Journal of Controlled Release 146 (2010) 175–181

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Complement activation cascade triggered by PEG–PL engineered nanomedicines and carbon nanotubes: The challenges ahead

S.M. Moghimi [a,*], A.J. Andersen [a], S.H. Hashemi [a], B. Lettiero [a], D. Ahmadvand [a], A.C. Hunter [b], T.L. Andresen [c], I. Hamad [d], J. Szebeni [e]

[a] Centre for Pharmaceutical Nanotechnology and Nanotoxicology, Department of Pharmaceutics and Analytical Chemistry, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark
[b] Molecular Targeting and Polymer Toxicology Group, School of Pharmacy, University of Brighton, Brighton BN2 4GJ, UK
[c] Department of Micro- and Nano-Technology, DTU Nanotech, Technical University of Denmark, Frederiksborgvej 399, DK-4000 Roskilde, Denmark
[d] Department of Pharmaceutical Sciences and Pharmaceutics, Faculty of Pharmacy, Applied Sciences University, Amman, Jordan
[e] Nanomedicine Research Centre, Bay Zoltán Foundation for Applied Research, Semmelweis Medical University and Seroscience Kft, Budapest, Hungary

ARTICLE INFO

Article history:
Received 2 February 2010
Accepted 4 April 2010
Available online 11 April 2010

Keywords:
Carbon nanotube
Complement activation
Liposome
Micelle
Poly(ethylene glycol)
Pseudoallergy

ABSTRACT

Since their introduction, poly(ethylene glycol)–phospholipid (PEG–PL) conjugates have found many applications in design and engineering of nanosized delivery systems for controlled delivery of pharmaceuticals especially to non-macrophage targets. However, there are reports of idiosyncratic reactions to certain PEG–PL engineered nanomedicines in both experimental animals and man. These reactions are classified as pseudoallergy and may be associated with cardiopulmonary disturbance and other related symptoms of anaphylaxis. Recent studies suggest that complement activation may be a contributing, but not a rate limiting factor, in eliciting hypersensitivity reactions to such nanomedicines in sensitive individuals. This is rather surprising since PEGylated structures are generally assumed to suppress protein adsorption and blood opsonization events including complement. Here, we examine the molecular basis of complement activation by PEG–PL engineered nanomedicines and carbon nanotubes and discuss the challenges ahead.

1. Introduction

MethoxyPEG$_{2000-5000}$–phosholipid (mPEG–PL) conjugates are versatile molecules with numerous biomedical applications. These conjugates were initially incorporated into the liposomal bilayer for conferring longevity to vesicles in the systemic circulation; this eventually led to the development of Doxil® approved for treatment of HIV-related Kaposi's sarcoma and refractory ovarian carcinoma [1–3]. PEGylated vesicles, through prolonged circulation times in the blood, can ultimately target many vascular elements following conjugation of targeting ligands to the distal end of the projected PEG chains bearing a reactive functional group [1,2,4]. Other attempts include coupling of contrast agents and bio-sensors to the distal end of the PEG chain for engineering of multifunctional vesicles applicable to a wide range of pathologies [2,4–6].

PEG–phospholipid conjugates exhibit low critical micelle concentration values and form stable micellar structures of approximately 30 nm in size, allowing drug solubilization and are amenable for additional functionalization and parenteral administration [7]. The application of PEG–phospholipid conjugates in steric stabilization and generation of long circulating oil-in-water nanoemulsions is also noteworthy [8]. Finally, apart from nanomedicine design and development, PEG–phospholipids can further stabilize entities such as carbon nanotubes through surface adsorption and enhance their dispersion in aqueous media; such modifications also affect nanotube pharmacokinetics following intravenous injection for biological and mechanistic studies [9].

There are indications that certain PEG–phospholipid bearing nanomedicines may be Janus-faced; today, there are clinical reports of acute hypersensitivity reactions to infusion of long circulating regulatory-approved PEGylated liposomes in sensitive individuals [10–12]. These reactions, classified as pseudoallergy, are often associated with flushing and circulatory disturbances [13]. Recent studies in pigs have suggested that the cardiopulmonary distress caused by Doxil® and other liposomes strongly correlate with complement activation [14]. The complement system (Fig. 1), consisting of over 30 soluble plasma and cell-surface bound proteins, serves as an important effector of both innate and acquired immunity [15]. Accordingly, complement activation by PEGylated vesicles is an unexpected phenomenon since surface mPEG coverage is generally believed to dramatically suppress particle–protein interaction and blood opsonization events, including complement fixation [1,16]. Similar to the binding of allergens to IgE on the surface of mast cells and basophils, complement anaphylatoxins (and particularly C5a) can

* Corresponding author. Tel.: +45 35336528.
E-mail address: momo@farma.ku.dk (S.M. Moghimi).

