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Review

Nanoparticles and innate immunity: new perspectives on host defence

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ABSTRACT

The innate immune system provides the first line of defence against foreign microbes and particulate materials. Engineered nanoparticles can interact with the immune system in many different ways. Nanoparticles may thus elicit inflammation with engagement of neutrophils, macrophages and other effector cells; however, it is important to distinguish between acute and chronic inflammation in order to identify the potential hazards of nanoparticles for human health. Nanoparticles may also interact with and become internalised by dendritic cells, key antigen-presenting cells of the immune system, where a better understanding of these processes could pave the way for improved vaccination strategies. Nanoparticle characteristics such as size, shape and deformability also influence nanoparticle uptake by a plethora of immune cells and subsequent immune responses. Furthermore, the corona of adsorbed biomolecules on nanoparticle surfaces should not be neglected. Complement activation represents a special case of regulated and dynamic corona formation on nanoparticles with important implications in clearance and safety. Additionally, the inadvertent binding of bacterial lipopolysaccharide to nanoparticles is important to consider as this may skew the outcome and interpretation of immunotoxicological studies. Here, we discuss nanoparticle interactions with different cell types and soluble mediators belonging to the innate immune system.

1. Introduction

1.1. Engineered nanoparticles and innate immunity

The interaction of engineered nanomaterials (NMs) and nanoparticles (NPs) with the immune system and the possible induction of inflammation are of particular interest for two main reasons. First, we need to know more about the interaction of NPs with the immune system for understanding their potential health risks. Second, knowing how the immune system recognises and eliminates NPs will help with designing nanomedical products (*e.g.*, drug delivery systems) not only capable of modulating the immune responses, but also of escaping immune surveillance thereby exerting more effectively their therapeutic potential. Thus, in the current safe-by-design approach to nanotechnological and nanomedical products, assessing how NPs interact with the immune defences is a crucial issue in determining NP safety *versus* hazard for human as well as for environmental health [1–4].

Most of the efforts of immunonanotoxicology have been aimed at the innate immune system, since the innate defence effector cells and humoral factors are the first that come in contact with foreign materials introduced into the body. Innate immune defences are particularly enriched in the tissues at the interface with the external environment

Abbreviations: CNT, carbon nanotube; DAMP, damage associated molecular pattern; DC, dendritic cell; DPM, discoidal polymeric nanoconstructs; HA-PEI, hyaluronic acid-poly (ethyleneimine); HAMP, homeostasis-altering molecular process; LbL, layer-by-layer; LPS, lipopolysaccharide; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; MPO, myeloperoxidase; MPS, mononuclear phagocyte system; MSV, multistage silicon nanovectors; MWCNT, multi-walled CNT; NAcGlc, *N*-acetyl-D-glucosamine; GO, graphene oxide; NAMP, nanoparticle-associated molecular pattern; NE, neutrophil elastase; NET, neutrophil extracellular trap; NM, nanomaterial; NP, nanoparticle NK natural killer; NLRC4, NLR family CARD domain-containing protein 4; NLRP3, NOD- LRR- and pyrin domain-containing 3; NOD, nucleotide-binding oligomerisation domain; PAMP, pathogen-associated molecular pattern; PEG, polyethylene glycol; PIM, pulmonary intravascular macrophages; PLGA, poly(lactide-co-glycolide); PM, particulate matter; PMN, polymorphonuclear leukocyte; PRR, pattern recognition receptor; PTX, paclitaxel; RBC, red blood cells; ROS, reactive oxygen species; SPION, superparamagnetic iron oxide nanoparticle; SWCNT, single-walled CNT; TAM, tumour-associated macrophages; TLR, Toll-like receptor; QD, quantum dot; XL-MSN, mesoporous silica NP with XL pores

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(skin, mucosal linings of the respiratory and gastrointestinal tract), but NPs may also reach the blood, either upon transport and penetration through epithelial barriers, due to their small size, or, in the case of nanomedicines, because of intentional parenteral administration.

Interaction of NPs with the innate immune system can have different outcomes, mostly depending on the characteristics of the NPs [5–9]. The immune system reacts to foreign materials with defensive actions only if these are perceived as potentially dangerous. In the case of NPs, as for many other environmentally borne agents (such as dust particles and microorganisms), the body's biological barriers and elimination systems are in most instances sufficient for excluding them, and immune recognition mechanisms are not activated. In other cases, NPs may actually elicit an immune reaction that ends up in the destruction of the foreign material. The effector cells that are mainly involved in this reaction are phagocytes, both resident tissue macrophages and blood-borne monocytes and polymorphonuclear leukocytes (PMN), which eliminate the NPs by phagocytosing and degrading them. In this context, it is important to be aware of the fact that NPs coming in contact with the innate immune system are never pristine ("as synthesized"), and that once released in any environment they undergo chemical and physical changes. Thus, they can agglomerate (a reversible phenomenon) or aggregate (irreversible) depending on their surface chemistry and on the environmental conditions (ionic strength, non-specific protein adsorption, *etc.*) and interaction with other agents present in the same environment. Aggregated NPs are usually in the microscale, are detected very easily by phagocytes and readily ingested and, in general, bigger NPs are taken up better than smaller ones. In contact with body fluids, NPs can undergo significant surface changes. NPs can adsorb proteins and other plasma molecules and form a so-called bio-corona on their surface [10–12], with hydrophobic particles doing so more easily than hydrophilic NPs [13], and with a difference in the type of adsorbed proteins depending on the NP surface chemistry and hydration water [14]. The surface coating of NPs then changes their mode of interaction with innate immune cells, and we can identify three potential scenarios:

