This is an open access article published under a Creative Commons Non-Commercial No Derivative Works (CC-BY-NC-ND) Attribution License, which permits copying and redistribution of the article, and creation of adaptations, all for non-commercial purposes.

ACS AUTHORCHOICE

Chemical Research in Toxicology

pubs.acs.org/crt

Perspective

Nanomaterials and Innate Immunity: A Perspective of the Current Status in Nanosafety

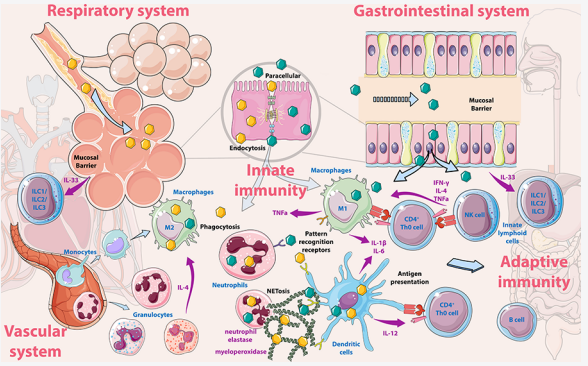
James G. Cronin,*,⊥ Nicholas Jones, Catherine A. Thornton, Gareth J. S. Jenkins, Shareen H. Doak, and Martin J. D. Clift*,⊥

Cite This: *Chem. Res. Toxicol.* 2020, 33, 1061−1073

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Human exposure to engineered nanomaterials (ENMs) is inevitable due to the plethora of applications for which they are being manufactured and integrated within. ENMs demonstrate plentiful advantages in terms of industrial approaches as well as from a consumer perspective. However, despite such positives, doubts remain over the human health implications of ENM exposure. In light of the increased research focus upon the potential effects of ENM exposure to human health in recent decades, questions still remain regarding the safety of these highly advanced, precision-tuned physical entities. The risk of short-term, high-dose exposure to humans is considered relatively low, although this has formed the direction of the hazard-assessment community since the turn of the 21st century. However, the possibility of humans being exposed repeatedly over a long period of time to a low-dose of ENMs of varying physicochemical characteristics is of significant concern, and thus, industry, government, academic, and consumer agencies are only now beginning to consider this. Notably, when considering the human health implications of such low-dose, long-term, repeated exposure scenarios, the impact of ENMs upon the human immune system is of primary importance. However, there remains a real need to understand the impact of ENMs upon the human immune system, especially the innate immune system, at all stages of life, given exposure to nanosized particles begins before birth, that is, of the fetus. Therefore, the purpose of this perspective is to summarize what is currently known regarding ENM exposure of different components of the innate immune system and identify knowledge gaps that should be addressed if we are to fully deduce the impact of ENM exposure on innate immune function.



CONTENTS

1. INTRODUCTION

Irrespective of the exposure type toward the human body, whether it is via inhalation, dermal application, the gastrointestinal (GI) tract, direct injection into the circulatory system, or even via the ocular, immune interactions with engineered nanomaterials (ENMs) will initially occur through the innate immune system. The innate immune system is a diverse array of tissue barriers and specialized cellular roles that function to limit the damage typically caused by "nonself" and to facilitate wound healing.[1]

Special Issue: Future Nanosafety

Received: February 7, 2020
Published: April 20, 2020

Downloaded via 81.151.220.198 on July 22, 2020 at 15:04:41 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

Traditionally, the study of immunology has been split into innate and adaptive (or acquired) immunity. For much of the 20th century, research focused upon identifying and characterizing components of the adaptive immune system with great strides made in the understanding of T and B cell biology. However, since the 1990s, there have been huge advances in our understanding of innate immunity. This has been achieved by the systematic identification and characterization of pattern recognition receptors (PRRs) and their corresponding ligands, primarily pathogen associated molecular patterns (PAMPs) but also host-derived danger-associated molecular patterns (DAMPs), revealing a blueprint for how infection and danger are sensed at the cellular and molecular level.

Anatomic and physiologic barriers constitute the first line of defense against not only pathogens but also foreign materials. When these barriers are overwhelmed or breached, cellular innate immunity is activated. Mucosal barriers, such as those of the lung or GI tract, are often the first to encounter exogenous xenobiotics such as ENMs. In the GI tract, the microbiome has a central role in maintaining host health by colonizing biological niches, in principle preventing the potential access of ENMs to the mucosa and underlying epithelia.[1] In the lung, ENMs are often cleared via robust mucociliary clearance, the primary innate defense mechanism of the respiratory tract.[2] While the integrity of the epithelium and its surfactant layer should restrict deposition of ENMs into the alveolar region, ENMs have been demonstrated to reach the underlying mucosal lamina propria and eventually enter the lymphatic and portal circulation.[3] This requires that ENMs overcome the physiological processes of the epithelium, where epithelial cell tight junctions (TJs) should contribute to blocking ENMs from reaching the underlying lamina propria.[4]

Epithelial cells also have a repertoire of PRRs. As part of the innate immune system, epithelial cells are supported by hematopoietic-derived cells, including monocytes, macrophages, dendritic cells (DCs), natural killer (NK) cells, mast cells, neutrophils and eosinophils, as well as by innate lymphoid cells (ILCs) and noncellular components such as cytokines/chemokines, complement proteins, pentraxins, and collectins. Macrophages and DCs also function as antigen presenting cells (APCs) linking innate and adaptive immune responses to, among other things, support the production of specific antibodies to enhance phagocytosis of foreign materials by the process of opsonisation on a subsequent encounter.[5]

PRRs are appositely placed in distinct cellular compartments to detect PAMPs and DAMPs, constituting an essential early warning system.[6] This range of PRRs includes Toll-like receptors (TLRs) and the cytosolic nucleotide-binding oligomerization (NOD)-like receptors (NLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors, and C-type lectin receptors (CLRs). It is important to remember that PRRs are activated not only by PAMPs but by a spectrum of DAMPs released as a result of cellular or tissue damage including endogenous proteins, such as high mobility group box 1 (HMGB1) protein, heat shock proteins, nucleic acids, and the cytokine interleukin (IL)-1α, or by exogenous materials themselves.[7]

The breaching of the dense protein/lipid layers (i.e., mucus/surfactant) at epithelial barriers by pathogens induces an immediate innate response. Yet, whether ENMs similarly initiate an innate immune response and what these mechanisms might be are largely unknown. Recent advances in nanotechnology are driving a logarithmic increase in the development and application of ENMs by industry.[8] Yet, understanding of the potential hazard of the inevitable human exposure to ENMs has not kept pace.[9] Reasons for this have been numerous, including the number and type of ENMs being manufactured, as well as the experimental approaches being adopted.[10] All of this has instigated ongoing hazard assessment of ENMs. These are focused predominantly on toxicological investigations, but elucidation of the long-term health effects of human ENM exposure requires insight into how nanomaterials interact with innate immune cells.

