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PERSPECTIVE

Rough around the Edges: The Inflammatory Response of Microglial Cells to Spiky Nanoparticles

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ABSTRACT The versatility of nanoparticle design has established nanotechnology as a potential "one-stop solution" to many biological and medical applications. The capacity to control nanoparticle size, shape, and surface chemistry has enabled their use as imaging contrast agents or carriers for drugs and other compounds. However, concerns of nanoparticle toxicity have surfaced that could limit their clinical translation. In order to overcome this challenge, researchers are starting to characterize how particle properties influence their interactions with biological systems. By identifying the specific nanoparticle parameters responsible for toxicity, it may be possible to engineer safer and nontoxic nanoparticles.

See the accompanying Article by Hutter *et al.* on p 2595.

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At the most basic level, a nanoparticle's geometric and surface design can change its cellular uptake, accumulation, biological response, and toxicity. Over the past two decades, substantial work has been conducted to understand the mechanism and capacity of cell–nanoparticle interactions. Arising from this research, it is now generally accepted that spherical nanoparticles with sub-100-nm diameters can be readily internalized by cells and will preferentially accumulate within the cell in the 40–60 nm size range.[1,2] The signaling response triggered by nanoparticles is usually related to the recognition of ligands on the nanoparticle surface by the cell's receptors. These preliminary studies have also determined that rod and spherical morphologies can enhance the rate of nanoparticle uptake,[3] while cationic surface chemistries yield enhanced cellular uptake over their neutral and anionic counterparts.[4,5]

Researchers have moved beyond *in vitro* cell culture into animal models in order to study the details of nanoparticle interactions. Studying the consequences of nanoparticle presence in each recipient animal will be more intricate and time-consuming than current *in vitro* cell studies. It has already become apparent that nanomaterials may exhibit different properties *in vitro* and *in vivo*. For example, the breakdown of CdSe quantum dots led to significant cell death in cultured liver hepatocytes[6] while showing no signs of toxicity in rat models.[7] Under these circumstances, animal models are likely more relevant in determining toxicity and optimizing design of nanostructures for human applications. To illustrate, one of the most studied nanoparticle applications is for targeting and delivering chemotherapeutic or contrast agents into tumors. The discovery of unique properties within the tumor microenvironment (*i.e.*, poor lymphatic drainage and immature vasculature) and the identification of cancer cell-specific surface markers (*e.g.*, EGFR, TfR, HER2) have provided nanotechnologists with specific objectives for their design parameters.[8,9] Such discoveries could not have occurred using cell culture models alone. These tumor-targeting nanoparticles have improved diagnostic sensitivity and therapeutic efficiency and have spawned a number of products that are now FDA-approved or pending approval.[8]

Studying the consequences of nanoparticle presence in each recipient animal will be more intricate and time-consuming than current *in vitro* cell studies.

Nanotoxicity. An important but controversial issue regarding nanotechnology developments is the potential toxicity of nanoparticles. Heavy metals and other compounds of known toxicity are typically used to achieve the desirable optical, electrical, and magnetic properties of nanoparticles. The bundling of these compounds into a compact nanoparticle could affect their delivery into organs and cells. Researchers have shown that *in vivo* biodistribution is dependent on size and surface chemistry[10] and that nanoparticle clearance *via* the kidney occurs when their size

is below 6 nm.[11] Elucidating the complex nanoparticle−biological interactions requires characterization of transport kinetics, clearance, and variations in gene expression in response to nanoparticle exposure in cell culture and animal models. Generally, nanoparticles may cause cell death in a number of ways including the production of reactive oxygen species that could modify protein function or DNA structure, which could ultimately alter the state, function, and response of cells.[12] While there have been significant research activities on this topic, it has been somewhat frustrating for researchers not to be able to say conclusively, "nanoparticle parameter(s) *x* causes effect *y*." Unlike small organic molecules, nanoparticles can be engineered controllably with well-defined geometric and chemical properties. By systematically evaluating a single parameter for each experiment, one should be able to establish precise correlations between nanoparticle design and biological interactions.

