Cancer nanomedicine and the complement system activation paradigm: Anaphylaxis and tumour growth

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A wide variety of nanocarriers and particularly cancer nanomedicines activate the complement system, which is the first line of the innate immune defence mechanism. Complement activation may induce inflammatory responses, but such responses arising from uncontrolled complement activation could be life threatening. Accordingly, the role of complement in initiation of adverse reactions to particulate and polymer therapeutics is receiving increasing attention. Furthermore, the involvement of complement-activation products in promoting tumour growth has also been indicated. This could be of serious concern for development of cancer nanomedicines and cancer nanotechnology initiatives. These concepts are reviewed with preliminary evidence that intra-tumoural accumulation of model long circulating nanoparticles could promote tumour growth.

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1. Nanoparticle-mediated complement activation: general consequences

The complement system comprises soluble proteins, membrane-bound regulators and receptors, and functions as a key effector of an innate and cognate immune system [1]. The complement system uses the ‘pattern recognition’ strategy to recognize danger signals such as the conserved microbial cell wall components and carbohydrate motifs as well as synthetic polymers [1,2]. Particulate matters, depending on their physicochemical properties (size, shape, surface characteristics) and structural transformation in biological fluids, may trigger complement cascade via three distinct pathways (classical, lectin and alternative) that converge at the third component of complement (C3), and reviewed recently [2]. Many consequences arise from nanoparticle-mediated complement activation (Fig. 1). As an integral part of the innate immune system, complement triggering primes the intruders’ surface for rapid recognition and clearance by phagocytic cells. This process is well observed with many engineered particulate drug delivery systems comprising liposomes, oil-in-water emulsions, micellar systems and polymeric nano- and microparticles [2–11]. Complement activation may further induce inflammatory responses through liberation of anaphytoxins (e.g., C3a and C5a), iC3b and the lytic C5b–9 complex, but such responses arising from uncontrolled complement activation could be life threatening (Fig. 2) [2,12,13]. Furthermore, C5a is a chemoattractant species and can modulate haemostasis and contribute to disease pathogenesis through immune cell recruitment (e.g., suppressive T cells, monocytes, neutrophils) [1,14]. Numerous reports have indicated that complement activation may be a contributing factor in eliciting acute allergic-like reactions to regulatory-approved particulate and polymeric medicines, including stealth entities, in some individuals. Notable examples are the so-called cancer nanomedicines such as Doxil® and Taxol™, and these were discussed in depth recently [7,12,13]. These observations are of prime concern for future design and engineering of nanomedicines for intravenous delivery, since nanomedicines are often composed of polymeric components and other patterned nanostructures [15], which will most likely increase the probability of interaction with complement pattern recognition molecules such as C1q, C-reactive protein, ficolins, mannose binding lectin and properdin. Earlier, my laboratory provided conceptual basis for design of safer stealth vesicles for cancer therapy, where the importance of linkage chemistry as well as vesicular curvature/membrane stress in complement activation was highlighted [4,7]. Another key finding was demonstration of the unexpected and remarkable affinity of complement pattern recognition molecules for the same surface projected polymeric structural determinants, but of different conformational states and geometry [6,10]. The latter observations have underlined limitations in nanoparticle surface engineering in generating nanotherapeutics compatible with the innate immune system (or at least the complement system). Alternative interventions are therefore needed, such as integration of endogenous or designer complement inhibitors into nanoparticle formulation, but these interventions will add complexity to the design and formulation stability and could increase production costs [16–18]. Nevertheless, it is rather remarkable that only few complement pattern-recognition proteins can recognize indefinite pattern combinations. It is plausible that this process is
Fig. 1. Key consequences arising from nanoparticle-mediated complement activation. The diagram shows the likely role of key complement activation products in opsonization, anaphylaxis/inflammation, chemotaxis and adaptive defence. In relation to anaphylaxis and inflammation, complement anaphylatoxins may crosstalk with Toll-like receptor signalling pathways (TLR), and particularly with TLR-2, 4 and 9 as well as with the endotoxin receptor (CD14), and regulate cytokine production. Schematic representation of complement activation pathways is presented elsewhere [2,6,7].

Fig. 2. Symptoms of acute infusion reactions to nanomedicines and polymer therapeutics. Activation of the complement system may be a contributing factor, but not a rate limiting factor in eliciting adverse reactions to nanomedicines in sensitive individuals.

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mediated by dynamics of hydration water to a large extent. Indeed, structured water has already been shown to regulate immune recognition, where water molecules bridge the major histocompatibility complex–peptide binding site through hydrogen bonding [19]. This non-specifically allows MHC to bind a large number of epitope sequences with high affinity.

