Toxicology and Applied Pharmacology 299 (2016) 78–89

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

# Current understanding of interactions between nanoparticles and the immune system

Marina A. Dobrovolskaia [a,*], Michael Shurin [b,c], Anna A. Shvedova [d,e,*]

[a] Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, NCI at Frederick, Frederick, MD 21702, USA
[b] Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
[c] Department of Immunology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
[d] Health Effects Laboratory Division, National Institute of Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV 26505, USA
[e] Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV 26506, USA

ARTICLE INFO

*Article history:*
Received 29 October 2015
Revised 24 December 2015
Accepted 26 December 2015
Available online 29 December 2015

*Keywords:*
Nanoparticles
Preclinical
Immunotoxicity
Immunology
Drug delivery

ABSTRACT

The delivery of drugs, antigens, and imaging agents benefits from using nanotechnology-based carriers. The successful translation of nanoformulations to the clinic involves thorough assessment of their safety profiles, which, among other end-points, includes evaluation of immunotoxicity. The past decade of research focusing on nanoparticle interaction with the immune system has been fruitful in terms of understanding the basics of nanoparticle immunocompatibility, developing a bioanalytical infrastructure to screen for nanoparticle-mediated immune reactions, beginning to uncover the mechanisms of nanoparticle immunotoxicity, and utilizing current knowledge about the structure–activity relationship between nanoparticles' physicochemical properties and their effects on the immune system to guide safe drug delivery. In the present review, we focus on the most prominent pieces of the nanoparticle–immune system puzzle and discuss the achievements, disappointments, and lessons learned over the past 15 years of research on the immunotoxicity of engineered nanomaterials.


## 1. Introduction

The immune system's function in the maintenance of tissue homeostasis is to protect the host from environmental agents such as microbes or chemicals, and thereby preserve the integrity of the body. This is done through effective surveillance and elimination of foreign and abnormal self cells and structures from the body. It is well known that certain environmental contaminants and xenobiotics, as well as other drugs, may alter the immune system's normal function. Therefore, screening for immunotoxicity is a generally accepted step in toxicological research related to both environmental factors and pharmaceutical products (Luebke, 2012).

The interactions between nanoparticles and various components of the immune system have become an active area of research in bio- and nanotechnology because the benefits of using nanotechnology in industry and medicine are often questioned over concerns regarding the safety of these novel materials. The past decade of research has shown that, while nanoparticles can be toxic, nanotechnology engineering can modify these materials to either avoid or specifically target the immune system. Avoiding interaction with the immune system is desirable when the nanoparticles are being used for medical applications not intended to stimulate or inhibit the immune system, as well as when they are used for industrial and environmental applications. Specific targeting of the immune system, on the other hand, provides an attractive option for vaccine delivery, as well as for improving the quality of anti-inflammatory, anticancer, and antiviral therapies (Mallipeddi and Rohan, 2010; Gonzalez-Aramundiz et al., 2012; Zaman et al., 2013; Tran and Amiji, 2015). Moreover, nanotechnology-based carriers can be used to reduce the immunotoxicity of traditional drugs (Libutti et al., 2010).

Some nanomaterials, metal colloids and liposomes, for example, were in use more than a decade ago (Gregoriadis et al., 1974), yet most active research in this field began in early 2000, fueled by the attention paid by regulatory agencies, such as the United States Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA), to the rapidly growing number of applications containing various types of engineered nanomaterials. The increase in submissions was expected since innovative research in this area had been progressing for years, culminated by the establishment of several breakthrough technologies that led to the discovery of fullerenes (Benning et al., 1992), carbon nanotubes (Ramirez et al., 1994), dendritic polymers (Tomalia, 1991; Newkome et al., 2002), and quantum dots (Takagahara, 1987). In 2005–2006, many worldwide initiatives were launched to improve the understanding of nanoparticle safety and included, among others, the establishment of the Nanotechnology Task Force by the FDA (http://www.fda.gov/ScienceResearch/SpecialTopics/

