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Research Paper

It takes two to tango: Understanding the interactions between engineered nanomaterials and the immune system

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ABSTRACT

The immune system represents our primary defense system against foreign intrusion, including pathogens as well as particles. In order to understand the potential toxicity of engineered nanomaterials of ever increasing sophistication, it is necessary to understand the sophistication of the immune system with its multiple, specialized cell types and soluble mediators. Moreover, it is important to consider not only material-intrinsic properties of the pristine nanomaterial, but also the acquired, context-dependent 'identity' of a nanomaterial in a living system resulting from the adsorption of biomolecules on its surface. The immune system has evolved to recognize a vast array of microbes through so-called pattern recognition; we discuss in the present review whether engineered nanomaterials with or without a corona of biomolecules could also be sensed as 'pathogens' by immune-competent cells.

1. Introduction

The ability to manipulate matter at the nano-scale enables many new properties that are both desirable and exploitable, but the same properties could also give rise to unexpected (if not entirely novel) toxicities that may adversely affect human health [1]. Delineating the physico-chemical properties that are driving the toxicity of nanomaterials remains a challenge [2]. However, being able to link material properties to toxicity would enable the prediction of nanomaterial hazards and facilitate the design of nanomaterials that retain their useful properties, but display reduced toxicity (i.e., safety-by-design). Automated, high-throughput screening of well-defined libraries of nanomaterials is likely to aid in this endeavor and data generated through this approach can be used for structure–activity relationship (SAR) modeling using *in silico* methods [3]. If one can delineate the nanomaterial 'properties of concern' then assays for screening of nanomaterials could be refined and patterns would begin to emerge that allow for grouping of nanomaterials. In addition, systems biology approaches whereby quantitative measurements of molecular and functional changes are determined using gene expression profiling or other omics-based methodologies combined with computational modeling of the molecular interactions may also aid in defining the interactions of nanomaterials and other chemicals with biological systems [4].

In this context, it may be pertinent to recall that the immune system has evolved to protect us from foreign intrusion, including bacteria, viruses, parasites as well as particles [5]. Indeed, viruses, may be viewed essentially as self-replicating, biological nanoparticles that 'hijack' the biological machineries of the host for their own purposes. Notably, immune cells belonging to the innate (or, 'primitive') arm of the immune system use so-called pattern recognition receptors, including Toll-like receptors (TLRs), to recognize conserved molecular motifs on the surface of microbes and this allows for the recognition of a multitude of different microorganisms through the recognition of a limited number of molecular 'signatures' [6]. Thus, there are important lessons to learn from the field of immunology in terms of understanding how nano- or micro-scale objects are perceived by the immune system. Conversely, as highlighted by Hubbell et al. [7] material sciences have a great deal to offer immunology and medicine; the purposeful design of vaccine platforms is one example, as we will discuss below. However, the aim of the present essay is not to provide a

Abbreviations: DAMPs, danger-associated molecular patterns; DCs, dendritic cells; EPO, eosinophil peroxidase; GO, graphene oxide; HMGB1, high-mobility group box 1 protein; LPS, lipopolysaccharide; MPO, myeloperoxidase; PAMPs, pathogen-associated molecular patterns; NAMPs, nanomaterial-associated molecular patterns; NASPs, nucleic-acid scavenging polymers; NETs, neutrophil extracellular traps; NLRP3, nucleotide-binding domain, leucine-rich family (NLR), pyrin-containing 3; PRR, pattern recognition receptor; ROS, reactive oxygen species; TLRs, Toll-like receptors; SPIONs, superparamagnetic iron oxide nanoparticles; SWCNTs, single-walled carbon nanotubes; TNF, tumor necrosis factor.

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litany of all relevant nanomaterials studied, but rather to extract illustrative examples from the literature.

Nanotoxicology may be viewed as the study of the undesirable interference between man-made nanomaterials and biological nano-scale structures [5]. Here, we will focus, in particular, on nanomaterial interactions with the innate and adaptive arms of the immune system. We shall also consider the notion that engineered nanomaterials may, in fact, be recognized as 'pathogens' by cells of the immune system, including macrophages and other phagocytes, and the finding that carbon-based nanomaterials are enzymatically degraded by different innate immune cells much like bacteria and fungi.

2. Nanotoxicology: understanding the identities of nanomaterials

Most nanomaterials that have been studied to date are relatively simple materials; however, future developments may incorporate several different nano-sized components into complex assemblies of nanomaterials and suitable methodologies with which to assess adverse effects of such composite structures are therefore needed. In addition, as pointed out recently [8], increasingly sophisticated materials, including active materials responding to environmental cues or self-assembling materials present new, dynamic risks that are currently not well understood. Nanomaterial-enabled products also need to be assessed for any potential hazard throughout the product life cycle.