The purpose of this perspective therefore is to highlight current knowledge about the effect of ENMs on the human innate immune system while indicating key research concepts for improving our understanding of how ENMs interact with human immune defense systems. It is important to note that this article will not cover the human adaptive immune system or nanomedicine applications toward modulating the innate immune system. For important reviews on these aspects, the authors highlight refs 11 and 12 (ENM adaptive immunity and nanosafety) as well as ref 13 (nanomedicine and the innate immune system), which adequately cover these areas.

2. BARRIER FUNCTION

It is well-known within the nanosafety community that ENMs readily interact with epithelial barriers.[14] Depending on their physicochemical properties, inhaled ENMs may deposit in the airway or alveolar region of the lung, the majority locating to the alveolar region.[15] As noted above, epithelial cell TJs play an important role in blocking ENMs from entering the underlying lamina propria[4] and regulating paracellular distribution. The formation of TJs depends on the extra-cellular matrix proteins such as laminins and collagens and the epithelial cellular formation expression of zonula occludins (e.g., ZO-1) and claudins.[16] However, as highlighted previously, ENMs can readily breach epithelial barriers often entering the systemic circulation.[17] In the gut, for example, translocation of ENMs conjugated with bacterial surface proteins, such as invasin, is sufficient to confer internalization in mammalian cells.[18] Furthermore, due to their hydrophobicity, such specific ENMs can directly interact with cell membrane lipids, potentially causing size-dependent, cytotoxic damage in epithelial cells.[19] Oral exposure to silver ENMs alters the expression of genes associated with GI epithelial TJs, including claudins and platelet endothelial cell adhesion molecules (PECAMs), adherens junctions, the NOTCH pathway, and hemidesmosomes, as well as being associated with an increase in the cytokine tumor necrosis factor α (TNFα).[20] Such a mechanism could underpin the association between increased exposure to ENMs and inflammatory bowel diseases such as ulcerative colitis, a condition associated with dysfunctional epithelial TJ formation.[21] The need to elaborate mechanisms such as this highlights the need for advanced *in vitro* systems.[22] As an example, alternative *in vitro* systems to study the implications of ENM exposure[22] can be created by coculture of the enterocyte-like, microvilli expressing Caco-2 cells and the goblet cell-like HT29 cell line, which secretes mucus. These cell lines when cocultured are capable of forming TJs and display a dense mucus layer representing an epithelial barrier. While several studies have used this model to investigate the effect of ENMs, for example, titanium dioxide, these have yielded conflicting results,[23−25] and so further emphasis is needed to understand the usefulness of these sophisticated models.[26]

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

Underlying the mucosa is a plethora of immune cells and it has been realized relatively recently that mucosa function depends on the interaction between, and communication of, epithelial and stromal cells with tissue-resident immune cells, such as macrophages and ILCs, which shape barrier function, tissue remodelling, and homeostasis.[27,28] The discovery of ILCs has shifted our understanding of the innate immune response. ILCs are predominantly abundant at the mucosal surfaces of the lung and intestine and mirror key functions of T lymphocytes and, although on the whole being beneficial, their dysregulation is known to contribute to inflammation and disease pathology.[28]

ILCs, like tissue-resident macrophages, are likely seeded during fetal development and maintained in the tissue long-term. While tissue-resident macrophages, as discussed later, are continuously replenished by the vasculature, ILCs are seemingly not,[29] although more recent evidence challenges this concept.[30] Although ILCs display a lack of antigen-specific receptors, ILCs adapt to environmental cues using receptors for cytokines that are released during tissue damage and inflammation. ILCs are divided into three main groups, ILC1, ILC2, and ILC3s, and can be regarded as the innate immune system's equivalent of T helper cell subsets Th1, Th2, and Th17, respectively.[30] Similar to natural killer (NK) cells, ILC1s secrete the cytokine interferon-γ (IFNγ). Accordingly, natural killer cells are generally grouped with ILC1s; however, for this review, NK cells will be discussed separately.[27]

There is limited work on the interaction of ENMs with ILCs (apart from NK cells). However, there is some evidence of their impact upon ILC function. For instance, the perinatal period is a critical time for seeding of innate immune cells, such as ILCs and tissue-resident macrophages, within developing organs. Upon prenatal exposure to carbon black nanoparticles, the postnatal number of $CD4^{-}CD8^{-}$ cells, that is, non-B or -T lymphocytes, increased in the thymus of male offspring.[31] This cellular population included ILC2s, which produce traditional Th2 type cytokines (including IL-4, IL-5, and IL-13) in response to IL-33 and have been associated with pathology that develops from allergic inflammatory diseases, for example, asthma.[32−34] ILCs are partly regulated by IL-33 from epithelial cells including their potential recruitment from the bone marrow.[30] ENMs, such as titanium, silica, and zinc (di)oxide carbon nanotubes, are known to exacerbate allergy in mouse models of allergic airway disease.[35−38] Multiwalled carbon nanotubes (MWCNTs) have been shown to induce epithelial cells to produce IL-33, which purportedly recruits ILCs to airways and stimulates production of the Th2 type cytokine IL-13.[39] In addition, pulmonary exposure titanium dioxide is associated with increased pulmonary ILC2s and a systemic innate Th2 type response.[40] ILC2 immune responses are characterized by recruitment of important innate effector cells in allergic disorders such as mast cells, basophils, and eosinophils.[41] Furthermore, epithelial and stromal cells of the mucosa selectively express the receptor (restricted to cells of a nonhematopoietic origin) for IL-22, a cytokine produced by ILC3s complementing the role of Th22 T helper cells. IL-22 may be protective but can also play a pathogenic role in chronic inflammatory diseases and is associated with rheumatoid arthritis, inflammatory bowel diseases, and psoriasis.[42] Generally, the role ILCs play in shaping the innate immune response to ENMs is currently underexplored. Interestingly, absence of ILCs in patients has been shown to have no apparent clinical effects including susceptibility to infection. Therefore, ILCs were proposed to be redundant in animals with a functioning adaptive immune system (under conditions of modern medicine and hygiene).[43] Nevertheless, as discussed here, ILCs appear to have roles in the pathology of sterile inflammatory diseases. Thus, more research is required to delineate the importance of ILCs in the response to ENMs and how these responses may have a role in driving pathology.

