficolins or the collectin 11. Activation of the alternative pathway depends on spontaneous hydrolysis of the internal thioester bond found in protein C3 that can be triggered by nucleophilic groups found on the intruder surface. The three pathways converge to generate the C3 convertase and the terminal membrane attack complex (MAC) C5b-9. Activation of the CS also leads to the release of many types of anaphylatoxins which contribute to the inflammatory process (Carroll and Sim, 2011).

Interactions of proteins of the CS with colloidal particles can trigger the complement cascade (Bertholon et al., 2006a; Dobrovolskaia and McNeil, 2013; Ilinskaya and Dobrovolskaia, 2016a,b; Moghimi and Farhangrazi, 2013; Lorenze-Abalde et al., 2016; Szebeni, 2014; Szebeni et al., 2012; Vorup-Jensen and Boesen, 2011). Then, the production of opsonins such as C3b leads to a rapid capture of nanocarriers by the MPS resulting in a short circulation time in the blood. While this effect can compromise the efficacy of delivery systems, another consequence of the activation of the complement system concerns its safety when it gives rise to hypersensitivity reactions called complement activation-related pseudoallergy (CARPA) (Andersen et al., 2012; Szebeni, 2014, 2012; Szebeni et al., 2012).

Nanoparticles based on biodegradable poly(alkylcyanoacrylate) polymers were widely investigated as drug delivery systems (Couvreur and Vauthier, 2006; Nicolas and Couvreur, 2009; Nicolas et al., 2013; Nicolas and Vauthier, 2011; Vauthier et al., 2007). These colloidal carriers have shown many advantages in terms of drug protection, long stability, biocompatibility, biodegradability and drug delivery potential. Different preparation methods can be used to generate poly(isobutylcyanoacrylate) PIBCA nanoparticles having hydrophilic coronas of various compositions and molecular architectures modulating their capacity to activate the complement system and their biodistribution (Vauthier, 2015). A series of PIBCA nanoparticles with different hydrophilic coronas made of dextran was thoroughly characterized in previous works (Bertholon et al., 2006b,c; Chauvierre et al., 2004; Labarre et al., 2005; Vauthier et al., 2011, 2009). These nanoparticles could serve as models to improve our understanding of the influence of the molecular feature of the hydrophilic polymers of the nanoparticle corona on biological mechanisms involved in efficacy and safety of nanoparticles. Such a work would be consistent with the need to develop systematic studies providing results required to improve our capacity to design efficient and safe nanoparticulate drug delivery systems on a more rational basis (Clogston et al., 2016; Szeto and Lavik, 2016; Truel and Nienhaus, 2013; Xiang et al., 2013).

The goal of the present work was to evaluate how molecular characteristics of the corona of dextran-coated PIBCA nanoparticles could interfere in the activation of the complement system, including the resolution of the involved pathway(s). Activation of the complement cascade was acknowledged in a systematic way by evidencing protein C3 cleavage using a high-throughput serial 2D immunoelectrophoresis method (Coty et al., 2016). Pathways were differentiated incubating nanoparticles and serum with calcium and magnesium to highlight complement activation triggered through all pathways and with magnesium only to evidence the contribution to activation of the alternative pathway that is a calcium independent pathway (Des Prez et al., 1975; Hamad et al., 2010; Labarre et al., 1993). Results were discussed in the light of our knowledge on the molecular characteristics of the nanoparticle corona.

2. Materials and methods

2.1. Materials

Isobutylcyanoacrylate (IBCA) was synthesized and provided by Orapi (Saint-Vulbas, France). Chemicals purchased from Sigma (Saint-Quentin-Fallavier, France) were tricine, Tris base (Sigma 7–9®), sodium chloride, EDTA sodium salt, dextran 66.7 kDa, and bromophenol blue. Dextran 17.7, cerium (IV) ammonium nitrate and hydrochloric acid were supplied by Fluka (Saint-Quentin-Fallavier, France). Nitric acid was purchased from Prolabo (Paris, France). Calcium lactate, glacial acetic acid, coomassie brilliant blue R-250 were supplied by Thermo Fisher Scientific (Villebon-sur-Yvette, France). Gel-Fix™ for agarose (265 × 150 mm) was obtained from Serva Electrophoresis (Heidelberg, Germany). Nafamostat mesylate (NM) was obtained from Abcam (Cambridge, UK). All chemicals were of reagent grade and used as purchased.

Polyclonal anti-human C3 antibody raised in goat was purchased from Fitzgerald antibodies (Acton, USA). Serum was prepared from human plasma obtained from healthy donors from Etablissement Français du Sang (EFS, agreement # 14/EFS/041) (Rungis, France). Heat-aggregated gamma globulin (HAGG) and Cobra Venom Factor (CVF) were purchased from TECO Medical (Rambouillet, France) to be used as positive controls for the activation of the complement system.

2.2. Preparation of nanoparticles by redox radical emulsion polymerization (RREP)

Three types of RREP nanoparticles were prepared according to the method described by Bertholon et al. (2006c). R1 and R2 were prepared as follow using dextran 66.7 kDa and dextran 17.7 kDa respectively at a concentration of 1.3%. Dextran (0.1356 g) was dissolved in 8 mL of 0.2 N nitric acid. After 10 min bubbling with argon at 40 °C, the polymerization was initiated adding successively and under vigorous stirring 2 mL of a solution of cerium (IV) ammonium nitrate (8×10^{-2} M) in 0.2 N nitric acid and 0.5 mL of IBCA. The reaction was allowed to continue for 1 h at 40 °C. At the end, the dispersion was cooled down in an ice bath.

R3 was prepared following the same method using a concentration of dextran 66.7 kDa of 0.5%. Dextran 66.7 kDa (0.0502 g) was dissolved in 9.3 mL of 0.2 N nitric acid following the above described protocol. The polymerization was started by adding successively 0.7 mL of a solution of cerium (IV) ammonium nitrate (8×10^{-2} M) in 0.2 N nitric acid and 0.5 mL of IBCA. At the end of the polymerization the dispersion was cooled down at 4 °C in an ice bath.