Numerous studies in recent years have shown that engineered nanomaterials may display toxicities *in vitro* and *in vivo*, even though it has been argued that many of these studies are fraught with shortcomings related to the use of unrealistically high doses, or the lack of reference materials with which to benchmark the results, and so on [9,10]. Moreover, it is important to determine whether toxicities of nanomaterials are 'novel' or merely scalable (and therefore, in principal, predictable, based on the study of larger particles or fibers). Indeed, it has been argued that "the final common pathways for pathological effects, that is, oxidative stress, inflammation, and genotoxicity are entirely shared by both nanoparticles and conventional particles and no novel pathogenic pathways are anticipated" [11]. Yet, it remains possible that the proximal events leading to the initiation of a common downstream program of cellular demise and subsequent organ dysfunction may nonetheless be related to the size of the offending particle. For instance, nanoparticles of a certain size have been shown to interject themselves into the inter-endothelial cell adherens junction thereby eliciting a size-dependent toxicity, manifested in an *in vivo* model as vascular leakiness [12]. Moreover, as noted recently, future materials of ever-increasing sophistication are more likely to resemble the complexity of natural nano-scale machineries rather than the apparent simplicity of chemicals [8]. This means that the assays used to assess for toxicity need to be sophisticated too, and that care should be taken to exclude assay interferences related to the nanoparticles themselves. Furthermore, nanoparticles that come into contact with biological fluids are thought to be rapidly covered by biomolecules forming a 'corona' that, in turn, interfaces with biological systems [13]. Indeed, it has been suggested that the interactions of engineered nanomaterials with cells and tissues are determined by the combination of material physico-chemical properties (the 'synthetic identity') and the context-dependent properties arising from the bio-corona the composition of which depends on the biological compartment in question [2]. This may be of particular relevance when considering interactions with the immune system as macrophages and other professional phagocytic cells of the immune system are equipped with receptors that recognize opsonized microorganisms and particles (opsonization is the process whereby a pathogen is marked for engulfment through coating with a substance, such as a protein).

2.1. The intrinsic, synthetic identity of nanomaterials

The importance of material-intrinsic properties, such as size, shape, surface charge and colloidal stability, for toxicological effects of nanomaterials cannot be overstated [2]. Together, these properties constitute the 'synthetic identity' of a material. Size is important as size can affect, for example, cellular uptake or the ability of particles to traverse biological barriers. In a seminal study, Choi et al. [14] followed the fate of intratracheally instilled near-infrared (NIR) fluorescent nanoparticles that were varied systematically in size, surface modification and core composition and determined that nanoparticles with hydrodynamic diameter less than 34 nm with non-cationic surface charge translocate rapidly from the lungs to regional lymph nodes in rats following intratracheal instillation. Furthermore, nanoparticles with a hydrodynamic diameter less than 6 nm were found to traffic from the lungs to lymph nodes and the bloodstream, ultimately being cleared from the body through the kidneys [14]. Moreover, nanoparticle behavior was found to depend strongly on surface coating which affects protein adsorption in body fluids; hence, for charged nanoparticles, nonspecific adsorption of endogenous proteins, mostly albumins, resulted in a large increase in hydrodynamic size, and this affected the biodistribution of the nanoparticles [14]. It is noteworthy that particles of the same chemical composition can elicit different responses depending on their shape. Hence, using highly stable, polymer micelle assemblies known as filomicelles, Geng et al. [15] compared the transport and trafficking of filamentous particles (filomicelles) with spherical particles of similar chemistry in an animal model. The filomicelles persisted in the circulation up to one week after intravenous injection which is about ten times longer than their spherical counterparts. Using a flow chamber with immobilized phagocytic cells, long filomicelles were found to flow past the cells, while smaller micelles and spheres were captured [15]. Surface charge also impacts on the interaction of nanoparticles with cells; higher toxicity of positively charged nanoparticles is generally correlated to their enhanced cellular uptake [16]. It has been suggested that the adsorption of a bio-corona of proteins may effectively equalize the surface charge of different nanoparticles [17]. However, it remains possible that the bio-corona is stripped off inside the cell, for instance in lysosomes [18], thereby revealing the intrinsic surface charge of the nanoparticle itself. Dissolution (of metal or metal oxide nanoparticles) and other forms of biotransformation may also occur in a living system and this has been shown to drive the toxicity of various nanoparticles (see [19] for a recent review). Finally, surface coating or functionalization impacts on the interaction of nanoparticles with cells. For instance, functionalization of carbon nanotubes (CNTs) and so-called carbon nano-onions, i.e., spherical carbon nanoparticles, with benzoic acid has been shown to diminish their inflammogenic properties, as assessed by decreased secretion of IL-1β and reduced recruitment of neutrophils and macrophages after intraperitoneal injection into mice [20]. Similarly, Li et al. [21] showed that functionalization determines pulmonary toxicity of multi-walled CNTs insofar as strongly cationic, polyetherimide (PEI)-modified CNTs induced significant lung fibrosis while anionic functionalization (carboxylation) decreased the extent of fibrosis in mice. These differences could be attributed to differences in cellular uptake and lysosomal damage leading to inflammasome activation (see below for a further discussion). Gao et al. [22] showed that the recognition of CNTs by macrophage-differentiated THP.1 cells can be regulated through surface chemistry modifications leading to a 'switch' from mannose receptor-mediated to scavenger receptor-mediated