3. COMPLEMENT SYSTEM

The fate of ENMs in any biological environment is correlated closely with the formation of a protein corona.[44−46] For example, as ENMs circulate in the blood, their outer surface rapidly adsorbs biomolecules, such as serum proteins (i.e., albumin), which affects the interaction of ENMs with innate immune cells and their subsequent clearance from the bloodstream.[46,47] Although the formation of a "hard" corona around the surface of ENMs can be positive in terms of their potential cytotoxicity,[48,49] serum proteins can also "shield" functionalized nanoparticles from binding to targeted receptors, causing loss of specificity.[50,51]

The complement system, an essential humoral component of innate immunity, was originally characterized for its role in the destruction and removal of pathogens via the activation of innate and adaptive immune cells.[52] The three pathways of the complement system, known as the alternative, classical, and lectin pathways, converge at component C3. This complement protein is one of the most well reported proteins in the area of ENM−protein interactions/complex formation.[53] Since the role of complement is to aid the clearance of microbes/damaged cells via phagocytosis, it is clear that the innate immune system recognizes ENMs as foreign objects. The association of C3 has been reported to be a commonly observed protein on the surface of superparamagnetic iron oxide nanoparticle (SPION) nanoworms.[54] Upon cleavage of C3, the protein fragment iC3b binds to nonself surfaces and is an activating ligand of the Type 3 complement receptor (CR3) on innate immune cells.[55] CR3-dependent uptake of opsonized ENMs may require this activation step. Larger ENMs, such as latex ENMs, are ingested more readily by IL-4-induced multinucleated giant cells (MGCs) than unfused macrophages.[56] Other components of plasma, including IgG and IgM, can also bind to nonself surfaces and are critical factors in complement activation.[51,57] Material science, through polymer chemistry, has enabled the coating of ENMs with different polymers, most often polyethylene glycol (PEG) or zwitterionic polymers,[58−65] to form a repulsive barrier to the immune system, namely phagocytic cells. Nevertheless, adsorption of proteins to coated ENMs still readily occurs, and this might actually be useful in avoiding immunosurveillance.[62]

4. PHAGOCYTOSIS

Binding of C3 to ENMs initiates recognition by phagocyte receptors that signal for phagocytosis, by neutrophils and macrophages in particular, and is important for ENM-induced cytotoxicity.[66] The physicochemical properties of ENMs are well documented to influence the form of active uptake used by phagocytic cells. Specifically, ENMs with anionic or cationic surface charges more readily enter phagocytes than ENMs with a neutral charge.[67] Macrophages display increased ENM uptake upon activation, for example, prestimulation using lipopolysaccharide (LPS; a prototypic PAMP derived from Gram negative bacteria), although irrespective of LPS

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

stimulation, they play a fundamental role in engulfing and clearing ENMs.[68] Within the nanosafety community, the interaction between ENMs and phagocytes, especially macrophages, has been well described in recent years.[69] However, the impact of ENM exposure on neutrophils is relatively unknown. While neutrophils have been widely used as a marker of inflammation following ENM exposure,[70] these studies do not consider the impact of the ENM encounter on neutrophil function. As highlighted below, this indicates that further focus upon how neutrophils interact with ENMs is critical to determine relevance of the prolonged inflammation and the long-term hazard of ENMs. Alas, even in macrophages, the specific innate immune pathways that ENMs activate remain all but undefined. It is important to note, however, that an inflammatory response initiated by the innate immune response is primarily a defensive response against a foreign object (e.g., pathogen) and does not indicate the onset of a pathological impact. The concept of a prolonged inflammatory response, above baseline levels, is one that should be considered as a negative impact and thus one that the nanosafety community must focus on in terms of ENM hazard implications.

While it is known that the kinetics of active interaction plays a significant role, alongside the ENM surface and adsorbed proteins (as just discussed), the true mechanism of active entry, as well as intracellular trafficking, which have both been associated with the hazard noted from ENM-phagocyte exposure,[67] is not fully deduced. Further, it is not a matter of "read-across" between ENM types since such interaction and associated impact are considered to be highly specific to the physicochemical characteristics of ENMs (i.e., size, geometry, surface coating/chemistry, surface charge).[71−76]

5. NEUTROPHILS

Neutrophils are the most abundant phagocyte in blood and, along with tissue-resident macrophages, are considered the first line of defense once barriers have been penetrated by ENMs. Granulocytes, such as neutrophils, immobilize and sequester ENMs, occasionally leading to granuloma formation[6] following, for example, *in vivo* exposure to MWNCT.[77] *In vitro*, neutrophils display size-dependent, rapid internalization of ENMs[40] particularly of polymer coated ENMs adsorbed with human serum albumin, which increases their entry into neutrophils two-fold.[78] Neutrophils are also activated by titanium dioxide *in vitro*,[79] and *in vivo* this is characterized by a marked neutrophil infiltrate and neutrophilia.[80−82]

Neutrophils can also form neutrophil extracellular traps (NETs) in response to exogenous and endogenous DAMPs, with NET formation (also known as NETosis) dependent on reactive oxygen species (ROS) formation, and granular enzymes such as neutrophil elastase (NE) and myeloperoxidase (MPO).[83,84] Notably, MPO is able to degrade ENMs,[85] indicating a potential protective effect by the innate immune system to biodurable/biopersistent ENMs. NETs are formed by membrane disruption and the translocation of NE and MPO to the nucleus, initiating histone degradation and the decondensation and release of chromatin and granular proteins. NETosis is triggered by ENMs in a size-dependent manner, initiated by damage to the plasma membrane as well as lysosomal membranes, resulting in membrane instability. Neutrophils exposed to ENMs rapidly rupture and release NETs, for example, within 15 min of contact with gold ENMs.[86] Carbon or polystyrene ENMs induce a size-dependent formation of NET-like structures. Polyhedral oligomeric silsesquioxanes (POSS) are readily taken-up by cells, and their small size and high charge induce NET formation in a dose-dependent manner.[87] Furthermore, NETosis is not inhibited by caspase inhibitors; therefore, it might be an apoptosis-independent process.[88] A study by Hwang et al. demonstrated that NETosis, induced by solid lipid ENMs, was dependent on ROS and calcium.[89] NETs, while initially promoting a pro-inflammatory response, are also involved in the resolution of chronic inflammation.[90] In the setting of ENM exposure, this might be of benefit as an immediate short-term inflammatory response triggers NETosis trapping ENMs, reducing inflammation and limiting tissue damage *in vivo*.[19] Nanodiamonds induce cellular membrane damage *in vitro* and *in vivo*,[19] and the resolution of nanodiamond-induced inflammation measured as paw edema in mice was dependent on ROS production and NETosis. Mice unable to generate NADPH (nicotinamide adenine dinucleotide phosphate)-dependent ROS failed to initiate NETosis or resolve inflammation.[91] Furthermore, aggregated NETs also limit inflammation by degrading cytokines and chemokines.[90] However, elucidation of the effects of ENM on neutrophil structure and function is limited.