* Corresponding authors.
*E-mail addresses:* marina@mail.nih.gov (M.A. Dobrovolskaia), ats1@cdc.gov (A.A. Shvedova).

http://dx.doi.org/10.1016/j.taap.2015.12.022
0041-008X/

Nanotechnology/ucm2006658.htm), several nanotechnology research programs by the EPA (http://www2.epa.gov/chemical-research/research-evaluating-nanomaterials-chemical-safety), the E56 committee by the American Society for Testing and Materials (ASTM) International (http://www.astm.org/COMMITTEE/E56.htm), and the TC229 Nanotechnologies technical committee by the International Organization for Standardization (ISO) (http://www.iso.org/iso/iso_technical_committee?commid=381983). In addition to these efforts, the U.S. National Cancer Institute established the Nanotechnology Characterization Laboratory (NCL) to accelerate the translation of nanotechnology-based concepts intended for medical applications in the area of cancer diagnosis and therapy from bench to bedside (http://ncl.cancer.gov/). One of the initial goals of the NCL was to support the nanotechnology community by developing a so-called assay cascade that would include, among other tests, a battery of immunological assays. This assay cascade contributed to the initial understanding of the interactions between nanoparticles and the immune system and created a framework for stimulating discussions in the area of nano-immunotoxicology (Dobrovolskaia and McNeil, 2007; Marx, 2008; Dobrovolskaia et al., 2009a; Pantic, 2011; Smith et al., 2013). Recently, the European Commission has established the European Nanomedicine Characterization Laboratory (EU-NCL), which shares several objectives with those of the NCL (https://ec.europa.eu/jrc/en/news/eu-ncl-launched).

The rapid growth of this field becomes obvious when one compares the number of publications searchable in PubMed using the key words "nanoparticles" and "immune system" between years 2000 and 2015 (Fig. 1). Reviewing these data reveals many advances, as well as disappointments. Moreover, delving into the mechanisms of nanoparticle immunotoxicity uncovered many challenges in material characterization. Due to the wide variety of nanomaterials available, the characterization of their physicochemical properties is directed toward addressing parameters specific to certain type of particles (e.g. porosity is applicable to silicon nanoparticles, but is not informative for liposomes and dendrimers). The grand challenge in the particle characterization that precedes immunotoxicity studies relates to the estimation of immunoreactive contaminants, such as synthesis byproducts (e.g. iron catalysts in carbon nanotubes, cetyltrimethylammonium bromide [CTAB] in gold nanorods), and bacterial endotoxins, as well as excipients (e.g. Cremophor EL, polysorbate 80), and linkers (e.g. certain linkers used to attach poly(ethylene glycol) [PEG] to the nanoparticle surface) (Crist et al., 2013).

The challenges related to the physicochemical characterization (Clogston and Patri, 2013) and estimation of endotoxin contamination have been recently reviewed elsewhere (Crist et al., 2013; Dobrovolskaia, 2015).

The immunotoxicity of environmental materials has also been reviewed elsewhere (Kagan et al., 2010b).

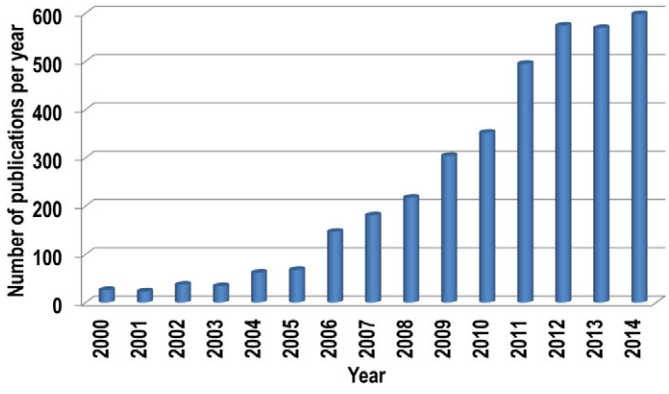


**Fig. 1.** Publications statistics. The PubMed data base was searched using the keywords "nanoparticles" and "immune system" for the years 2000–2015. The data for 2015 were excluded from the analysis because the publication year was incomplete at the time of the search. Each bar shows the total publication number per year.