0168-3659/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2010.04.003

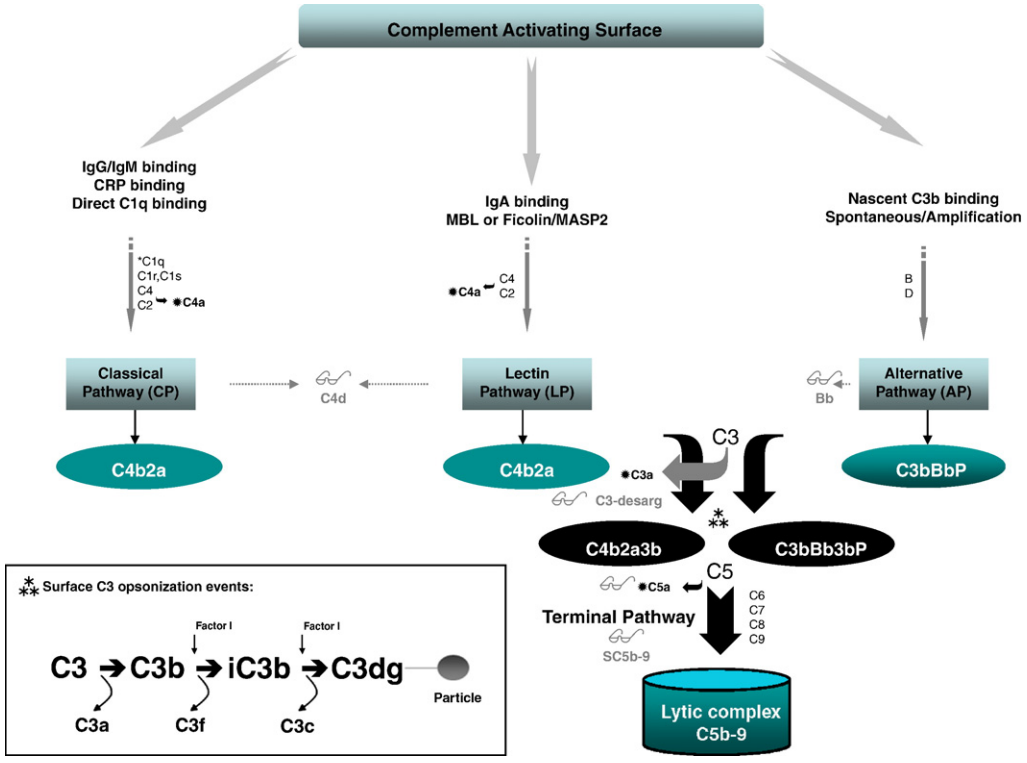

Fig. 1. Complement activation pathways. C4d, Bb and SC5b-9 are established activation markers (⌒) for calcium-sensitive (CP and LP), AP and terminal pathways, respectively. Activation markers for anaphylatoxins (✸) are also shown.

trigger immediate release of a plethora of pro-inflammatory mediators from these cells and macrophages. This cascade of secondary mediators substantially amplifies effector immune responses and may induce anaphylaxis in sensitive individuals [13,17]. In addition to PEGylated vesicles, unexplained acute adverse reactions such as ataxia, restlessness and trembling, respiratory abnormalities, frothing at the mouth, collapse and even death have also surfaced in veterinary scenarios (cattle, sheep and swine) following administration of intravenous medicines containing high PEG content [18]. Anaphylaxis has further been reported in some patients and animals who have received intravenous formulations containing the block co-polymer poloxamer 188, which is structurally similar to PEG [19,20]. Remarkably, these polymers (at concentrations relevant to their designated applications) also activate the complement system [18,20]. These observations raise the question as to what extent PEG and PEG–PL aggregates or micelles could initiate complement activation; it is of note that micelles are usually present in PEGylated liposomal preparations.

It is the purpose of this article to examine the molecular basis of complement activation by PEG–PL engineered nanomedicines and other entities (e.g., PEGylated carbon nanotubes), highlighting the modulatory role of interfaces in which PEG–PL molecules are located, and discuss the challenges ahead.

2. The porcine model of anaphylaxis

Intravenous administration of minute amounts of Doxil® (0.1 mg lipid/kg body weight) induces reproducible haemodynamic changes and ECG alterations in the porcine model as depicted in Fig. 2a. These include an abrupt drop in systemic arterial pressure (SAP) that is associated with massive pulmonary hypertension, decreased cardiac output and decreased end-tidal PCO_2 [14]. During the nadir of the blood pressure curve (lasting ~4 min), a transient tachyarrhythmic episode followed by ST depression and T-wave elevation is noticeable. However, the ECG is normalized after 12–15 min. A more severe reaction (a deeper and longer hypotensive period) occurs by doubling the Doxil® dose (Fig. 2a). An interesting feature of the systemic pressure response is that the reduction of systolic pressure is greater compared with that of diastolic pressure, resulting in a substantial reduction of pulse pressure amplitude. These changes can be associated with severe bradycardia (which is not of sinus origin), arrhythmia and the presence of incomplete as well as complete atrioventricular block with asystole. The ECG traces suggest that the bradycardia is a reflection of slowed atrioventricular conduction. Since the physiological baroreflex to hypotension is tachycardia, the bradycardia associated with hypotension represents 'paradoxical bradycardia'. Follow up studies suggested that acute adenosine release within the heart could explain the paradoxical bradycardia via A_1 receptors [14]. Larger liposome doses (0.5 mg lipid/kg body weight) are lethal in the porcine model; ventricular fibrillation and cardiac arrest occurs within 3 min of liposome injection [14].

Activation of mast cells in the coronary arterial intima, and perivascularly, in close proximity to myocytes can induce cardiac anaphylaxis [21,22]. These cells express high-affinity receptors for anaphylatoxins C3a and C5a, and triggering of anaphylatoxin receptors induces the release of a variety of inflammatory mediators and vasoactive molecules from mast cells [13]. For instance, eicosanoid release (particularly thromboxane A2) could induce pulmonary and coronary vasoconstriction, which may be combined with microthrombus formation and microembolization of capillaries by neutrophil–platelet aggregates [13]. In addition, C3a can further activate platelets, enhancing their aggregation and adhesion, but C5a is 100-fold more potent than C3a, and enhances blood thrombogenicity through upregulation of tissue factor and plasminogen activator inhibitor-1 expression on various cell types [23,24]. Overall, the resultant falls in cardiac preload and coronary flow lead to myocardial ischemia, decreased contractibility and reduced cardiac output and hypotension. If not resolved spontaneously, this may lead to circulatory collapse and death [14].

Recent studies have provided further evidence for the role of complement-derived anaphylatoxins in Doxil®-induced anaphylaxis