6. PATTERN-RECOGNITION RECEPTORS (PRRS)

ENMs may possess pathogen-mimicking properties, resulting in the activation of PRRs.[92] PRRs were initially characterized for their role in sensing and orchestrating the response to exogenous pathogens, distinguishing "self" from "nonself". However, PRRs have now been firmly established for their role in detecting DAMPs generated by sterile inflammation, stress responses, and cell death. PRRs vary in their cellular localization and binding affinity for DAMPs. TLRs and CLRs are localized to cellular membranes, including TLRs on internal endosomal membranes, whereas RLRs or NLRs are localized to the cytosol. The ten TLRs identified in humans exist as hetero- or homodimers, composed of ligand-binding leucine-rich repeats and a toll/IL-1 receptor-like domain (TIR), which activates cell signaling pathways that culminate in the production of pro-inflammatory cytokines, TJ components, and increased mucus secretion from epithelial cells.[93] The NLR family of PRRs activates innate immunity in response to pathogens, metabolic stress, or sterile inflammation. For example, NLRP6 regulates goblet cell mucus secretion at the colonic host−microbial interface,[94] whereas NLRP3 is activated due to disruption of cellular membranes by, for example, asbestos, silica, or by sensing extracellular ATP released by injured tissues and cells.[95,96] Furthermore, coactivation of TLRs and NLRP3 leads to the processing of the cytokine pro-IL-1β into its mature form, a procedure that requires tightly controlled regulation, due to the tissue-damaging effects of IL-1β.[96]

DAMPs, such as nucleic acids, sugars, and lipids, are likely to adsorb to corona proteins on the surface of ENMs and interact with PRRs.[97] For example, graphene oxide can activate TLR4 in macrophages, inciting a classical inflammatory response and gold ENMs activate endosomal TLR9 signaling pathways.[98,99] This highlights the heterogeneity of ENM interaction with PRRs, which can have significantly different downstream effects on the immune response. Further to this single-walled CNTs (SWCNTs) are neither recognized nor phagocytosed by phagocytic cells.[100] However, other studies report that energy-independent intracellular trafficking of functionalized CNTs

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

occurs after a few hours contact with immune cells, even under conditions where endocytosis is inhibited.[101] Moreover, the nature of the functional group does not influence whether CNTs are internalized,[101] although coating SWCNTs with phosphatidylserine reportedly facilitates their uptake by alveolar macrophages *in vitro* and *in vivo*.[100] Mass spectrometry imaging has revealed that the majority of SWNCTs locate to the kidney and the macrophage rich red pulp region of the spleen.[102] Moreover, a computational study suggests that CNTs might be recognized as pathogens.[103] Extrapolation from studies of airborne particulate matter (PM), about which much is already known, might provide insight into the effects of ENMs. For example, PM exposure is associated with lung inflammation and chronic respiratory disease and has been shown to induce a TLR2/TLR4-dependent inflammatory response in the lung *in vivo*.[104,105]

ENM internalization may result in inflammatory responses initiated by the inflammasome group of protein complexes. Following from above, airborne PM induced TLR2/TLR4-dependent inflammatory responses are accompanied by inflammasome activation and ROS signaling.[106] NLRP3 inflammasome activation, triggered by ROS generated by the NADPH oxidase system, can result in the production of (pro-)inflammatory cytokines IL-1β and IL-18 from the direct activation of caspase-1.[107] Inflammasomes have been reported to be activated by both larger particles and ENMs, including silica,[108] amino-functionalized polystyrene ENMs[109] and CNTs,[110,111] as well as a range of plasmonic/metal oxide ENMs, including silver, iron oxide, and titanium dioxide.[95,112−117] Generally, it is unlikely that inflammasome activation is a result of discernment of particular ENMs but rather via the induction of cell stress responses.[107,118−120] Thus, ENMs pose a potential indirect effect upon the inflammasome. Wang et al. demonstrated, *in vitro* and *in vivo*, that glutathione depletion in macrophages exposed to cellulose nanofibers was involved in ROS generation and a (pro-)inflammatory response from lung innate immune cells, that is, epithelial cells.[121,122] It has also been reported that fiber-like aggregates formed by MWCNTs induce the phenomenon known as "frustrated phagocytosis" in macrophages resulting in the production of ROS, NLRP3 activation, and IL-1β secretion *in vitro* in a manner similar to asbestos.[123,124] Such results should be taken with extreme caution, particularly when comparing MWCNTs in general with asbestos fibers. However, from *in vitro* and *in vivo* studies, it has been reported that ROS-dependent NLRP3 activation has a role in lung fibrosis.[125] NADPH oxidase null mice showed a marked increase in neutrophils and apoptotic cells in the lungs in response to SWCNT[126] revealing NADPH to have an important role in orchestrating the transition from acute inflammation to chronic fibrosis in the lung. Nickel contamination of MWCNTs was associated with more robust inflammatory responses in *in vitro* and *in vivo* studies. The nickel contaminated MWCNTs disrupted alveolar macrophage phagolysosomes inducing NLRP3 activation and the release of cathepsin B and HMGB1, an endogenous stress DAMP.[127,128] Gold ENMs were found to bind to HMGB1 inside lysosomes, while carbon ENMs stimulated HMGB1 release from macrophages.[99,129] All of this indicates that ENMs on direct interaction with innate immune cells, namely macrophages, can promote the development of chronic inflammation, which is a significant concern for deducing the safety profile of ENMs.

