

Complement monitoring of carbon nanotubes

To the Editor — In their survey describing the promises and challenges of carbon nanotubes in imaging and therapeutics, Kostarelos and colleagues¹ state that "...chemically functionalized [carbon] nanotubes have been shown by many groups to be more biocompatible (no immune or acute inflammatory responses) than pristine nanotubes." We point out below *in vitro* and *in vivo* studies that show surface-modified carbon nanotubes can trigger immune responses, notably activation of the complement system^{2–4}.

The complement system is a network of over thirty different proteins that orchestrate innate and acquired immunity. Microbial invaders and nanoparticulate systems, depending on their surface properties, size and shape, may trigger the complement cascade through any of the three established classical, alternative and lectin pathways, and more than one pathway may be involved in many cases. Activation of the complement system primes the surface of the invaders with complement proteins for rapid recognition and ingestion by phagocytic cells. Complement activation further generates anaphylatoxins and chemoattractants, and these may potentially trigger the release of a variety of secondary mediators that could, in turn, initiate pseudoallergic responses in sensitive individuals⁵. These responses are often associated with cardiopulmonary disturbance and other related symptoms such as skin reactions, and have been noted in some patients receiving regulatory-approved nanomedicines, such as liposomes and micelles, and contrast agents^{5–7}. The incidence of pseudoallergic responses was recently estimated to be as high as 77% of all immune-mediated hypersensitivity reactions. Therefore, understanding the molecular basis of complement activation is relevant to the design of safer nanomedicines and carbon nanotubes.

Authors' reply — In our Progress Article¹, we defined 'pristine' carbon nanotubes as unmodified; 'coated' as surface modified by non-covalent coating with lipid, polymers or surfactants; and 'functionalized' as chemically modified by either acid

Sim and colleagues² demonstrated *in vitro* that pristine single-walled carbon nanotubes trigger the complement system by the classical pathway, whereas double-walled carbon nanotubes are capable of doing this by both the classical and alternative pathways. Carbon nanotubes that are covalently functionalized with either ϵ -caprolactam or L-alanine were further shown to activate the complement system but to a lesser extent than pristine nanotubes, and it was suggested that chemical functionalization may afford some degree of protection against complement activation³. However, it is important to note that from the immunological point of view even a small amount of complement activation may lead to adverse reactions in over-sensitive individuals.

For many proposed biomedical applications, carbon nanotubes require prolonged circulation times in the blood, and this is achieved by coating the surface with certain polymeric materials such as poly(ethylene glycol) (PEG) and poloxamers^{5,8}. PEG-phospholipid conjugates in both monomeric and micellar forms do not activate the complement system⁷. Yet, in our studies⁴, we found that single-walled carbon nanotubes non-covalently coated with amino- and methoxy-PEG₅₀₀₀-phospholipid (average length 250 nm and 1–5 nm wide) activated the complement cascade in human sera through the lectin pathway, and induced complement activation *in vivo*.

In our opinion, current surface-modification methodologies (covalent or non-covalent) do not necessarily prevent the activation of the complement system, but rather switch activation from one pathway to another. We believe that the inherent property of the carbon-nanotube surface plays a role in modulating complement activation because current surface-modification strategies do not uniformly cover the nanotube surface.

treatment or other covalent conjugation reactions. With this clarification in mind, the statement by Moghimi and Hunter points out the considerable differences in complement activation reported for pristine and coated carbon nanotubes^{2,3}

Understanding the molecular basis of complement events, however, may provide a viable platform for designing safer carbon nanotubes with possible applications in medicine. Nevertheless, the chances for preventing complement-activation-related pseudoallergy might be improved using complement inhibitors⁶.

Single-walled carbon nanotubes that have been covalently functionalized with a neutralizing viral coat protein have been shown *in vivo*⁹ to generate high levels of protective and virus-neutralizing antibodies. Although this means nanotubes could potentially deliver candidate vaccine antigens to peripheral dendritic cells and therefore act as particulate adjuvants, the adjuvanticity of the peptide-functionalized carbon nanotubes is consistent with their ability to activate the complement system². In conclusion, the interaction of nanomaterials (including carbon nanotubes) with the immune system deserves special attention. □

References

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note here that controlling surface coverage in functionalized carbon nanotubes is indeed difficult because it depends on several parameters including type of reaction, reaction conditions and the nature of the nanotubes⁵.

To date, there are no published *in vivo* data describing activation of the complement system by chemically functionalized carbon nanotubes. Moghimi and colleagues³ previously reported that carbon nanotubes coated with poly(ethylene glycol) (PEG)-phospholipids can activate the complement system *in vitro*, however, the *in vivo* (rat) model used showed inconsistent responses. It was suggested that the inconclusive nature of the rat model would require further studies using more sensitive complement activation models such as pig or dog. Therefore, the *in vivo* activation of the complement system even, for pristine or coated carbon nanotubes (which have exhibited reactivity *in vitro*), remains unknown.

The *in vivo* study by Pantarotto and co-workers⁶ describes an enhanced immune response by peptide-functionalized carbon nanotubes. It was shown that an

immunogenic peptide (derived from a viral coat protein), when covalently bound to nanotubes that have been chemically modified with maleimido groups, can generate virus-neutralizing antibodies. In that study, serum samples from mice containing antibodies against the peptide did not show cross-reactivity with the maleimido-functionalized tubes, suggesting that the derivatized tubes (without the peptide) were immunologically inert⁶. No further experimental evidence has since been reported on the adjuvanticity of chemically functionalized carbon nanotubes or on their effects on complement activation *in vivo*.

The issue of acute complement activation on administration is important for the clinical development of carbon nanotubes, as has been the case for other nanoparticle-based therapeutics such as liposomes (for example, Doxil)⁷. Clinical experience based on liposome therapeutics indicates that PEGylated lipids can induce complement activation in up to 10% of treated patients⁸. Our Progress Article did not highlight the clinical relevance of such

studies for carbon nanotubes because none are currently available in the literature. More preclinical studies are warranted and will surely appear. □

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