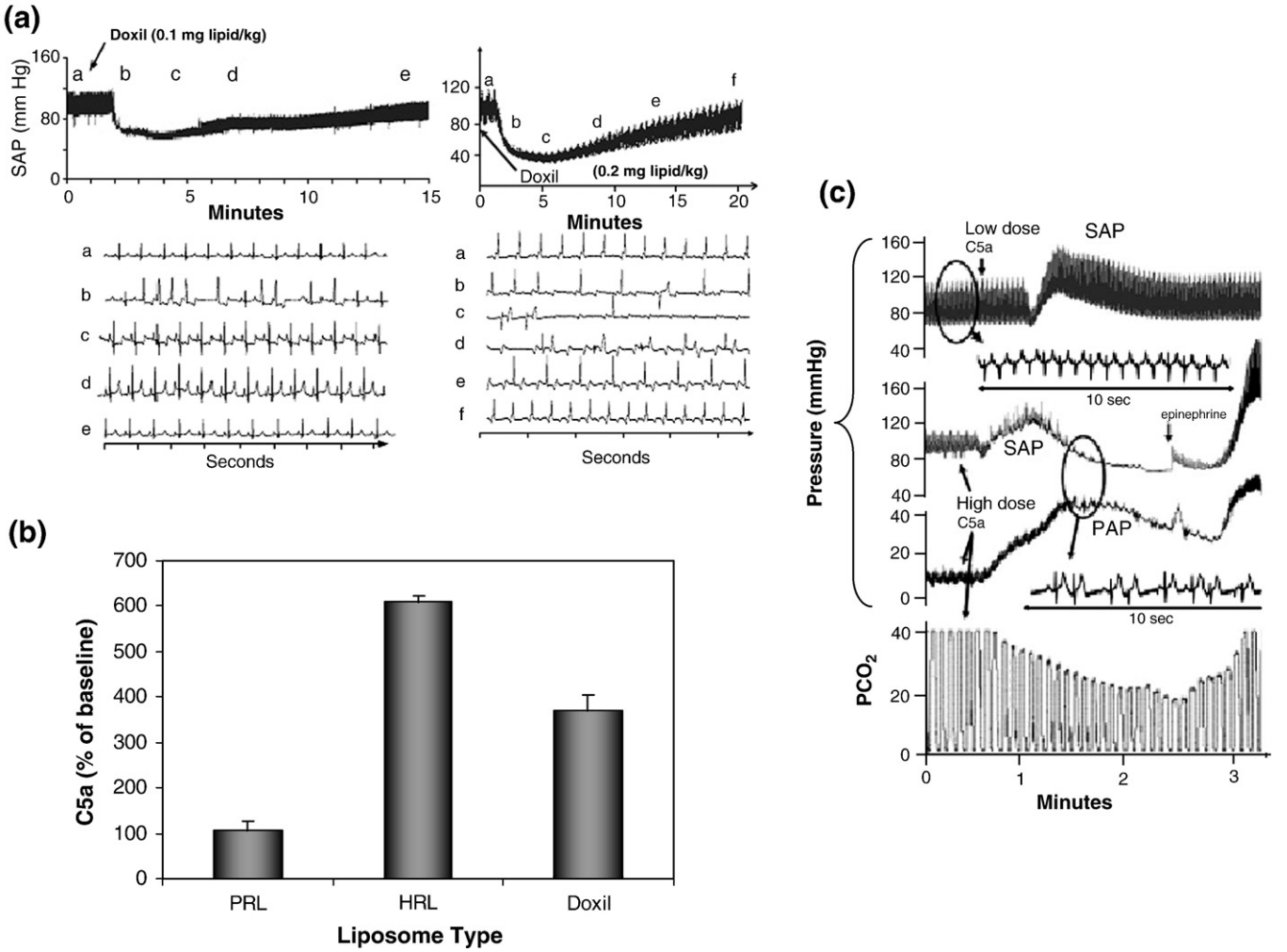

Fig. 2. Cardiopulmonary and electrocardiogram changes in pigs after bolus injection of Doxil® (a) or recombinant human C5a (c) and C5a production in pig plasma following liposome challenge (b). SAP = systemic arterial pressure; PAP = pulmonary arterial pressure; PRL = standard poorly reactive liposomes (they do not induce significant haemodynamic and cardiorespiratory abnormalities) in pigs; HRL = highly reactive liposomes (multilamellar vesicles formed from DMPC:DMPG:cholesterol, mole ratios 50:5:45 that induce dramatic and extended declines in SAP, pulse amplitude, end-tidal PCO_2 and major rise in PAP) in pigs.
Modified after Szebeni et al. [14] with permission.

[14]. Firstly, in heparinized (10 IU/ml, which has no major impact on controlling complement activation) pig plasma, Doxil® addition was able to elevate C5a levels dramatically above the baseline level (Fig. 2b), thus confirming complement activation [14]. In comparison, multilamellar vesicles composed of DMPC:DMPG:cholesterol (50:5:45 mole ratios) not only generated more C5a in the pig serum than Doxil® treatment (Fig. 2b), but also induced more severe cardiac abnormalities in the porcine model that were similar to those induced by administration of zymosan (a potent complement-activating agent). These observations support the notion that the anaphylatoxin C5a plays a causal role in the cardiac abnormalities caused by liposome administration, and severe liposome reactions may involve a considerable rise of plasma C5a in pigs. Indeed, bolus administration of recombinant human C5a (rhC5a) at a dose of 330 ng/kg (normal pig plasma C5a level is ~20 ng/ml and the plasma volume is ~33 ml/kg) led to a mild reaction with transient reduction of pulse pressure and slight reversible hypertension (Fig. 2c). In sharp contrast, higher doses of rhC5a (e.g., equivalent to 600- to 700-fold higher than the baseline C5a level in pig) caused a short-lived transient hypertension followed by massive hypotension in association with bradyarrhythmia, pulmonary hypertension and marked decrease of end-tidal PCO_2 (Fig. 2c). This treatment mimicked the severe cardiac abnormalities associated with multilamellar vesicles and zymosan. Furthermore, the severity of liposome-induced cardiac abnormalities in the porcine model can be significantly reduced with complement inhibitors such as the recombinant truncated soluble form of complement receptor type-1 and anti-porcine C5a antibody GS1 [14]. However, we cannot exclude a possible and significant role for other complement-derived anaphylatoxins (e.g., C3a) in the most severe or lethal reactions.

3. Doxil®-mediated complement activation in human serum

Doxil® was shown to trigger complement and generate complement opsonic fragments from radiolabelled C3 in human serum [16,25]. As shown in Fig. 3 C3b deposition and degradation (65 and 40/43 kDa fragments) reach the plateau within 5 min. This further confirms that surface-bound mPEG molecules do not interfere with C3b inactivation by factors H and I [16]. Another notable feature is generation of high molecular weight C3b- and iC3b-containing complexes (C3-X), a phenomenon typical of complement activation by immune aggregates. Doxil® has also been shown to activate complement in 21 out of 29 cancer patients, as reflected by significant elevation of SC5b-9 (the terminal complex activation marker of complement system) levels in plasma within 10–30 min of infusion [12]. In this study [12], acute allergic reactions were reported in 13 patients, where 12 had elevated