Particulate systems are receiving increasing attention to enhance the immunogenicity of subunit vaccines through both antigen protection and targeting to antigen-presenting cells as well as immunostimulation [20,21]. The latter is often mediated through a complement system, since complement activation products C3d and C3dg can induce B cell activation (Fig. 1) [22]. Indeed, C3d lowers the threshold for cognate B lymphocyte activation through interaction with complement receptor 2 in conjugation with the B cell antigen receptor complex and CD19 [22].

Contrary to the conventional belief that complement can kill cancer cells (e.g., complement-dependent cell cytotoxicity), a recent study demonstrated that intra-tumoural complement activation could help tumour growth and progression [14]. This unexpected finding has important bearings on the development and the use of cancer nanomedicines and nanotechnologies, which I discussed in 2009 [23]. Because of the leaky nature of tumour microvasculature and the lack of functional lymphatics, long-circulating cancer nanomedicines extravasate from the blood and accumulate passively at tumour interstitial spaces [24–27]. Bearing in mind that the currently available clinical cancer nanomedicines are capable of triggering complement, the outstanding issue is whether such nanoparticles can induce complement activation in the tumour tissue and subsequently promote tumour growth.

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2. Nanoparticle-mediated tumour growth: cause for concern

Generation of C5a in a tumour microenvironment was shown to enhance tumour growth by promoting the recruitment of myeloid-derived suppressor cells into malignant tumours, thereby deregulating or suppressing cytotoxic CD8+ T cells [14]. Accordingly, extravasated nanoparticles in tumour interstitium may trigger complement activation and liberate C5a, and eventually help tumour growth. To test whether complement-activating nanoparticles can induce tumour growth, three types (A, B & C) of engineered nanoparticulate systems with different surface properties were selected (Fig. 3a). This selection was based on a recently described model polystyrene nanoparticles bearing different levels of poloxamine 908 coat, which is a star-shaped block copolymer of polyethylene oxide (PEO)/polypropylene oxide (PPO). The copolymer adsorbs strongly onto the surface of polystyrene nanoparticles through its central POP segments, while allowing for PEO segments to extend from the surface [6]. As demonstrated earlier, with Type A nanoparticles (Fig. 3a) the surface projected PEO chains of the poloxamine coat assume ‘mushroom’ configuration [6]. On intravenous injection, hepatic Kupffer cells and the splenic macrophages rapidly extract circulating Type A nanoparticles (similar to uncoated nanoparticles) obtained from different regions of the poloxamine adsorption isotherm [6]. The thickness layer (δ) of the adsorbed poloxamine is shown for each nanoparticle (determined by dynamic laser light scattering) [6]. Circulation half-lives measured using [125I]-labelled polystyrene nanoparticles [6] following intravenous injection into C57BL/6 mice (0.5 mg polystyrene/animal, n = 3). Nanoparticle-mediated complement activation was performed in human serum (CP = classical pathway, AP = alternative pathway, AP(P) = alternative pathway activation through direct properdin binding, LP = lectin pathway). For full experimental details, the reader should refer to earlier publications [6,25,30]. Panel (b) shows the extent of tumour accumulation of the three types of poloxamine 908-coated nanoparticles in syngeneic male immunocompetent C57BL/6 mice (n = 3) bearing TC-1 tumour. Nanoparticles were injected intravenously at day 15 post tumour implantation and tumour accumulation of nanoparticles was determined 24 h later.

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Type A nanoparticles is short (Fig. 3a). With Type C nanoparticles, surface projected PEO chains are in ‘brush-like’ configuration, whereas Type B nanoparticles bear PEO chains in an intermediate ‘mushroom-brush’ architectural arrangement (Fig. 3a) [6]. The altered conformation of the PEO chains makes both Type B and C nanoparticles resistant to interference by the macrophages of the reticuloendothelial system and, therefore, long circulatory [6]. It was also established that the conformational states of surface-projected PEO chains modulate complement activation differently (Fig. 3a) [6]. Alteration of block copolymer architecture on nanoparticles from mushroom to brush configuration not only switches complement activation from C1q-dependent classical to lectin pathway, but also reduces the level of generated C5a [6]. Furthermore, different conformational states of surface projected PEO chains enhance alternative pathway turnover of the complement system differently [6]. Therefore, these collections of complement activating poloxamine-coated nanoparticles can serve as excellent models to test the abovementioned hypothesis. Since Type A nanoparticles are not long circulating and accumulate in tumours poorly, their administration will allow for assessing whether nanoparticle-mediated complement activation in the blood can induce tumour growth. In contrast, both Type B and C nanoparticles accumulate in tumours through extravasation (Fig. 3b), and since they activate complement differently [6], they could serve as good models for testing the tumour growth hypothesis through intra-tumoural complement activation, which in turn may also depend on the pathway of complement activation.