7. MACROPHAGES

As evident from the above, the role of the macrophage in the innate immune response to ENMs is extensive. While they have deservedly received increased attention in the last two decades, they still command efforts to understanding immune interaction with and response to ENMs. Macrophage plasticity means they can change their function in response to a wide range of environmental cues, giving rise to subpopulations with distinct functions. Macrophages are prodigious phagocytic cells and, as part of their homeostatic role, are responsible for removing cellular debris and clearing erythrocytes and apoptotic cells. These immunologically silent homeostatic processes occur independently of their role in innate immunity.[130] However, cellular debris that occurs as a result of tissue trauma or stress is often "unsilenced" by the release of endogenous danger signals, that is, DAMPs, such as nuclear proteins, heat-shock proteins, histones, and nucleic acids.[7] Macrophages detect DAMPs via surface and intracellular PRRs, and the IL-1 receptor (IL-1R).[7] Under these scenarios, endocytosis by macrophages leads to changes in cell surface proteins and the induction of inflammatory cytokines and mediators.[130]

Macrophages are commonly classified as either classically activated (M1) or alternatively activated (M2).[131] This M1/M2 classification of macrophages is an oversimplification of the spectrum of possible phenotypes and should be employed cautiously as macrophages display dramatic differences in their physiology and biochemistry[132] and can evolve to exhibit characteristics shared by more than one classification.[133] Nevertheless, classically activated M1 macrophages are generally characterized by increased secretion of inflammatory mediators and can be induced from monocytes by cytokines interferon-γ (IFN-γ) and TNF-α.[132] In tissue, IFN-γ is produced at an early stage of the innate immune response by natural killer (NK) cells, causing macrophages to produce reactive oxygen and nitrogen species and inflammatory cytokines.[134] *In situ*, TNF-α will typically be induced by PRR signaling pathways from the macrophages themselves, activating macrophage populations in an autocrine or paracrine manner.[133] The importance of TNF-α is apparent in macrophages as stimulation solely with IFN-γ results in much less effective clearance of microorganisms. To induce a more robust response, macrophages can be stimulated with exogenous TNF-α or PAMPs such as LPS. Activation of PRRs induces transcription factor pathways, including nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STATs), and mitogen activated protein kinases (MAPKs).[135] The activation of M1 macrophages should be tightly controlled as classically activated macrophages produce potentially tissue-damaging cytokines such as IL-1β, IL-6, and IL-23, which can result in the expansion of T-helper (T_H)17 lymphocytes, which subsequently secrete IL-17, inducing tissue-recruitment of neutrophils, leading to tissue damage.[136] Alternate granulocytes, including basophils and mast cells, are important for the production of IL-4, often following tissue injury. Early release of IL-4 promotes a pool of macrophages, commonly referred to as alternatively activated macrophages, that function to promote production of extracellular matrix and wound healing.[131] Macrophages are an important link to adaptive immunity via antigen processing and presentation. Macrophages treated with IL-4 (and/or IL-13) *in vitro* fail to present antigen to T-lymphocytes cells and produce little ROS or

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

inflammatory cytokines. Alternatively activated macrophages can also be detrimental to the host, being implicated in IL-4 driven fibrosis and experimental asthma.[137,138] Macrophage polarization, however, does not seem to impact their interaction with ENMs.[139] Polarization of macrophages toward the classically activated M1 phenotype reportedly enhanced ENM uptake,[68] whereas Hoppstadter et al. demonstrated that alternatively activated differentiated monocytes (dTHP-1) showed enhanced endocytosis of ENMs.[140] Differential uptake by polarized macrophages might be determined by ENM dose or the presence of a protein corona.[141,142]

Functionalized ENMs might even serve to reprogram macrophage polarization.[143] At this time, it is not understood how ENMs could differentially interact with different macrophage phenotypes. This is a key area for future research, in terms of not only of their safety to human health but also the potential medical application of ENMs.

Macrophages either originate from embryo progenitors or differentiate from bone marrow-derived monocytes that circulate in the blood.[49] The majority of the tissue macrophage cache, including embryo-derived, is replenished over time.[49,50] Monocytes migrate from the blood and replenish long-lived tissue-specific macrophages, known as the mononuclear phagocyte system (MPS), including osteoclasts (bone), microglial cells (central nervous system), alveolar cells (lungs), Kupffer cells (liver), and also within the spleen and GI tract. However, the frequency at which these pools are replenished depends on the tissue type. For example, the turnover of embryo derived alveolar macrophages in the lung may take months to years, whereas in the gut, macrophages are turned over rapidly, within days to weeks.[132] There is also evidence that local proliferation of tissue-resident macrophages occurs, sustaining populations of mature macrophages in tissue.[144] This leads to the question of whether tissue macrophage function changes as the macrophage population is replenished and more critically for the purposes of this review how ENMs might impact upon such potentially changed macrophage populations.

8. DENDRITIC CELLS

Although it is clear that further research is needed in terms of deducing the role of macrophage−ENM interactions, the same can be said for DC−ENM interactions. Notable reviews about DCs and their interaction with ENMs have been published recently.[145] However, as these cells are critical in bridging the innate and adaptive immune systems, it is essential to note some key findings here. DCs exist in an immature state in the periphery, becoming mature DCs by the recognition of components of the innate immune system, that is, antigens/PAMPs/DAMPs, loss of their endocytic and phagocytic receptors, and migration to the lymphoid organs for T cell priming.[146] ENMs, such as gold ENMs, readily accumulate in DCs of the spleen after intravenous injection.[147] Different surface modifications of gold ENMs affected DC viability and their inflammatory response.[148] In contrast, DCs exposed to silver ENMs have increased ROS production, decreased viability, but no change was found in inflammatory cytokine production.[149] Negatively charged PEG coated iron (II, III) oxide ENMs activate the peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) pathway in DCs, inducing mitochondrial instability via decreased autophagy.[150] Furthermore, NOD ligands encapsulated into poly(lactic acid) (PLA) biodegradable nanocarriers were efficiently taken up by DCs inducing maturation and inflammatory cytokine secretion.[151] This again highlights how the physicochemical characteristics of ENMs determine how ENMs are recognized and drive the functional effects on the innate immune system. Whether DCs identify and treat ENMs as antigens for processing and presentation to T cells remains to be determined. This is particularly important when considering the many approaches encompassed under the umbrella term of nanomedicine.