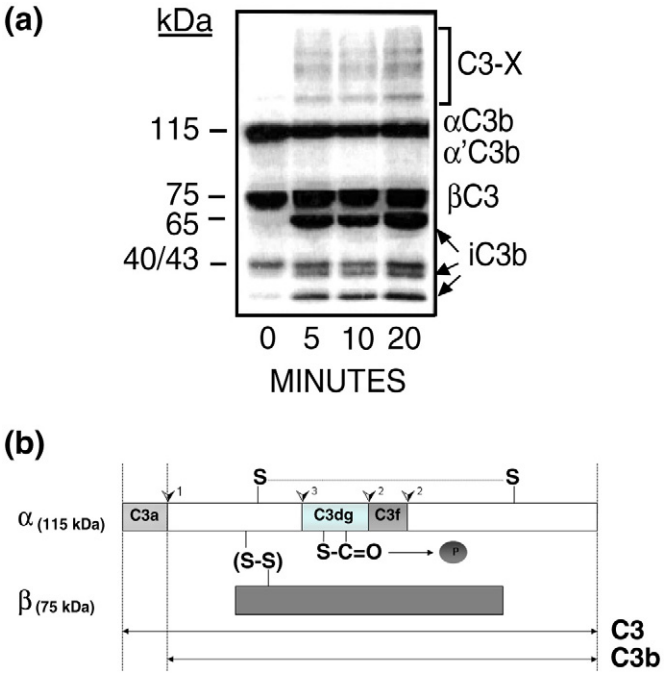

Fig. 3. Doxil®-mediated complement activation in human serum. For clarity and interpretation of the SDS-PAGE profile in (a), the schematic structure of C3 is also presented (b). C3 convertases initially cleave the α chain at arrow head 1 (b), generating anaphylatoxin C3a and a conformationally changed C3b fragment (α′ chain and the uncleaved β chain), where the reactive thiolester moiety in C3dg portion can form covalent bonds with the complement-activating surface/particle P. Only a small portion of C3b binds to the complement-activating surface and the thiolester of the majority of C3b reacts with water to form iC3b. C3b is sequentially catalyzed (two stages) by factor I (arrow heads 2 and 3), resulting in the release of C3f (and the formation of iC3b) and C3c. This leaves C3dg bound to the target (see also Fig. 1).
Modified after Moghimi and Szebeni [16] with permission.

plasma SC5b-9 levels. However, among the 16 non-responding individuals, 9 had elevated plasma SC5b-9 levels.

Vesicles of the same size and composition as Doxil®, but without encapsulated doxorubicin, also trigger complement in human serum through both classical and alternative pathways, but the extent of complement activation is less than that of Doxil® [25]. The mechanisms of complement activation by PEGylated liposomes, however, are poorly understood. Surface projected mPEGs may trigger complement through the classical pathway in the presence of reactive antibodies to PEG. Indeed, some 25% of the general populations are believed to be positive for IgG2 and IgM antibodies that recognize four to five repeat ethoxy units [26,27]. In our hands, none of the tested human sera were positive for anti-PEG antibodies, therefore complement activation by PEGylated liposomes can proceed in the absence of such antibodies. Nevertheless, anti-PEG antibodies through complement activation may play an important role in liposome clearance from the blood in protocols involving repeated administration of PEGylated vesicles [28,29].

Alternatively, PEG may act directly on complement proteins (e.g., C3) or indirectly through water activity to further enhance fluid phase complement turnover [18]. It is well established that the extent of water clustering increases with PEG size; the hydration increases from two molecules of water per PEG monomer for a tetramer to five molecules of water per PEG monomer for 45-mer [30,31]. Therefore, if C3 binds to or become trapped between surface PEG 'brushes', C3 hydration and conformational changes ("C3 tickover") may become accelerated, leading to the assembly of fluid phase C3Bb convertases (Fig. 1). However, our studies with endotoxin free near monodispersed PEG_{2000} at a final concentration of 2.5 mg/ml in serum (this PEG concentration far exceeds that of surface attached PEG in complement activation studies with PEGylated liposomes) did not raise serum levels of complement activation products C4d (a marker of calcium-sensitive classical and lectin pathways), Bb (a marker of the alternative pathway) and SC5b-9 above the background [32]. However, complement activation by PEG is concentration-dependent; for PEG_{2000} this translates to a concentration of ≥10 mg/ml but with higher molecular weight PEGs complement activation proceeds with lower concentrations [18]. The role of alternative pathway and lectin pathway in PEG-mediated triggering of complement cascade has been demonstrated [18]. These observations are therefore relevant to scenarios where intravenous PEG is used as a therapeutic agent; examples include spinal cord injury and traumatic axonal brain injury, where PEG is believed to seal the membrane of damaged axons through membrane fusion [33,34].

We further determined that mPEG–PL conjugates in micellar form were incapable of activating complement [32]. Thermodynamically, micellar solution is at equilibrium, the concentration of monomers being equal to the critical micelle concentration. This further suggests that mPEG–PL monomers are also ineffective in triggering complement. Remarkably, methylation of the phosphate-oxygen moiety of mPEG–lipid prevented complement activation by PEGylated vesicles [32]. This is illustrated in Fig. 4 with mPEG–prodrug ether lipid conjugate-based liposomes of different sizes and is further confirmed in vivo, using the rat model. Methylation not only removes the anionic charge on the phosphate-oxygen (anionic liposomes, and depending on their phospholipid head group, are complement activators [15,35]), but may also sterically block the simultaneous binding of naturally occurring anti-phospholipid antibodies to both liposomal phospholipid head group and the phosphate-oxygen moiety of the PEGylated conjugate. Additionally, methylation may interfere with spatial organization of surface-bound antibodies for correct recognition by the three modules of the globular C1q domain. On the basis of these observations, we ascribe the inability of mPEG–PL micelles to activate complement to their small hydrodynamic size and other geometrical factors that restrict the surface assembly of complement convertases. Indeed, complement convertases are in a similar size range (~20 nm) to these micelles. In marked contrast to this statement, we have also shown complement activation by poloxamer 188 and 407 micelles, which share similar size ranges to mPEG–PL micelles [16,20]. We are currently investigating the responsible mechanisms with these micelles. Collectively, these observations demonstrate the importance of interface characteristics, in which PEG–PL molecules are accumulated, resulting in triggering the complement cascade. Therefore, it would be interesting to examine the complement-activating properties of methylated mPEG–PL incorporated liposomes containing anionic phospholipids.

Complement-fixed PEGylated liposomes interact poorly with macrophage complement receptors (CRs) [32]. This is most likely due to steric hinderance by the projected mPEG chains to the binding of C3b- and iC3b-opsonized vesicles to their corresponding receptor (CR3) [32]. This process is most likely responsible for the blood longevity of PEGylated liposomes, which are opsonized.

4. Complement policing of carbon nanotubes

There is interest among material scientists to utilize non-biodegradable carbon nanotubes for site-specific drug delivery (as in targeting of solid tumours and nucleic acid delivery) and other biological interventions, which partly arise from their unique spectroscopic and thermal properties [36]. These entities can be single-walled or multi-walled, but for biological studies carbon nanotubes must be solubilized. This may be achieved either by covalent functionalization of their surface with different chemical groups or by adsorption of amphiphilic molecules, such as PEG–lipids.