For tumour development syngeneic male immunocompetent C57BL/6 mice (n = 6, 8–10 weeks old) were injected with 1.5 × 10⁶ TC-1 tumour cells (American Type Culture Collection) into the right flank. The tumor cell line, TC-1, is derived from primary lung epithelial cells of C57BL/6 mice [28]. These cells are immortalized with the amphotropic retrovirus vector LXSN16E6E7 and subsequently transformed with the pVEJB plasmid expressing the activated human c-Ha-ras oncogene [28]. The transformed cells are selected with G418 and Hygromycin B and are positive for the expression of HPV-16 E7 [28].

Nanoparticle injection (0.5 mg polystyrene/animal) [29] began on day 15 when tumour volume was 74 ± 65 mm³. As shown in Fig. 4a, two injections of Type A nanoparticles had no influence on tumour growth compared with control (saline injections). Similarly, multiple injections of type A nanoparticles (on days 15, 18, 21 and 24) had no effect on tumour growth and tumour volume was comparable with control saline injections (data not shown). However, a single injection of long circulating Type B nanoparticles significantly promoted tumour growth compared with saline injection (Fig. 4b), and the tumour growth was further enhanced when animals received two injections of nanoparticles at interval of 9 days (Fig. 4c). This interval was chosen since 9–10 days after the first injection of nanoparticles, the second dose still behaves as long circulatory, while at shorter intervals (3–7 days) Type B nanoparticles will be cleared rapidly by macrophages of the liver and the splenic due to poloxamine-mediated macrophage activation [30]. Interestingly, with Type C nanoparticles, tumour growth become significant from day 24 onwards compared with control and with Type B nanoparticles (Fig. 4d). Nevertheless, it appears that nanoparticle-mediated tumour growth is dependent on tumour accumulation.

CS-deficient mice (C5KO) [31] with implanted TC-1 tumour cells were used subsequently to determine whether nanoparticle-mediated tumour growth is complement dependent. When the tumour volume was 510 ± 300 mm³, mice received two doses of Type B nanoparticles (at intervals of 9 days) and tumour volume was monitored. Nanoparticle injections had no effect on tumour volume compared with saline injections (Fig. 5a). When animals received intravenous injections of human C5 (3 μg/g body weight) 10 min before the first and second nanoparticle (or saline) injections (afterwards C5 administration continued every second day at a dose of 1.5 μg/g body weight and the experiment was terminated 15 days post injection) (Fig. 5a). When animals received intravenous injections of human C5 (3 μg/g body weight) 10 min before the first and second nanoparticle (or saline) injections (afterwards C5 administration continued every second day at a dose of 1.5 μg/g body weight and the experiment was terminated 15 days post injection) (Fig. 5a).
was detected 6 days post injection of Type B nanoparticles compared to saline injection (two injections of each in total). Afterw...n of C5 (3 μg/g body weight) 10 min before each nanoparticle or saline injections (two injections of each in total). Afterwards, C5 administration continued every second day at a dose of 1.5 μg/g body weight and the experiment was terminated two weeks after the first nanoparticle/saline injection. Panel (b) compares tumour volume between C5aR KO and the control wild-type mice (C5aR-WT), whereas panel (c) shows the effect of NP-B treatment on tumour volume in C5aR-KO mice compared with saline injection. *p < 0.05, **p < 0.01 (two-way ANOVA).

Fig. 5. The effect of nanoparticle injection on TC-1 tumour volume in C5 knock-out (CSK0) and C5a receptor knock-out (CSaR-KO) mice. Panel (a) shows tumour volume in mice that received two doses of saline or Type B nanoparticles, NP-B, (nanoparticle description is shown in Fig. 3) with 9 day interval. Injections were performed when TC-1 tumour volume was 510 ± 300 mm³. Finally, tumour volume was measured 15 days after the first injection. In a second set of experiments animals received intravenous injections of human C5 (3 μg/g body weight) 10 min before each nanoparticle or saline injections (two injections of each in total). Afterwards, C5 administration continued every second day at a dose of 1.5 μg/g body weight and the experiment was terminated two weeks after the first nanoparticle/saline injection. Panel (b) compares tumour volume between C5aR-KO and the control wild-type mice (C5aR-WT), whereas panel (c) shows the effect of NP-B treatment on tumour volume in C5aR-KO mice compared with saline injection. *p < 0.05, **p < 0.01 (two-way ANOVA).
layer(s) of the adsorbed poloxamine is shown for each nanoparticle (determined by dynamic laser light scattering) [6]. Circulation half-lives were measured using [125I]-labelled polystyrene nanoparticles [6] following intravenous injection into C57BL/6 mice (0.5 mg polystyrene/animal, n = 3). Nanoparticle-mediated complement activation was performed in human serum (CP = classical pathway, AP = alternative pathway, AP(P) = alternative pathway activation through direct properdin binding, LP = lectin pathway). For full experimental details, the reader should refer to earlier publications [6,29,30]. Panel (b) shows the extent of tumour accumulation of the three types of poloxamine 908-coated nanoparticles in syngeneic male immunocompetent C57BL/6 mice (n = 3) bearing TC-1 tumour. Nanoparticles were injected intravenously at day 15 post tumour implantation and tumour accumulation of nanoparticles was determined 24 h later.