Herein, we focus on the most prominent pieces of the nanoparticle–immune system puzzle, discussing what worked, what didn't, and what has been learned over the past 15 years of research on nanomaterials engineered for biomedical applications. A summary of achievements, disappointments, and lessons learned is presented in Fig. 2, and is further discussed below.

## 2. Achievements

### *2.1. Structure–activity relationship*

The physicochemical properties of nanoparticles determine their interactions with proteins in biological matrices (e.g. blood plasma and alveolar fluid) and with the immune cells. The structure–activity relationships between the most prominent physicochemical properties of nanoparticles and their effects on the immune system that lead to the most common types of immunotoxicity are summarized in Fig. 3. Below, we review several examples.

Nanoparticles with cationic surfaces, or those that carry cationic ligands, interact with biological membranes electrostatically. This leads to cellular damage, which triggers hemolysis, platelet activation, and aggregation, and to the induction of leukocyte procoagulant activity (PCA) and disseminated intravascular coagulation (DIC) (Greish et al., 2012; Jones et al., 2012a; Jones et al., 2012b; Ziemba et al., 2012). For example, cationic dendrimers of different architecture and size (generation five [G5] and generation four [G4] poly (propylene imine) [PPI] dendrimers [Bhadra et al., 2005; Agashe et al., 2006], G4 polyamidoamine [PAMAM] dendrimers [Bhadra et al., 2003; Asthana et al., 2005], generation three [G3] PAMAM and G3 PPI dendrimers [Malik et al., 2000], as well as G4 poly-L-lysine [PLL] dendrimers [Agrawal et al., 2007]) were shown to be hemolytic both in vitro and in vivo. The in vitro percent hemolysis varied from 14 to 86% in whole blood from human donors and various animal species, and was dependent on the density of the surface groups. Likewise, cationic PAMAM dendrimers, but not their anionic and neutral counterparts, altered key platelet functions and perturbed plasma coagulation, which culminated with DIC (Greish et al., 2012; Jones et al., 2012a; Jones et al., 2012b). The particle size, surface charge, and conformation of the polymer coating are important determinants of particle clearance by the mononuclear phagocytic system (MPS) in that smaller particles (100–200 nm) with unprotected surfaces and surfaces coated with a hydrophilic polymer in a "mushroom" configuration are primarily cleared by Kupffer cells in the liver; larger particles are eliminated by red pulp macrophages in the spleen. The addition of a hydrophilic polymer coating in a "brush" configuration protects particles from immune recognition, while increasing the particle size above 300 nm provides no protection, regardless of the polymer conformation (Gbadamosi et al., 2002). Exposure to high aspect ratio particles (e.g. carbon nanotubes, titanium nanobelts, cellulose nanofibers), as well as certain metallic particles (e.g. Si), results in inflammasome activation and the induction of proinflammatory cytokine interleukin (IL)-1β. These particles, as well as certain cationic and carbon-based particles, can exaggerate endotoxin-mediated inflammation (Baron et al., 2015).

The immunotoxicity of a nanoparticle is also influenced by the therapeutic payload it carries. For example, the induction of cytokines and type I interferons, the inflammatory reaction, the prolongation of plasma coagulation time, and complement activation are common dose-limiting toxicities of therapeutic nucleic acids (Levin, 1999). These toxicities are also commonly observed with nanoformulated nucleic acids, and this limits their translation into clinical use (Dobrovolskaia and McNeil, 2015a; Dobrovolskaia and McNeil, 2015b). Cytotoxic DNA-intercalating drugs used to treat cancer (e.g. doxorubicin, daunorubicin, and vincristine) are known to induce PCA and DIC (Wheeler and Geczy, 1990; Swystun et al., 2009; Kim et al., 2011). Formulating these drugs using nanotechnology carriers may help avoiding the toxicity. However,