Non-functionalized carbon nanotubes, whether single- or multi-walled, are activators of the human complement system [37]. For instance, with single-walled entities complement activation is exclusively due to adsorption of C1q and is independent of antibodies

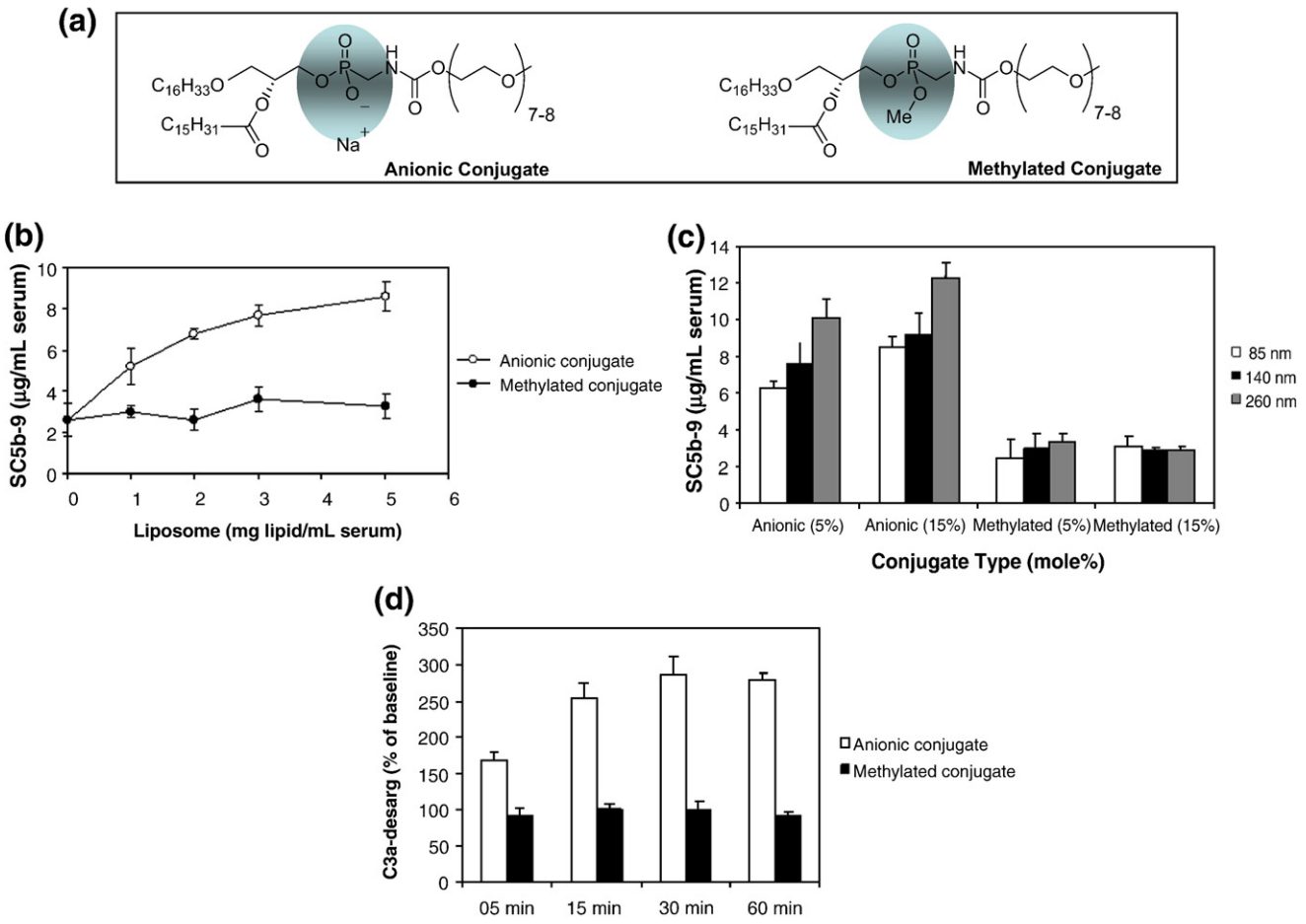

Fig. 4. Effect of PEGylated liposome composition and size on complement activation in human serum. Liposomes were composed of DPPC and designated mPEG–lipids (a) in molar ratios of 95:5, unless stated otherwise.
Modified after Moghimi et al. [32] with permission.

[37]. Complement activation also occurs with covalently functionalized nanotubes (e.g., ε-caprolactam or L-alanine functionalized) albeit to a lesser extent when compared with non-functionalized entities (uncoated nanotubes) [38].

Coating of carbon nanotubes (250 nm in length and 1–5 nm in width) with PEG_{5000}–phospholipid conjugates affords excellent solubilization and stability in biological milieu [9]. These non-biodegradable entities potentially provide another interesting platform/model to assess the role of PEG–PL interfaces in modulating the activity of complement system and compare them to PEGylated nanomedicines. Accordingly, through a series of functional studies involving healthy and C1q-depleted sera we showed that PEG–PL-stabilized carbon nanotubes can activate the human complement system through lectin pathway (Fig. 5). Indeed, nanotube-mediated complement activation was blocked by N-acetylglucosamine, a substrate for mannose-binding lectin (MBL) and ficolin, as well as in the presence of antibodies against MBL-associated serine protease-2, MASP-2 (the zymogen associated with MBL/ficolin) [39]. Mixed responses in complement activation were noted in rats following nanotube administration (Fig. 5). These observations were based on thromboxane B2 measurements (an established and direct marker for thromboxane A2) [39]. Complement anaphylatoxins induce thromboxane A2 release from blood cells and demonstration of increased serum thromboxane B2 levels provides evidence of in vivo complement activation. The observed mixed responses may be due to differences in the blood concentration of lectin pathway components (e.g., mannose-binding lectin and/or ficolin; MASP-2 activity) among different rats. Nevertheless, our findings [39] are consistent with deposition of a significant fraction of intravenously injected PEGylated carbon nanotubes in hepatic Kupffer cells [9], which is presumably complement-mediated. Finally, the demonstrated adjuvanticity of carbon nanotubes (whether uncoated or surface-modified) [40] is also consistent with their complement-activating nature, bearing in mind that C3 split-products like C3d can induce B lymphocyte activation [41].