9. NATURAL KILLER CELLS

Another critical cell type linking the innate and adaptive immune systems is a group 1 ILC, the NK cell.[152,153] NK cells readily interact with DCs, macrophages, T cells, and epithelial and endothelial cells. NK cells are abundant in blood but exert their function primarily in tissues including secondary lymphoid organs. NK cells are important sentinel immune cells at mucosal surfaces, including the nasal cavity and GI tract, and make up around 10% of lymphocytes in the lung.[154,155] NK cells can induce direct antibody-independent destruction of stressed, abnormal, or virally infected cells via release of granule-dependent cytotoxic mediators such as granzyme B and perforin or via the Fas ligand-mediated (death receptor) pathway or engagement of TNF receptors, leading to apoptosis.[156] NK cell activation is finely tuned resulting in the release of immune activating cytokines, such as IFN-γ, IL-4 and TNF-α, which can act on other innate immune cells, including DCs[157] and epithelial cells[158] and on adaptive immune cells, such as T cells.[156] NK cell activation is orchestrated by surface expression of inhibitory and activating receptors or cytokines including IL-2, IL-12, IL-15, and IL-18. The major inhibitory receptors are specific for human leukocyte antigen (HLA) class I molecules (i.e., major histocompatibility complex (MHC)), preventing NK cells from attacking autologous cells.

NK cells can be grouped into cytokine secreting or cytotoxic subgroups by the expression of the cell adhesion receptor CD56 or the cytotoxic receptors CD16, respectively.[159] Because of their abundance in blood and location at mucosal surfaces, NK cells, in principle, can rapidly interact with exogenous ENMs. Exposure of blood-derived NK cells to silver ENMs reduced their viability and cytotoxic potential. This seemed to be by changing the relative abundance of inhibitory and activating receptors, increased expression of inhibitory CD159a, which binds to HLA class I molecules, and activating CD314, which binds to ligands on epithelial cell surfaces, accompanied by decreased expression of activating CD16.[160] A similar phenotype, reduced viability and reduced CD16 expression, was seen when NK cells were exposed to uncoated zinc oxice ENMs.[161,162] However, *in vivo* studies with silver ENMs conflict with these results, but this might reflect varied experimental approaches, that is, routes of administration and types of ENMs.[163,164] Nonetheless, in the study by Muller et al., silver ENMs did not induce the oxidative stress or the inflammation observed in lung cells *in vitro*.[165] However, Andersson-Willman et al. showed no effect of titanium dioxide on NK cells. *In vivo*, lung exposure to titanium dioxide induced a transient increase in an NK cell population, marked by an increase in T cell-activating cytokine profile, without associated lung pathology of epithelial injury or lung fibrosis.[166] This may indicate that early ENM exposure initiates an innate immune response to which NK cells contribute, potentially culminating in a T cell adaptive immune response. As with all of the cell types discussed herein, there is a fundamental need for more research about NK cell interaction with ENMs.

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

10. MODELS OF THE INNATE IMMUNE SYSTEM

As within the nanosafety community as a whole, primary research into this area previously has focused upon *in vivo* models (i.e., rodents) or *ex vivo* cell systems (i.e., PBMCs) with few assessments using alternative models (i.e., *in vitro* systems). However, recent years have seen more *in vitro* studies on the innate immune response conducted, albeit using cell lines, that is, A549/Calu-3 (lung), Caco-2 (GI tract),[167] or HepG2 (liver),[168] or continuing with the *ex vivo* or modeled "primary" cell-type (e.g., monocyte-derived macrophages). As across the nanosafety arena, it is important to focus upon alternative models and approaches to deduce the mechanistic, biological implications of ENM exposure. However, using only one cell type does not replicate the complex physiology of tissue barriers or the innate immune system *in vivo*. Although advanced *in vitro* systems are available, it is important to note that many multicellular systems often neglect to include important immune cell populations, although key examples where this has been achieved are available.[169] The interaction between different innate immune cells is relatively unexplored yet requires attention to determine how the interplay of these cells upon ENM exposure and how this might affect other cell types within the biological system/environment. There are also novel systems focusing on specific sections of organ anatomy that have been largely ignored to date. For example, there have been attempts to create an *in vitro* model of the nasal epithelium since this will be the first tissue interacting with ENMs following their inhalation.[170] Several approaches are being attempted to establish advanced, nonstatic alternative models to assess the innate immune response to ENMs. Recently, for example, Li et al. have developed a perfusable 3D human microvessel network within a microfluidic device, which partly portrays the physiological response of human vessels.[82] These advances are imperative if we are to fully elucidate the effects of ENM exposure on the innate immune system and thereby human health.

11. SUMMARY AND FUTURE CONSIDERATIONS

In summary, the innate immune system is one of the key pillars of host defense toward foreign bodies that includes xenobiotics and pathogens. It intelligently directs specific cell subsets to interact with, and if necessary null these threats across all organs of the entire human body. It is, as highlighted throughout this perspective, pivotal to any adaptive immune response and thus governs such a response meticulously to provide an efficient and effective response to these foreign, nonself objects in the human body. Alas, despite the clear growth within the nanosafety field of understanding the impact of ENMs upon the innate immune system, many knowledge gaps remain. Specific examples of these knowledge gaps relate to:

- The precise manner in which ENMs interact/bind with key innate immune cells. It is understood that the manner of entry into (immune) cells can contribute toward the hazard noted. To date, the precise entry mechanism has not been defined for ENMs, albeit elucidated for some. Nonetheless, the receptor-mediated approaches are not fully deduced. How ENMs may interact/bind with specific PRRs, on specific innate immune cells, will allow not only the entry mechanism to be noted but also the pathway and signaling cascades that might be relevant to how the cell will, potentially, process the ENM. Thus, entry mechanisms are simply not enough, but specific receptor-binding knowledge is the next step forward in understanding the ENM–immune cell interaction.
- Understanding how ENMs and specifically how their different physicochemical characteristics may impact upon the structure and function of key innate immune cells (i.e., macrophages, neutrophils, DCs, and NK cells).
- Further research must be given toward understanding the above focus points on the currently less studied leukocyte populations (i.e., lymphocytes (both T- and B-cells), eosinophils, basophils, and mast cells) and how ENMs interact with them (potentially impacting their structure and function).
- Deducing how the different (innate) immune cells interplay with one another, in different organs, following ENM exposure/interaction, and how they respond to ENM exposure. This approach befits advanced *in vitro* approaches using multicellular models as well as dynamic, fluid-flow systems to allow effective mimicking of the *in vivo* scenario.
- In combination with the above approach, understanding the complete signaling cascade, following ENM exposure, is essential. Measuring one soluble mediator (e.g., cytokine/chemokine) is not sufficient. Knowledge as to how the immune response adapts to the initial ENM exposure over time, and relative to dose, and then how this impacts upon cell/tissue/organ structure and function are now necessary requirements to deduce the complete impact of ENMs upon the innate immune system.

