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Complement activation turnover on surfaces of nanoparticles

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Abstract

The complement system is an important component of the innate immune system, which contributes to non-specific host defence. Particulate matters, such as invading pathogens and nanomedicines, in the blood may activate the complement system through classical, lectin and alternative pathways. Complement activation can aid recognition and clearance of particulate matters by immune cells, but uncontrolled complement activation can inflict damage and be life threatening. Plasma proteins on adsorption to surfaces of nanoparticles also play a significant role in complement activation and particularly through the alternative pathway. This process is continuous and changeable in vivo; protein-complement complexes are formed on the nanoparticle surface and then released and the cycle repeats on further plasma protein deposition. This complement activation turnover poses a challenge for design of immune-safe nanomedicines.

Keywords

Adverse injection reactions; Complement system; Protein adsorption; Stealth therapeutic nanoparticles

Intravenously injected nanoparticles of organic and inorganic origins are finding increasing applications in diagnostic and therapeutic medicine [1]. Macrophages (a type of immune cell that engulfs cellular debris, microbes and other foreign particulate matters) of the liver and the spleen rapidly intercept blood-borne particles and this is problematic if the designated target for therapeutic nanoparticles resides outside these organs [2,3]. Surface camouflaging with synthetic polymers or alterations in particle geometry or both, however, can modulate nanoparticle pharmacokinetics and delay their recognition and clearance by macrophages in contact with blood (e.g., Kupffer cells in the liver and macrophages located in the marginal zone and the red-pulp regions of the spleen) [1,3]. It is generally believed that polymer coating may suppress or prevent deposition of opsonic blood proteins on nanoparticles [1,3]. The opsonic blood proteins, which aid particle recognition by macrophage receptors, include

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fibronectin, immunoglobulins (e.g., IgG and IgM) and some components of the complement system [4]. The complement system, comprising of more than 30 proteins, is an important part of the innate immunity that contributes to non-specific host defence. A number of complement proteins bind to foreign surfaces and react with one another. Some of these proteins are proteases (an enzyme that performs proteolysis) that are themselves activated by proteolytic cleavage.

On complement activation, the third complement protein (C3) is enzymatically cleaved and this generates the two opsonic complement fragments known as C3b and iC3b [4]. Nanoparticles and particulate intruders tagged with C3b and iC3b are recognized by macrophages through their corresponding complement receptors (complement receptors 1 and 3, respectively) [2,4]. Nanoparticles (including the so-called stealth entities), depending on their physicochemical properties such as size, morphology and surface patterns may activate the complement system through three different main pathways. These are known as classical, lectin and alternative complement pathways and are reviewed in detail elsewhere [5]. Activation of the complement system further releases biologically active complement peptides C4a, C3a and C5a (referred to as anaphylatoxins) through proteolytic cleavage of their parent molecule (C4, C3 and C5, respectively) [4,5]. Anaphylatoxins cause the release of histamine from mast cells (a type of immune cell that participate in allergic reactions); enhance vascular permeability and smooth muscle contraction [6]. There are efficient and rapid control mechanisms of anaphylatoxin activity under physiological conditions [6]. However, uncontrolled anaphylatoxin release may promote life threatening inflammatory reactions, chemotaxis (an attribute of motile cells moving toward or away from a chemical signal) and cardiopulmonary distress (e.g., difficulty in breathing, acute fluctuations in blood pressure, chest pain, decreased cardiac output, cardiac arrest) [7]. Accordingly, there is a great interest in understanding of the mechanisms by which diagnostic and therapeutic nanoparticles activate complement system, as this may improve immune safety and efficacy of therapeutic nanoparticles through better design and engineering approaches.

Here, we limit our discussion to the alternative pathway of the complement system and its activation by nanoparticles. Initiation of the alternative pathway of the complement system usually proceed as a result of spontaneous hydrolysis of the thiolester in C3 to form $C3(H_2O)$, which subsequently bind complement factor B to form $C3(H_2O)B$ [4,5]. The bound factor B is susceptible to cleavage and activation by complement factor D to form $C3(H_2O)Bb$, which is known as the fluid-phase C3 convertase. This convertase cleaves C3 into C3a (the anaphylatoxin) and C3b (the opsonic molecule) [4,5]. The latter can covalently bind to hydroxyl and amino groups presented on surfaces of nanoparticles [5]. Surface-bound C3b can bind factor B, which is in turn cleaved by factor D to form C3bBb. This complex is the unstable alternative pathway C3 amplification convertase [4,5]. C3bBb is stabilized by another complement protein known as properdin (P) to form the convertase C3bBbP, which also cleaves C3 into C3a and C3b. This establishes a positive feedback amplification loop causing more C3b deposition on the nanoparticle surface [4,5]. The turnover of this amplification loop is regulated by complement factors H and I. It should also be emphasized that $C3(H_2O)$ may also deposit directly (non-covalently) on surfaces and initiate C3 cleavage through subsequent binding of factors B and D [8]. Recently, we showed that dextran and poly(ethylene glycol) (PEG) stabilized nanoparticles can efficiently