Gold Nanoparticles as a Model. Spherical gold nanoparticles are perhaps the most studied nanoparticles for biological applications. They have been used as probes for imaging and *in vitro* diagnostics[13] and hyperthermia therapy[14] and can be used as carriers for a variety of small-molecule drugs such as siRNA[15] or doxorobucin.[16] Studies on gold nanoparticle toxicity and their interactions with blood components, cells, and tissues are a crucial step in their translation into the clinic. Beyond their potential use in medicine, gold nanoparticles are excellent models for characterizing how the size, shape, and surface chemistry of nanostructures impact cell behavior. These nanoparticles are easy to synthesize in a wide variety of sizes and shapes, their surface can be modified with a wide range of chemistries, and they can be quantified using conventional techniques such as atomic emission spectroscopy and ultraviolet/visible spectrophotometry.

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***In Vivo* Transport and Immune Response of Metal Nanoparticles.** It has become apparent that nanoparticles do not diffuse freely throughout tissues and organs. Instead, their accumulation appears to be governed by their size and functional surface coating.[10] While blood-filtering organs such as the liver and spleen typically sequester the majority of administered nanoparticles, penetration into organs such as the brain can be difficult on account of the highly selective blood−brain barrier (BBB). Phagocytic cells such as macrophages, dendritic cells, and monocytes are located in various tissues and will nonspecifically take up nanoparticles. Nanoparticles are first engulfed by the cellular membrane and then internalized during the phagocytosis process. Current studies have provided evidence that increases in diameter[17,18] and aspect ratio of nanoparticles can impact phagocytic kinetics.[19,20] Of note, phagocytosis appears to increase when the diameter of a nanoparticle is increased or when the tangent angle at the point of contact between the cell membrane and nanoparticle is decreased.[19,20]

Macrophages are an important part of the reticuloendothelial system, are involved in the clearance and metabolism of foreign particles such as bacteria and viruses, and can stimulate lymphocytes and other immune cells in response to foreign particles. These processes could significantly influence the inflammatory response, where chemical mediators called cytokines released by the macrophages induce accumulation of fluid and immune cells. The intensity and location of the inflammation will determine if cell death and tissue damage occurs in a specific organ. By profiling the cytokines released after nanoparticle exposure, one can determine whether a process is pro- or anti-inflammatory. Examples of inflammatory nanoparticles include cobalt and nickel, which induce the release of tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2 cytokines subsequent to phagocytosis in cell culture.[21] In contrast, cerium oxide nanoparticles suppress macrophage production of free radical nitric oxide and dampen the inflammatory response.[22] Gold nanoparticles are neither immunosuppressive nor inflammatory.[21] *In vivo* experiments also reveal a wide range of immune responses when alveolar macrophages are exposed to inflammatory ferric oxide nanoparticles[23] or immunosuppressive carbon nanotubes.[24] It will be necessary in future studies to single out specific nanoparticle properties that alter immune response in a desirable manner.

Interactions of Non-uniform Nanoparticles with Brain Cells. In this issue's article by Hutter *et al.*,[25] the authors attempt to understand the effect of nanoparticle geometry on biological response in the central nervous system (CNS). The CNS is difficult to study mainly because of the restricted transport of nanoparticles across the BBB. The researchers introduced one of three

different nanoparticle geometries (spheres, rods, or urchins) to multiple CNS-associated cells. Although a diverse number of cell types reside in the brain, the researchers characterized uptake in two cell types: microglia and neurons. The authors demonstrated that nanoparticles with thin protrusions, known as nanourchins, are preferentially taken up by microglia, while only rods were internalized by neurons, suggesting that cells display selectivity toward certain particle geometries. Since all nanoparticles had the same surface chemistry, they propose that morphological differences in nanourchins promoted phagocytosis by microglia. Neurons relay chemical and electrical signals to and from the CNS, while microglia cells are the resident macrophages of the brain and spine. Neurons typically internalize small molecules such as proteins *via* endocytic mechanisms, whereas microglia phagocytose irregularly shaped particulates. Since nanoparticle morphology preference may correlate with cell function, the differences in uptake between these cell types should not be surprising.