1 The bio-corona facilitates recognition and elimination by immune cells. This is the case, for instance, of NPs opsonised with immunoglobulins or complement components.
2 The bio-corona masks the NP "foreign" surface, and as a consequence there is poor recognition by immune cells or factors (a dysopsonic effect) [15].
3 Adsorption on the NP surface may alter the folding of the plasma proteins, which are then recognised by innate immune cells as danger signals, thereby inducing a significant inflammatory reaction.

The modes of interaction of various NPs with different players of the innate immune system, and their sequelae in terms of the potential hazards for human health, are discussed in the present review.

1.2. Nanoparticle contamination with LPS

Toxicological studies are performed either *in vitro*, on human or animal-derived primary or transformed cells, or *in vivo* in different animal models. Models are by definition not perfect, and both *in vitro* and *in vivo* models have advantages and limitations. In immunonanotoxicology, it is very important to be aware of the model's limitations so as to avoid overinterpreting or even misinterpreting the results [16].

One of the examples of possible misinterpretation of nanotoxicological results is the undetected presence of endotoxin in NP preparations. Contamination with bacterial lipopolysaccharide (LPS), or endotoxin, is frequently encountered in NP preparations, due to the high surface area and surface reactivity of NPs, and the ubiquitous presence of endotoxin. It is being appreciated in the field of nanotoxicology that this is a major cause for false-positive reports about the inflammatory properties of NPs [17–20]. The difficulties are compounded by the fact that the available LPS tests are subject to artefacts [17,19,21] and that "cryptic" LPS can be transiently shielded by common buffer ingredients and detergents [22]. Recent studies suggested that certain carbon-based nanomaterials may interfere with conventional endotoxin assays and alternative monocyte or macrophage activation tests may be required [23].

Human primary monocytes and dendritic cells (DCs) are extremely sensitive to LPS and show significant responses to as little as 10 pg/mL of LPS, a concentration that is considered "endotoxin-free" in some commercial products [17,24]. Unintentional contamination of NPs with minute amounts of LPS can induce a powerful inflammatory response in monocytes, which can be fully ascribed to the contaminant [25]. Thus, one mechanism by which NPs can impact innate immunity may be their propensity to unintentionally carry over and deliver LPS. Researchers must be aware of this issue when designing experiments and interpreting the data.

In addition to the classical LPS receptor complex, based on TLR4, MD2 and CD14 [26], an intracellular LPS receptor/sensor has been described [27–29]. It is now accepted that LPS is recognised within the cytosol by caspases 4 and 5 in humans and by caspase-11 in the mouse [30]. The presence of these cytosolic LPS sensors may be a factor to explain why a phase-III clinical trial with a TLR4 inhibitor failed to decrease mortality in patients with severe sepsis [31].

The LPS-sensing caspases are expressed in immune cells from humans and mice [32]. There are so far no reports that analyse whether NPs, or LPS transported into the cell by NPs, can interact with these cytosolic LPS sensors. Since the LPS signal mediated by caspases leads to the activation of the inflammasome [30], this could, in principle, be another mechanism by which NPs can contribute to inducing an inflammatory phenotype in monocytes and DCs.

All these observations and considerations call for great caution when attributing inflammation-inducing effects to NPs both *in vitro* and *in vivo*, as the presence and effects of LPS and of other bystander contaminants, including other pathogen-associated molecular patterns (PAMPs), needs to be carefully controlled.

1.3. Inflammation: a double-edged sword

Inflammation is an adaptive response involving multiple cell types and soluble mediators that is triggered in response to infection, trauma, ischemia, toxic or other injury. The process normally leads to recovery and healing; however, chronic inflammation can lead to persistent tissue damage. Inflammation is therefore a double-edged sword. The distinction between acute and chronic inflammation is important as the outcomes are vastly different. Yet, in the toxicological literature, this distinction is sometimes lost, perhaps due to the paucity of chronic studies, and a lack of information on the long-term impact of nanomaterial exposure as well as resolution of the inflammatory response, with tissue repair, or perpetuation of inflammation and tissue damage. Furthermore, it is important to note that inflammation is not synonymous with infection; in the latter case, cells of the innate immune system are engaged to combat a microbial challenge, but inflammation can also be "sterile" and occur in the absence of microbial pathogens. Hence, while inflammation is vital for the host defence against invading pathogens, an inflammatory response can also be triggered by non-microbial signals. Importantly, cells of the innate immune system may use the same pattern recognition receptors (PRRs) to sense PAMPs and host-derived damage-associated molecular patterns (DAMPs). PRRs, such as the Toll-like receptors (TLRs) and the nucleotide-binding oligomerisation domain (NOD)-like receptor NLRP3 (NOD-, LRR- and pyrin domain-containing 3) are used by immune cells to sense microorganisms, and these receptors are also activated by DAMPs and particulate matters [33]. Indeed, one may ask whether nanoparticles are sensed directly by innate immune cells and/or whether proteins and