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Complement activation by carbon nanotubes and its influence on the phagocytosis and cytokine response by macrophages

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Abstract

Carbon nanotubes (CNTs) have promised a range of applications in biomedicine. Although influenced by the dispersants used, CNTs are recognized by the innate immune system, predominantly by the classical pathway of the complement system. Here, we confirm that complement activation by the CNT used continues up to C3 and C5, indicating that the entire complement system is activated including the formation of membrane-attack complexes. Using recombinant forms of the globular regions of human C1q (gC1q) as inhibitors of CNT-mediated classical pathway activation, we show that C1q, the first recognition subcomponent of the classical pathway, binds CNTs via the gC1q domain. Complement opsonisation of CNTs significantly enhances their uptake by U937 cells, with concomitant downregulation of pro-inflammatory cytokines and up-regulation of anti-inflammatory cytokines in both U937 cells and human monocytes. We propose that CNT-mediated complement activation may cause recruitment of cellular infiltration, followed by phagocytosis without inducing a pro-inflammatory immune response.

Key words: Carbon nanotubes; Complement; C1q; Cytokines; Macrophage

Introduction

The unique physical and chemical properties of carbon nanotubes (CNTs) make them very desirable materials in a range of biomedical applications.[1,2] CNT-mediated drug delivery has generated special interest.[1,3–11] By functionalising the outer walls of the nanotubes via attaching target-specific molecules (e.g. antibodies), drugs can be delivered to specific targets. Iron filled CNTs (Fe-MWNTs), filled with a ferromagnetic material, promise magnetic drug delivery and hyperthermia therapy.[3,12,13]

Due to their hydrophobicity and length, making stable dispersions of CNTs in physiological buffers, essential for drug delivery, is a common obstacle. Thus, non-covalent and covalent modifications of the CNT surfaces that include pre-coating with proteins, surfactants, nucleic acids, or introducing new functional groups on the external walls, are used. The biocompatibility of CNTs can be significantly improved when their surface is functionalized.[1,14–16]

Understanding the interactions between nanoparticles and immune system would facilitate their strategic and specific *in vivo* delivery.[17] The innate immune system plays a key role in protection against microorganisms and synthetic particles including CNTs.[18–21] Activation of the complement system, a major

Abbreviations: CNTs, carbon nanotubes; gC1q, globular head regions of human C1q; MWNTs, multi walled CNTs; Fe-MWNTs, iron filled multi walled CNTs; SWNTs, single-walled CNT; DWNTs, double-walled CNT.

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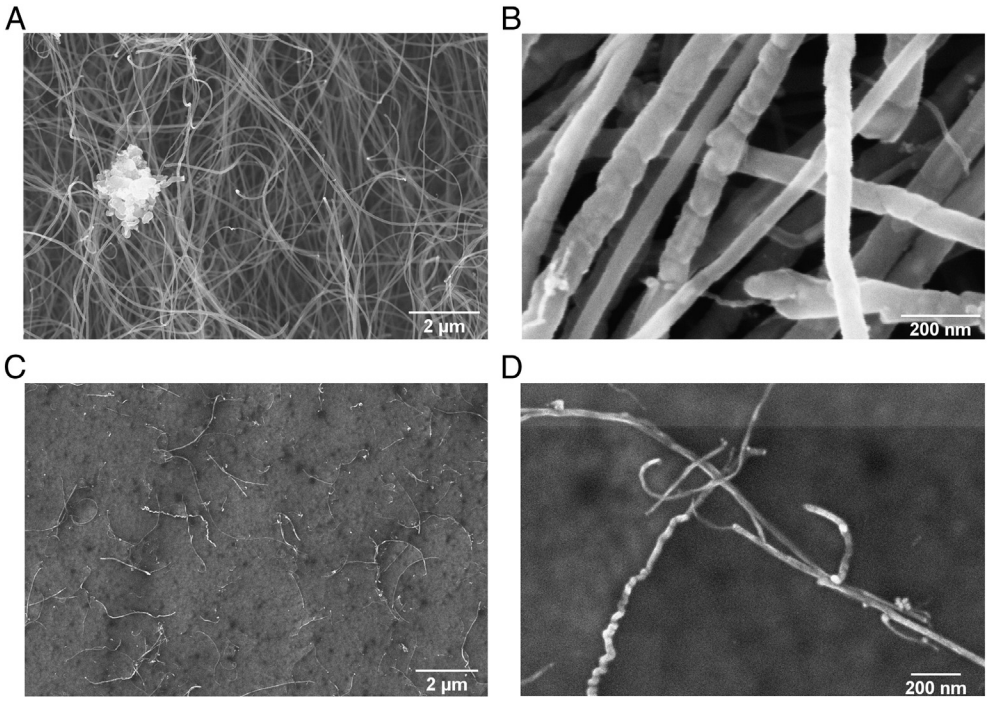