The exact mechanism for PEG–lipid stabilized carbon nanotubes-mediated triggering of lectin pathway remains unclear. Both mannose-binding lectin and ficolin express affinity for sugars with N-acetylated groups, but these structures are absent from native nanotubes. We suggest that complement activation either arises from direct binding of mannose-binding lectin and/or ficolin to unprotected regions of PEGylated nanotubes (and indeed, atomic force microscopy studies have indicated that surface coating is not homogenous [39], Fig. 6) or indirectly via adsorption of mannose-rich serum components such as certain apo-lipoproteins. Our recent studies (unpublished observations) have further shown that nanotubes with covalently grafted mPEG molecules can still trigger the complement cascade and therefore resolving nanotube-mediated complement activation remains a challenge.

5. Concluding remarks

We have noted three modes of complement-related responses with PEG–phospholipid engineered entities (Fig. 6) and discussed the likely driven forces and interfacial determinants that trigger complement. Comprehensive mapping of these events is expected to pave the way toward design and development of immunologically safer

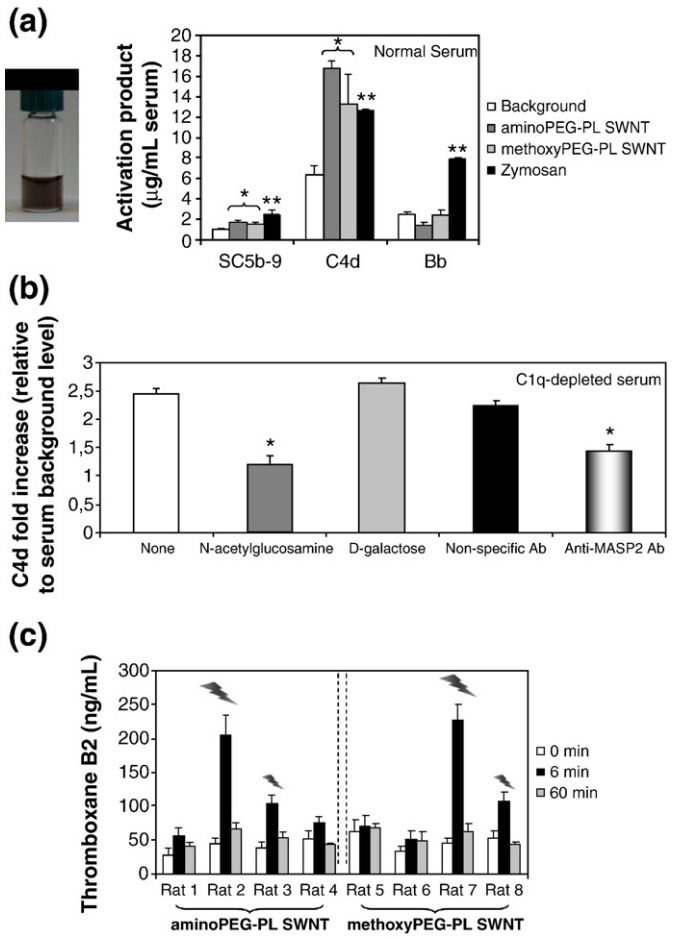

Fig. 5. Complement activation by single-walled carbon nanotubes (SWNT) in human serum and in vivo in rats. Detection of complement activation markers following nanotubes challenge is shown in (a) and the role of lectin pathway is depicted in (b) where nanotube-mediated complement activation was examined in C1q-depleted serum in the presence of designated additives. Nanotube-mediated generation of thromboxane B in rats is represented in (c).
Modified after Hamad et al. [39] with permission.

nanomedicines for clinical use. This has already begun with liposomes and micellar systems; from nanoengineering and 'structure–activity' relationship studies to clinical practice (i.e., dosing regimen, infusion time, and desensitization with placebos). Indeed, mPEG–PL micelles seem to have an immunological safety edge for biomedical and therapeutic applications as they do not activate human complement; however, pharmaceutical aspects must also receive equal considerations in designing viable formulations. We are also paying attention to additional biological and design considerations that may trigger complement in different body compartments [42,43] using a wide range of bionanotechnology tools and methodologies such as quartz crystal microbalance [44]. Our attempts include the pathology and microenvironmental factors regulating local complement activation (e.g., as in solid tumours and Alzheimer's disease), and also the effect of coupling of potentially complement-activating ligands (such as monoclonal antibodies and virally-derived peptides) to both PEGylated and non-PEGylated nanomedicines.

Resolution of carbon nanotube-mediated complement activation is largely hindered by the poorly-defined surfaces of nanotubes and lack of their reproducible production [45]. However, a clear understanding of molecular mechanisms that orchestrate complement activation by both native and surface-modified carbon nanotubes will have impact in the nanotoxicology field. Indeed, complement activation may be relevant to reported pro-inflammatory reactions following environmental exposure to carbon nanotubes and related carbon particles and fibers [46–48].

Clinical evidence from PEGylated liposome administration clearly attests that complement activation per se cannot solely explain vesicle-induced pseudoallergy. The involvement of other biological factors in pseudoallergy must not be ignored and should be investigated. Among many factors, there may be genomic differences between sensitive and non-sensitive individuals and future immunogenomic studies are expected to have an impact in pseudoallergy research. However, parallel development of in vitro tests is still needed to differentiate between immunologically (at least from complement immunology point of view) "potent" and "safe" nanomedicines and/or sensitive and non-responding individuals [17]. This is especially relevant to clinical scenarios where cardiovascular stress is not acceptable.

Acknowledgments

Scientific contributions by many collaborators throughout the years are indeed acknowledged. We also gratefully acknowledge financial support from the following bodies:

The Danish Agency for Science, Technology and Innovation (Det Strategiske Forskningsråd, reference 2106-08-0081).
European Commission FP7, reference CP-IP 212043-2 NAD.
Faculty of Pharmaceutical Sciences, University of Copenhagen, for the PhD Studentship Award to AJA.
The National Office for Research and Technology (NKTH), Budapest, Hungary, grants NKFP 01 1-CARPA777, TUDA-1-2006-0035 and NanoMedi.