It is imperative that research is focused upon these areas since it will allow for the elucidation of vital information about both detrimental and beneficial effects of ENM exposure on host defense mechanisms and inform realistic low-dose, long-term, repeated exposure scenarios. Only then will it be possible to realize the advantages of ENMs across the plethora of different disciplines and applications, most notably nano-medicine.

AUTHOR INFORMATION

Corresponding Authors

James G. Cronin − *Immunometabolism & Cancer Research Group, Institute of Life Science, Swansea University Medical School, Swansea, Wales SA2 8PP, U.K.*; orcid.org/0000-0002-0590-9462; Phone: 0044 (0) 1792 606409; Email: j.cronin@swansea.ac.uk

Martin J. D. Clift − *In Vitro Toxicology Group, Institute of Life Science, Swansea University Medical School, Swansea, Wales SA2 8PP, U.K.*; orcid.org/0000-0001-6133-3368; Phone: 0044 (0) 1792 602742; Email: m.j.d.clift@swansea.ac.uk

Authors

Nicholas Jones − *Human Immunology Research Group, Institute of Life Science, Swansea University Medical School, Swansea, Wales SA2 8PP, U.K.*

Catherine A. Thornton − *Human Immunology Research Group, Institute of Life Science, Swansea University Medical School, Swansea, Wales SA2 8PP, U.K.*

Gareth J. S. Jenkins − *In Vitro Toxicology Group, Institute of Life Science, Swansea University Medical School, Swansea, Wales SA2 8PP, U.K.*

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

Shareen H. Doak − *In Vitro Toxicology Group, Institute of Life Science, Swansea University Medical School, Swansea, Wales SA2 8PP, U.K.*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chemrestox.0c00051

Author Contributions

[⊥]JGC and MJDC equally contributed to this work. JGC and MJDC were responsible for conceiving the subject idea and writing the manuscript. All authors contributed toward iteration of the manuscript. All authors have acknowledged and approved the final submitted version of the manuscript.

Funding

The authors would like to acknowledge the EU Horizon2020 Project PATROLS (Grant Agreement No. 760813).

Notes

The authors declare no competing financial interest.

Biographies

James Cronin obtained a BSc in Medical Microbiology from King's College London, an MSc from the School of Medicine, Cardiff University, and was awarded a PhD from Swansea University Medical School for investigating the role chronic inflammation plays in throat cancer progression. James has keen interests in the roles the innate immune system and metabolism play in disease progression. In particular, James' group focuses on the aberrant cell signaling pathways that lead to chronic inflammatory diseases and resistance to chemotherapy in cancer.

Nick Jones is a lecturer within the Human Immunology research group at Swansea University Medical School. Nick received his PhD in 2017 from Swansea University, where he has molded his research focus upon the role of metabolism within innate immunity.

Cathy Thornton is a Professor of Human Immunology, and Deputy Head of Swansea University Medical School (SUMS). Her research interest focuses upon pregnancy and early childhood. This centers on identifying how antenatal environmental determinants affect the inflammatory response in the placenta and the development of immune function in the fetus for later effects on child health. Cathy has authored >80 original peer-reviewed papers and attracted > £6million of research income. She embeds public and patient involvement in her research that aims to benefit pregnant women, their families, and healthcare service provision to improve the health of future generations.

Gareth J. S. Jenkins is a Professor of Genetic Toxicology at SUMS and has extensive expertise in DNA mutation and cancer. He has been studying DNA mutation in vitro for over 20 years and has published over 100 peer-reviewed papers on this topic. He is also pursuing DNA mutation as a biomarker in early cancer diagnosis. He coleads the IVTG and has sat on the UK Government Committee on Mutagenicity (COM) since 2009. He is a long-standing member of both the UK Environmental Mutagen Society (Current Chair) and the British Association for Cancer Research and is currently a Senior Editor of the journal *Mutagenesis*.

Shareen H. Doak is a Professor of Genotoxicology and Cancer in Swansea University Medical School's *In Vitro* Toxicology Group. Shareen is a UK and EUROTOX Registered Toxicologist, Fellow of the Royal Society of Biology (FRSB), and Fellow of the Learned Society of Wales (FLSW). Her research interests focus on genotoxic profiles of engineered nanomaterials, mechanisms underlying their DNA damaging potential, and subsequent human health consequences. Her interests extend to development of advanced 3D culture models and mechanism-based bioassays for safety assessment to reduce the need for animal testing. Shareen sits on the UK Government Committee on Mutagenicity (COM) and is *Mutagenesis* Editor-in-Chief.

Martin J. D. Clift is an Associate Professor of *in vitro* (nano)particle toxicology at SUMS. His research focuses upon the development of advanced *in vitro* models to understand the human health hazard associated with inhalation exposure to nanomaterials. Martin has >120 publications and has received > £4.5 million in research funding. He is Editor-in-Chief for *Fibers*, an Associate Editor for *Journal of Nanobiotechnology*, and an editorial board member for *Particle and Fibre Toxicology*. Martin is Chair of the UK *In Vitro* Toxicology Society (IVTS) and is a member of the British Toxicology Society (BTS) Sub-Scientific Committee and of the Scientific Board of Animal Free Research (AfR).

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the collaborative discussions across the different research groups upon this topic and how these facilitate crosstalk between disciplines to gain a more encompassing and considered approach toward deducing the impact of ENMs on the human immune system.