Whether in the CNS or in other tissues, it is important to address whether accumulated nanoparticles will eventually activate the tissue's resident phagocytes causing inflammation. Hutter *et al.* investigated whether nanoparticle uptake up-regulated inflammatory molecules such as Toll-like receptor-2 (TLR-2) on microglia. This receptor is commonly expressed on phagocytes and recognizes multiple pathogen-associated molecular patterns found on the surface of bacteria, yeast, or parasites.[26] Binding of this receptor enhances phagocytosis rates and triggers the release of several proinflammatory cytokines. *In vitro* experiments revealed that nanourchins and nanorods both upregulated TLR-2 expression in microglia, whereas nanospheres did not (Figure 1). More detailed analysis revealed differential inflammatory cytokine expression in response to each nanoparticle. Nanourchins provoked increased interleukin-1α (IL-1α) production while suppressing granulocyte macrophage colony stimulating factor (GM-CSF). In contrast, poly(ethylene glycol)-coated (PEG) gold spheres and rods both increased the production of GM-CSF. These differences in nanoparticle-induced cytokines suggest a unique microglia-mediated immune response for each morphology *in vivo*.

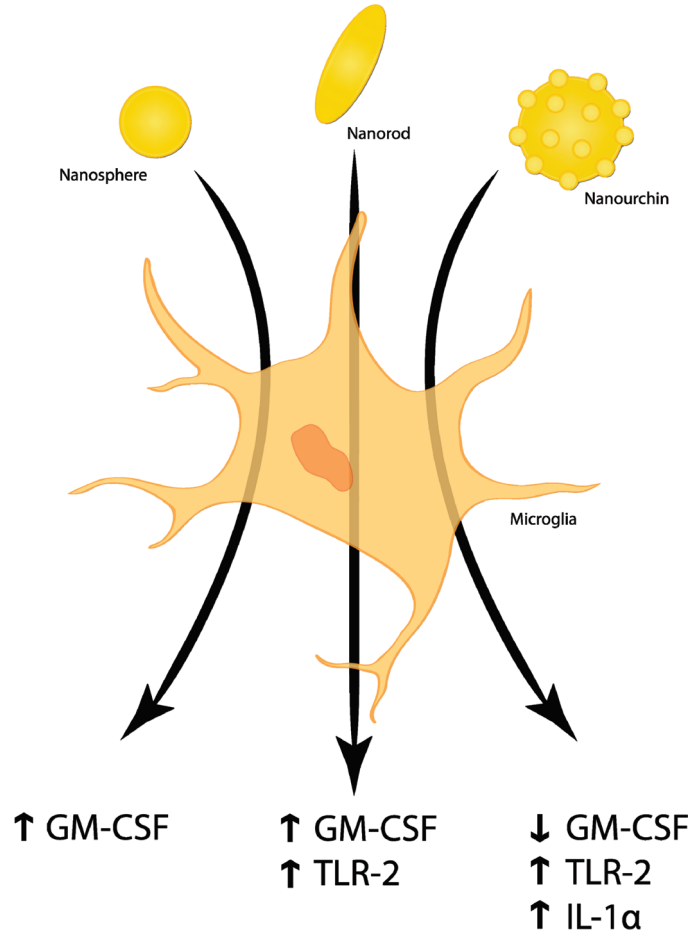

Figure 1. Differential release of chemical mediators called cytokines in response to nanoparticle geometry.

> The differences observed by Hutter *et al.* in nanoparticle-induced cytokines suggest a unique microglia-mediated immune response for each morphology *in vivo*.

CONCLUSIONS

In the last 5 years, there has been a significant interest in elucidating the interactions of nanoparticles with biological systems.[27] While the task is quite daunting considering the number of nanoparticle chemical compositions, sizes, shapes, and surface chemistries and the overall complexity and intricacy of physiological systems, these studies are indispensable. To be able to evaluate completely the importance of each parameter in a

biological system can take several decades. Hutter *et al.* described in their conclusions that many other nanoparticle parameters must be studied in order for them to develop guidelines for engineering nanoparticles as contrast agents for imaging the morphology and cells of the CNS. The success of such a large-scale endeavor will be beneficial to the field of nanotechnology since the outcome of these studies will provide important design parameters to engineer safe nanomaterials for a wide variety of medical applications. As an example, rapid clearance of nanoparticles by uptake into macrophages will lead to reduced accumulation in tumors, possibly rendering nanoparticles ineffective as imaging agents or drug carriers. This problem was solved by the discovery of the antifouling properties of the polymer PEG for coating nanoparticles.[28] The PEGylation chemistry is now an important part of the nanotoolbox. Fundamental studies on the interactions of nanoparticles with biological systems are important to the advancement of nanotechnology for medical applications.

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