References

[1] S.M. Moghimi, A.C. Hunter, J.C. Murray, Long-circulating and target-specific nanoparticles: theory to practice, Pharmacol. Rev. 53 (2001) 283–318.
[2] S.M. Moghimi, A.C. Hunter, J.C. Murray, Nanomedicine: current status and future prospects, FASEB J. 19 (2005) 311–330.
[3] T.M. Allen, P.R. Cullis, Drug delivery systems: entering the mainstream, Science 303 (2004) 1818–1822.
[4] V.P. Torchilin, Recent advances with liposomes as pharmaceutical carriers, Nat. Rev. Drug Discov. 4 (2005) 145–160.
[5] S.M. Moghimi, T. Kissel, Particulate nanomedicines, Adv. Drug Deliv. Rev. 58 (2006) 1451–1455.
[6] V.P. Torchilin, Multifunctional nanocarriers, Adv. Drug Deliv. Rev. 58 (2006) 1532–1555.
[7] V.P. Torchilin, Structure and design of polymeric surfactant-based drug delivery systems, J. Control. Rel. 73 (2001) 137–172.
[8] F. Liu, D. Liu, Long-circulating emulsions (oil-in-water) as carriers of lipophilic drugs, Pharm. Res. 12 (1995) 1060–1064.
[9] Z. Liu, C. Davis, W. Cai, L. He, X. Chen, H. Dai, Circulation and long-term fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 1410–1415.
[10] B. Uziely, S. Jeffers, R. Isacson, K. Kutsch, D. Wei-Tsao, Z. Yehoshua, E. Libson, F.M. Muggia, A. Gabizon, Liposomal doxorubicin: activity and unique toxicities during two complementary phase I studies, J. Clin. Oncol. 13 (1995) 1777–1785.
[11] D.S. Alberts, D.J. Garcia, Safety aspects of pegylated liposomal doxorubicin in patients with cancer, Drugs 54 (1997) 30–45 Suppl.
[12] A. Channan-Khan, J. Szebeni, S. Savay, L. Liebes, N.M. Rafique, C.R. Alving, F.M. Muggia, Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions, Annal. Oncology 14 (2003) 1430–1437.
[13] J. Szebeni, Complement activation-related pseudoallergy: a new class of drug-induced acute immune toxicity, Toxicology 216 (2005) 106–121.
[14] J. Szebeni, L. Baranyi, S. Savay, M. Bodo, J. Milosevits, C.R. Alving, R. Bunger, Complement activation-related cardiac anaphylaxis in pigs: role of C5a anaphylatoxin and adenosine in liposome-induced abnormalities in ECG and heart function, Am. J. Physiol. Heart Cir. Physiol. 290 (2006) H1050–H1058.
[15] S.M. Moghimi, I. Hamad, Liposome-mediated triggering of complement cascade, J. Liposome Res. 18 (2008) 195–209.
[16] S.M. Moghimi, J. Szebeni, Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties, Prog. Lipid Res. 42 (2003) 463–478.
[17] J. Szebeni, S.M. Moghimi, Liposome triggering of innate immune responses: a perspective on benefits and adverse reactions, J. Liposome. Res. 19 (2009) 85–90.
[18] I. Hamad, A.C. Hunter, J. Szebeni, S.M. Moghimi, poly(ethylene glycol)s generate complement activation products in human serum through increased alternative

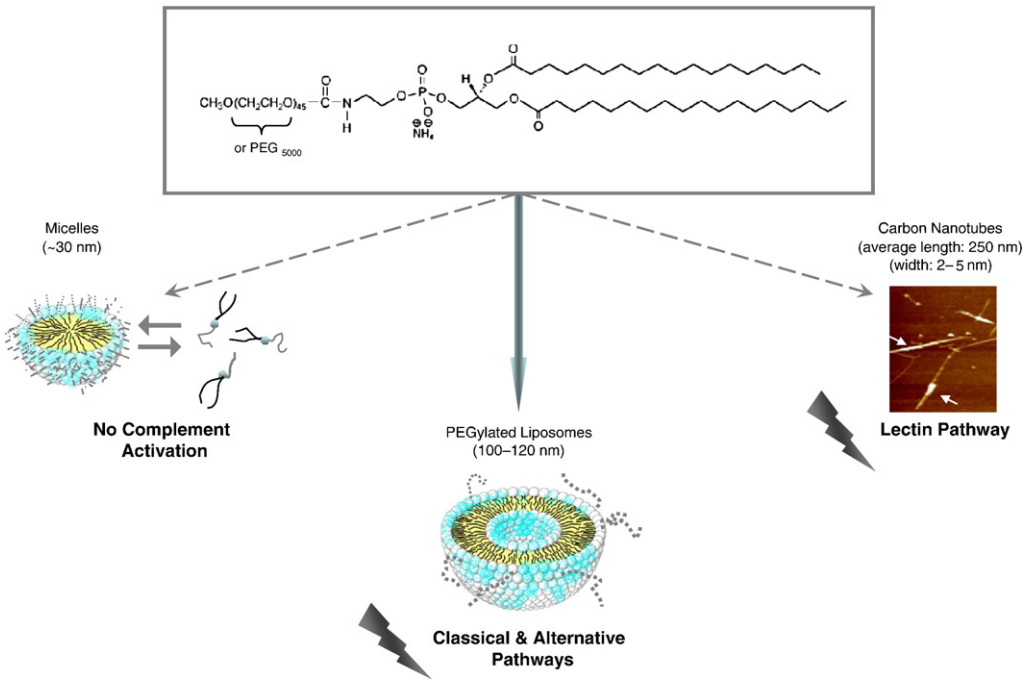

Fig. 6. Summary of complement cascade triggering by PEG–PL engineered nanomedicines and carbon nanotubes.