trigger the complement system in human blood through the alternative pathway [9]. However, the adsorbed blood proteins on the surfaces of nanoparticles, but not the polymer coat, inadvertently and predominantly caused activation of the alternative pathway [9] (Fig. 1). On surface adsorption plasma proteins may undergo thermodynamic and conformational changes and as a result expose reactive groups that may become susceptible to C3b attack. Eventually, this forms C3bBb and C3bBbP convertases bound to surface-adsorbed proteins. This process may be viewed as a non-specific global mechanism by which nanoparticles, regardless of their chemical composition and make up, could trigger the alternative pathway of the complement system. We further noticed that protein-C3b and protein-C3 convertases are continuously formed and released from the surfaces of the nanoparticles [9]. This dynamic process may therefore limit the action of complement regulatory proteins factor H and I to stop complement activation. This means that such nanoparticles in the blood may continuously activate the alternative pathway of the complement system [9]. Since C3b is also an opsonic molecule, continuous C3b binding and release (in the form of protein-C3b) may explain why long-circulating PEGylated and dextran-stabilized nanoparticles are slowly recognized and cleared from the blood by the liver and the spleen macrophages [3].

It is well known that long-circulating nanomedicines may passively accumulate in interstitial spaces of solid tumours [10]. However, the majority of clinically approved long-circulating anticancer nanomedicines have shown limited therapeutic efficacy in humans [11,12]. Although many factors may account for poor clinical efficacy [11,12], our observations may also offer an additional explanation. It is well known that the immune system can eliminate as well as promote malignancy [11,13]. With respect to latter, there are several types of immune cells (e.g., regulatory T cells, alternatively activated macrophages, neutrophils) that infiltrate tumour and harbor immunosuppressive activities. These cells not only foster tumour development through expression and release of potent protumour mediators (e.g., proangiogenic molecules), but also suppress the activity of antitumour immune responses [11,13,14]. In the tumour environment, accumulated nanoparticles activate complement [14] and complement activation may further proceed through non-specific protein adsorption. Since complement activation liberates C5a, continuous nanoparticle-mediated complement activation will generate a C5a gradient resulting in recruitment of immune cells with immunosuppressive activities into the tumour site [11,13,14].

Therefore, the grand challenge is how to design nanoparticles with super protein repelling properties as to avoid protein “corona”-mediated complement activation, since the well-known PEGylation technology (as well as surface functionalization with related polymers) cannot fully inhibit protein deposition [1,15,16]. With polymer-coated nanoparticles, the polymer type, its molecular mass, conformation, surface density and distancing as well as interactive forces such as Van der Waals force of attraction and hydrogen bonding may all control protein deposition or protein intercalation into polymer chains. Here, the role of hydration waters (solvation patterns) must also be taken into account, which may be rate limiting. Indeed, interfacial waters may fill cavities; this may mediate hydrogen bonding between a protein and a surface (e.g., hydrated PEG chains), enhancing affinity without contributing to specificity [17,18]. On surface deposition, solvation patterns of a protein may change (e.g., interior water molecules may escape); this could promote interaction with the nearby proteins, resulting in protein build-up, or alternatively causes protein destabilization

and/or desorption. It has been suggested that keeping long chain methoxyPEG molecules ~10–15 Å apart is the ideal spacing for better protein exclusion [19,20]. However, achieving such precision nanometer-scale patterning, for controlling spatial and architectural arrangements of immobilized polymers on the surfaces of nanoparticles, may be difficult with currently available technologies. Empirically, surface modification and void filling by polymer pairing/matching (combinations of short, medium and long-chain hydrophilic polymers of same or alternative classes) may be a viable approach in modulating surface energetic phases (e.g., elastic and osmotic components) and minimise or overcome statistical protein binding [21,22]. Nevertheless, such attempts should also consider the role of particle shape, deformability and the range of Gaussian curvatures in conformational state of surface projected polymers. Within this context, it should be emphasized that alterations in surface projected polymer configuration (e.g., a shift from mushroom to a brush-like configuration) have marginally reduced the extent of complement activation and C3b binding [23,24]. Instead, such approaches have shifted complement activation from one pathway to another [23]. Another possible strategy for future exploitation is to design and engineer super-hydrophobic nanoparticles that could repel proteins under shear flow conditions [25]. Innovations in materials science may aid such design initiatives, but the material of choice (and its degradation products) must be biological safety.

Finally, cells and virulent pathogens present examples of naturally evolved strategies that overcome complement sensing and activation [2,26]. Some of these strategies are finding their way into nanoparticle engineering [27,28], however, there are still challenges in tuning nanoparticle pharmacokinetics. Considering the complexities surrounding nanoparticle design and surface engineering, attention must also be paid to pharmaceutical reproducibility, scaling up and manufacturing processing as well as issues with clinical practice. Nevertheless, we believe a better mechanistic understanding of interfacial events could open the path for “simple” and “immune-safe-by-design” innovations in nanomedicine and related fields.