Nanoparticles (0.5 mg/20 g body weight) were injected intravenously at designated sites into syngeneic male immunocompetent C57BL/6 mice (n = 6) bearing TC-1 tumour. Control animals received saline (same volume as nanoparticles). NP-A, NP-B and NP-C refer to poloxamine 908-coated Type A, B and C nanoparticles as shown in Fig. 3. *p < 0.05; **p < 0.01 (two-way ANOVA).

Panel (a) in Fig. 5 shows tumour volume in mice that received two doses of saline or Type B nanoparticles, NP-B. (nanoparticle description is shown in Fig. 3) with 9 day interval. Injections were performed when TC-1 tumour volume was 510 ± 300 mm³. Finally, tumour volume was measured 15 days after the first injection. In a second set of experiments animals received intravenous injections of human CS (3 μg/g body weight) 10 min before each nanoparticle or saline injections (two injections each in total). Afterwards, CS administration continued every second day at a dose of 1.5 μg/g body weight and the experiment was terminated two weeks after the first nanoparticle/saline injection. Panel (b) compares tumour volume between C5aR-KO and the control wild-type mice (C5aR-WT), whereas panel (c) shows the effect of NP-B treatment on tumour volume in C5aR-KO mice compared with saline injection. *p < 0.05, **p < 0.01 (two-way ANOVA).

3. Greater implications and need for detailed fundamental research

More supporting information is still necessary to confirm a role for nanoparticle (particularly with long circulating liposomes)-mediated intra-tumoural complement activation in tumour growth. Most importantly, the role of infiltrating suppressive T cells as well as other immune cells such as monocytes and neutrophils in augmenting tumour growth must be mapped out and correlated with C5a liberation threshold. The current observations were based on model nanoparticles and in the absence of cytotoxic agents. Tumour responses may differ and could be far more complex with long circulating nanoparticles containing cytotoxic agents and polymer therapeutics (a wide range of polymers also incite complement). Following extravasation from the blood and accumulation in tumour interstitium, particulate cancer nanomedicine may gradually release their cargo and initiate tumour killing through apoptosis, necrosis, programmed necrosis as well as other modes of cell death processes. The type and the mode of cell death may further modulate tumour homeostasis by controlling immune responses and hence the overall therapeutic response. Indeed, apoptotic cells activate complement via various molecular mechanisms [34,35]. For instance, complement binding by apoptotic cells in normal human plasma occurs mainly to late apoptotic, secondary necrotic cells, and that the dominant mechanism involves classical pathway activation by IgM [34]. Others have shown direct binding of properdin to apoptotic T cells, which results in initiation of complement activation [35]. If intra-tumoural complement activation can induce tumour growth, then we should seriously consider whether cancer nanomedicines eventually shift the balance in favour of tumour growth (particularly in micrometastases and low-pressure regions in larger tumours) following intra-tumoural complement activation either directly or following cancer cell death or both. This provocative hypothesis may partly explain why cancer nanomedicines have shown rather disappointing efficacy with limited clinical progress [36]. The complement paradigm seems to add more complexity to strategies by which tumours can fend off body’s defences and improve their own survival. More complications may arise from the interplay between the complement and the coagulation cascades, whereby the complement system can amplify coagulation by enhancing local clotting [37,38]. Indeed, many human tumours are rich in blood clots. The activated clotting Factor XII can activate the classical complement pathway, and thrombin can directly cleave the third and fifth complement components (C3 and C5), respectively, thereby generating more chemoattractant C5a [37,38]. The effect of nanocarrier components on blood clotting pathways must therefore be considered and evaluated in detail. The scientific community should consider and evaluate these possibilities using appropriate and validated animal models, bearing in mind that a good fraction of inbred mice strains are deficient in selected complement proteins [39]. Future strategies, however, may embrace on therapeutic function of complement inhibition (e.g., C3a receptor antagonists) in the treatment of cancer [14,40]. Nanoparticles are expected to play important roles in development of functional and safe cancer medicines. These advances, however, must be based on detailed understanding of integrated biological processes, including those at the interface of tumour biology-immune system, and their rational translation, and should not be influenced by market forces and accelerated demands [41,42].

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