Figure 1. Scanning electron microscopy images of pristine **(A** and **B)** and CMC-coated **(C** and **D)** Fe-MWNTs showing high purity of the pristine samples and high dispersion of the functionalized samples. The dispersions are very stable (non-sedimenting).

component of the innate immunity,[22] can influence therapeutic activity and effectiveness of CNTs, as it can, in principle, cause nanoparticles to adhere to immune cells. In addition, activation of the complement system releases the bioactive peptides, C3a, C4a, C5a, which contribute to inflammation.[19–22]

The complement system is composed of a group of >40 plasma and cell surface proteins,[22] which recognises and clears non-self (microorganisms) and altered self (apoptotic and necrotic cells, aggregated proteins, etc.). It can be activated via three pathways: classical, alternative or lectin, all of which converge on the formation of a C3 convertase, a protease which activates C3 that gets cleaved into C3b.[23] The target-bound C3b and a degradation product, iC3b are powerful opsonins, i.e. they mediate binding of the target to phagocytic cells. In the classical pathway, C1q binds to charged or hydrophobic clusters on targets,[23] via its globular (gC1q) domain, followed by activation of two protease pro-enzymes, C1r and C1s, which, with C1q, form the C1 complex. This is followed by cleavage of C4 and C2, to form C4b2a, the C3 convertase. In the lectin pathway, Mannose-Binding Lectin (MBL) and Ficolins recognise neutral sugar and other uncharged features,[24] and form C4b2a via the MBL-associated serine proteases (MASPs). The alternative pathway involves a constant but slow hydrolysis of C3 in solution, which forms C3(H_2O). It forms a complex with Factor B, activated by Factor D (FD) to form C3(H2O)Bb (a C3 convertase).[25] C3b, which binds randomly and covalently to any nearby surface or particle, is stabilised by properdin, an upregulator of the alternative pathway. This stage is followed by generation of C5 convertase to cleave C5 to form C5a and C5b. C5b binds to C6, C7, C8 and C9 to form the C5b-9 complex, or membrane attack complex (MAC), which can insert into the lipid bilayer of the target and cause cell lysis.[25] The small fragments, C3a, C4a and C5a, promote inflammation as anaphylactic or chemotactic factors. Once C3b is deposited on a complement-activating particle, it is processed to form the products, iC3b and C3dg/C3d. These complement fragments are recognised by different complement receptors on various cell types, and the receptor–ligand interaction can cause the particle to adhere to the cell.[22] The receptors involved include Complement Receptor 1 (CR1) which binds C3b and C4b; CR2, which binds C3dg/C3d, CR3; and CR4 which bind iC3b and CRIg (C3b and iC3b).[26]

Non-functionalized SWNTs (single-walled) and DWNTs (double-walled), when placed in contact with human serum, activate complement via the classical and (to a lesser extent) the alternative pathway.[19] SWNTs, stabilised with several poly (ethylene glycol) derivatives, show no alternative pathway activation but C4 cleavage occurs, suggesting complement activation via the lectin pathway.[27,28] Functionalization (altering the surface properties of the CNTs) can increase or decrease the extent of complement activation,[15,18,27,28] while differing surface modifications can switch complement activation from one pathway to another. The mode of binding of the recognition subcomponents of the three pathways to CNTs, and whether they bind directly or via other deposited (serum) adaptor proteins, remains unclear. According to Ling et al, [29] C1q "crystallizes" on CNTs, but is not bound in a way that allows activation of the next step of the complement cascade. Thus, other serum proteins may form a stable layer on the CNTs, triggering indirect C1 binding and complement activation. Salvador-Morales et al,[18,19] however, observed direct binding of C1q to CNTs and subsequent complement activation.

In this study, we show several types of pristine and non-covalently functionalized CNTs activate the complement system