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Biographies

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Dmitri Simberg graduated from the Hebrew University of Jerusalem, Israel. Afterward he joined Burnham Institute, La Jolla, and later University of California at San Diego (UCSD) as a postdoctoral scientist. Dr. Simberg later joined the Center for Cancer Nanotechnology Excellence at UCSD as project scientist, where he developed his research program in nano-biointerface. In 2013, Dr. Simberg became a faculty member at University of Colorado and currently serves as co-director of the Colorado Center for Nanoscience and Nanomedicine. His current research interests are focused on the use of iron oxide nanoparticles and red blood cells for drug delivery and imaging, and in basic mechanisms of complement activation by nanomedicines.

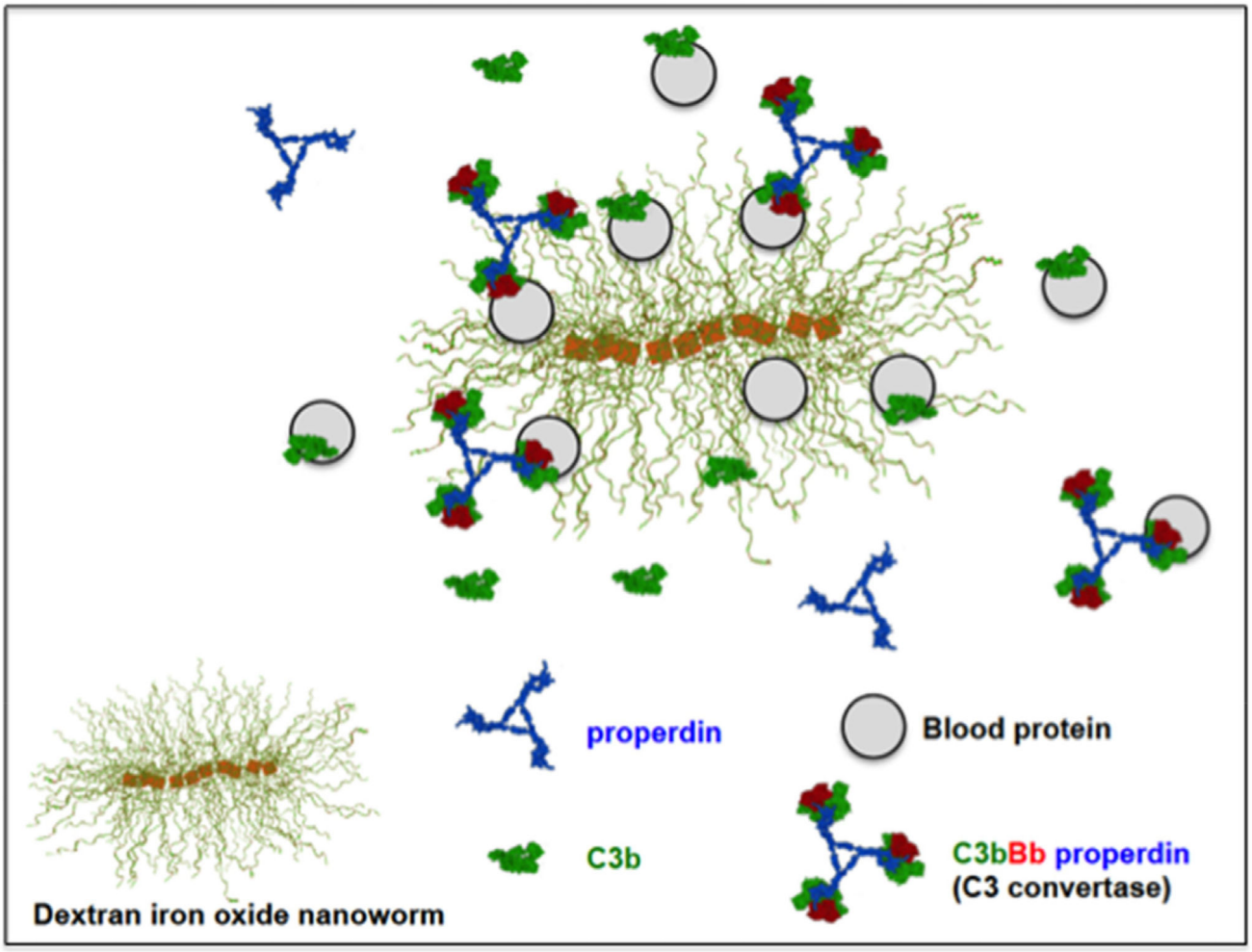

Fig. 1.

A schematic representation of the role of intercalated blood proteins in the dextran shell of iron oxide nanoworms in complement activation.