pathway turnover and a MASP-2-dependent process, Mol. Immunol. 46 (2008) 225–232.
[19] R. Lustig, N. McIntosh-Lowe, C. Rose, J. Haas, S. Kransnow, M. Spaulding, L. Prosnitz, Phase I/II study of Fluosol-DA and 100% oxygen as an adjuvant to radiation in the treatment of advanced squamous cell tumours of the head and neck, Int. J. Radiat. Oncol. Biol. Phys. 16 (1989) 1587–1593.
[20] S.M. Moghimi, A.C. Hunter, C.M. Dadswell, S. Savay, C.R. Alving, J. Szebeni, Causative factors behind poloxamer 188 (Pluronic F68, Flocor™)-induced complement activation in human sera. A protective role against poloxamer-mediated complement activation by elevated serum lipoprotein levels, Biochim. Biophys. Acta 1689 (2004) 103–113.
[21] G. Marone, M. Bova, A. Detoraki, A.M. Onorati, F.W. Rossi, G. Spadaro, The human heart as a shock organ in anaphylaxis, Novartis Found. Symp. 257 (2004) 133–149.
[22] G. Marone, V. Patella, G. de Crescenzo, A. Genovese, M. Adt, Human heart mast cells in anaphyaxis and cardiovascular disease, Int. Arch. Allergy Immunol. 107 (1995) 72–75.
[23] M.M. Markiewski, B. Nilsson, K.N. Ekdahl, T.E. Mollnes, J.D. Lambris, Complement and coagulation: strangers or partners in crime? Trend. Immunol. 28 (2007) 184–192.
[24] A.J. Andersen, A.H. Hashemi, T.L. Andresen, A.C. Hunter, S.M. Moghimi, Complement: alive and kicking nanomedicines, J. Biomed. Nanotechnol. 5 (2009) 364–372.
[25] J. Szebeni, L. Baranyi, S. Savay, H.U. Lutz, E. Jelezarova, R. Bunger, C.R. Alving, The role of complement activation in hypersensitivity to pegylated liposomal doxorubicin (Doxil), J. Liposome Res. 10 (2000) 469–481.
[26] J.K. Armstrong, G. Hempel, S. Koling, L.S. Chan, T. Fisher, H.J. Meiselman HJ, G. Garratty, Antibody against poly(ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients, Cancer 110 (2007) 103–111.
[27] A.W. Richter, E. Akerblom, Polyethylene glycol reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol modified allergens or placebo, and in healthy blood donors, Int. Arch. Allergy Appl. Immunol. 70 (1984) 124–131.
[28] A. Ludge, K. McClintock, J.R. Phelps, I. MacLachlan, Hypersensitivity and loss of disease site targeting caused by antibody responses to PEGylated liposomes, Mol. Ther. 13 (2006) 328–337.
[29] T. Ishida, S. Kashima, H. Kiwada, The contribution of phagocytic activity of liver macrophages to the accelerated blood clearance (ABC) phenomenon of PEGylated liposomes in rats, J. Control. Rel. 126 (2008) 162–165.
[30] V. Crupi, M.P. Jannelli, S. Magazu, G. Maisano, D. Majolino, P. Migliardo, R. Pontiero, Raman spectroscopic study of water in the poly(ethylene glycol) hydration shell, J. Mol. Struct. 381 (1996) 207–212.
[31] M. Kozielski, Conformational changes in the chains of polyoxyethyleneglycols, J. Mol. Liquid 128 (2006) 105–107.
[32] S.M. Moghimi, I. Hamad, T.L. Andresen, K. Jørgensen, J. Szebeni, Methylation of the phosphate oxygen moiety of phospholipid-methoxy(polyethylene glycol) conjugate prevents PEGylated liposome-mediated complement activation and anaphylatoxin production, FASEB J. 20 (2006) 2591–2593.
[33] P.H. Laverty, A. Leskovar, G.J. Breur, J.R. Coates, R.L. Bergman, W.R. Widmer, T.J. Toombs, S. Shapiro, R.B. Borgens, A preliminary study of intravenous surfactants in paraplegic dogs: polymer therapy in canine clinical SCI, J. Neurotrauma 21 (2004) 1767–1777.
[34] A.O. Koob, R.B. Borgens, Polyethylene glycol treatment after traumatic brain injury reduces β-amyloid precursor protein accumulation in degenerating axons, J. Neurosci. Res. 83 (2006) 1558–1563.
[35] S.M. Moghimi, A.C. Hunter, Recognition by macrophages and liver cells of opsonized phospholipid vesicles and phospholipid headgroups, Pharm. Res. 18 (2001) 1–8.
[36] K. Kostarelos, A. Bianco, M. Prato, promises, facts and challenges for carbon nanotubes in imaging and therapeutics, Nat. Nanotechnol. 4 (2009) 627–633.
[37] C. Salvador-Morales, E. Flahaut, E. Sim, J. Sloan, M.L. Green, R.B. Sim, Complement activation and protein adsorption by carbon nanotubes, Mol. Immunol. 43 (1996) 193–201.
[38] C. Salvador-Morales, E.V. Basiuk, V.A. Basiuk, M.L.H. Green, R.B. Sim, Effects of covalent functionalization on the biocompatibility characteristics of multi-walled carbon nanotubes, J. Nanosci. Nanotechnol. 8 (2008) 2347–2356.
[39] I. Hamad, A.C. Hunter, K.J. Rutt, Z. Liu, H. Dai, S.M. Moghimi, Complement activation by PEGylated single-walled carbon nanotubes is independent of C1q and alternative pathway turnover, Mol. Immunol. 45 (2008) 3797–3803.
[40] D. Pantarotto, C.D. Partidos, J. Hoebeke, F. Brown, E. Kramer, J.P. Briand, S. Muller, M. Prato, A. Bianco, Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses, Chem. Biol. 10 (2003) 961–966.
[41] D.T. Fearon, M.C. Carroll, Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex, Ann. Rev. Immunol. 18 (2000) 393–422.
[42] M.M. Markiewski, R.A. De Angelis, F. Benencia, S.K. Ricklin-Lichtsteiner, A. Koutoulaki, C. Gerard, G. Coukos, J.D. Lambris, Modulation of the antitumor immune response by complement, Nat. Immunol. 9 (2008) 1225–1235.
[43] S.M. Moghimi, T.L. Andresen, Complement-mediated tumour growth: implications for cancer nanotechnology and nanomedicines, Mol. Immunol. 46 (2009) 1571–1572.
[44] A.C. Hunter, Application of quartz crystal microbalance to nanomedicine, J. Biomed. Nanotechnol. 5 (2009) 669–675.
[45] M.C. Hersam, Progress towards monodisperse single-walled carbon nanotubes, Nat. Nanotechnol. 3 (2008) 387–394.
[46] C. Salvador-Morales, P. Towensend, E. Flahaut, C. Venien-Bryan, A. Vlandas, M.L.H. Green, R.B. Sim, Binding of pulmonary surfactant proteins to carbon nanotubes; potential for damage to lung immune defense mechanisms, Carbon 45 (2007) 607–617.
[47] L.A. Mitchell, F.T. Lauer, S.W. Burchiel, J.D. McDonald, Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice, Nat. Nanotechnol. 4 (2009) 451–456.
[48] C.A. Poland, R. Duffin, I. Kinloch, A. Maynard, W.A.H. Wallace, A. Seaton, V. Stone, S. Brown, W. MacNee, K. Donaldson, Carbon nanotubes introduced into the abdominal cavity of mice shows asbestos-like pathogenicity in a pilot study, Nat. Nanotechnol. 3 (2008) 423–428.