■ REFERENCES

(1) Thaiss, C. A., Zmora, N., Levy, M., and Elinav, E. (2016) The microbiome and innate immunity. *Nature 535*, 65−74.
(2) Bustamante-Marin, X. M., and Ostrowski, L. E. (2017) Cilia and Mucociliary Clearance. *Cold Spring Harbor Perspect. Biol. 9*, a028241.
(3) Kreyling, W. G., Hirn, S., Moller, W., Schleh, C., Wenk, A., Celik, G., Lipka, J., Schaffler, M., Haberl, N., Johnston, B. D., Sperling, R., Schmid, G., Simon, U., Parak, W. J., and Semmler-Behnke, M. (2014) Air-blood barrier translocation of tracheally instilled gold nanoparticles inversely depends on particle size. *ACS Nano 8*, 222−233.
(4) Rothen-Rutishauser, B., Muhlfeld, C., Blank, F., Musso, C., and Gehr, P. (2007) Translocation of particles and inflammatory responses after exposure to fine particles and nanoparticles in an epithelial airway model. *Part. Fibre Toxicol. 4*, 9.
(5) Gordon, S. (2016) Phagocytosis: An Immunobiologic Process. *Immunity 44*, 463−475.
(6) Chow, J., Franz, K. M., and Kagan, J. C. (2015) PRRs are watching you: Localization of innate sensing and signaling regulators. *Virology 479−480*, 104−109.
(7) Bianchi, M. E. (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. *J. Leukocyte Biol. 81*, 1−5.
(8) Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., and Danquah, M. K. (2018) Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein J. Nanotechnol. 9*, 1050−1074.
(9) Stone, V., Miller, M. R., Clift, M. J. D., Elder, A., Mills, N. L., Moller, P., Schins, R. P. F., Vogel, U., Kreyling, W. G., Alstrup Jensen, K., Kuhlbusch, T. A. J., Schwarze, P. E., Hoet, P., Pietroiusti, A., De Vizcaya-Ruiz, A., Baeza-Squiban, A., Teixeira, J. P., Tran, C. L., and Cassee, F. R. (2017) Nanomaterials Versus Ambient Ultrafine Particles: An Opportunity to Exchange Toxicology Knowledge. *Environ. Health Perspect. 125*, 106002.
(10) Krug, H. F. (2014) Nanosafety research–are we on the right track? *Angew. Chem., Int. Ed. 53*, 12304−12319.
(11) Pallardy, M. J., Turbica, I., and Biola-Vidamment, A. (2017) Why the Immune System Should Be Concerned by Nanomaterials? *Front. Immunol. 8*, 544.
(12) Fadeel, B. (2019) Hide and Seek: Nanomaterial Interactions With the Immune System. *Front. Immunol. 10*, 133.
(13) Halamoda-Kenzaoui, B., and Bremer-Hoffmann, S. (2018) Main trends of immune effects triggered by nanomedicines in preclinical studies. *Int. J. Nanomed. 13*, 5419−5431.

(14) Rothen-Rutishauser, B., Lehmann, A. D., Clift, M. J., Blank, F., and Gehr, P. (2010) Laser scanning microscopy combined with image restoration to analyse a 3D model of the human epithelial airway barrier. *Swiss Med. Wkly. 140*, No. w13060.
(15) Oberdorster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., Karn, B., Kreyling, W., Lai, D., Olin, S., Monteiro-Riviere, N., Warheit, D., and Yang, H. (2005) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part. Fibre Toxicol. 2*, 8.
(16) Rao, R. (2009) Occludin Phosphorylation in Regulation of Epithelial Tight Junctions. *Ann. N. Y. Acad. Sci. 1165*, 62−68.
(17) Bachler, G., Losert, S., Umehara, Y., von Goetz, N., Rodriguez-Lorenzo, L., Petri-Fink, A., Rothen-Rutishauser, B., and Hungerbuehler, K. (2015) Translocation of gold nanoparticles across the lung epithelial tissue barrier: Combining in vitro and in silico methods to substitute in vivo experiments. *Part. Fibre Toxicol. 12*, 18.
(18) Hussain, N., and Florence, A. T. (1998) Utilizing bacterial mechanisms of epithelial cell entry: invasin-induced oral uptake of latex nanoparticles. *Pharm. Res. 15*, 153−156.
(19) Munoz, L. E., Bilyy, R., Biermann, M. H. C., Kienhofer, D., Maueroder, C., Hahn, J., Brauner, J. M., Weidner, D., Chen, J., Scharin-Mehlmann, M., Janko, C., Friedrich, R. P., Mielenz, D., Dumych, T., Lootsik, M. D., Schauer, C., Schett, G., Hoffmann, M., Zhao, Y., and Herrmann, M. (2016) Nanoparticles size-dependently initiate self-limiting NETosis-driven inflammation. *Proc. Natl. Acad. Sci. U. S. A. 113*, E5856−E5865.
(20) Orr, S. E., Gokulan, K., Boudreau, M., Cerniglia, C. E., and Khare, S. (2019) Alteration in the mRNA expression of genes associated with gastrointestinal permeability and ileal TNF-alpha secretion due to the exposure of silver nanoparticles in Sprague-Dawley rats. *J. Nanobiotechnol. 17*, 63.
(21) Powell, J. J., Faria, N., Thomas-McKay, E., and Pele, L. C. (2010) Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract. *J. Autoimmun. 34*, J226−233.
(22) Burden, N., Aschberger, K., Chaudhry, Q., Clift, M. J. D., Fowler, P., Johnston, H., Landsiedel, R., Rowland, J., Stone, V., and Doak, S. H. (2017) Aligning nanotoxicology with the 3Rs: What is needed to realise the short, medium and long-term opportunities? *Regul. Toxicol. Pharmacol. 91*, 257−266.
(23) Brun, E., Barreau, F., Veronesi, G., Fayard, B., Sorieul, S., Chaneac, C., Carapito, C., Rabilloud, T., Mabondzo, A., Herlin-Boime, N., and Carriere, M. (2014) Titanium dioxide nanoparticle impact and translocation through ex vivo, in vivo and in vitro gut epithelia. *Part. Fibre Toxicol. 11*, 13.
(24) Dorier, M., Tisseyre, C., Dussert, F., Beal, D., Arnal, M. E., Douki, T., Valdiglesias, V., Laffon, B., Fraga, S., Brandao, F., Herlin-Boime, N., Barreau, F., Rabilloud, T., and Carriere, M. (2019) Toxicological impact of acute exposure to E171 food additive and TiO2 nanoparticles on a co-culture of Caco-2 and HT29-MTX intestinal cells. *Mutat. Res., Genet. Toxicol. Environ. Mutagen. 845*, 402980.
(25) Guo, Z., Martucci, N. J., Moreno-Olivas, F., Tako, E., and Mahler, G. J. (2017) Titanium Dioxide Nanoparticle Ingestion Alters Nutrient Absorption in an In Vitro Model of the Small Intestine. *NanoImpact 5*, 70−82.
(26) Burden, N., Aschberger, K., Chaudhry, Q., Clift, M. J. D., Doak, S. H., Fowler, P., Johnston, H., Landsiedel, R., Rowland, J., and Stone, V. (2017) The 3Rs as a framework to support a 21st century approach for nanosafety assessment. *Nano Today 12*, 10−13.
(27) Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., Koyasu, S., Locksley, R. M., McKenzie, A. N., Mebius, R. E., Powrie, F., and Vivier, E. (2013) Innate lymphoid cells–a proposal for uniform nomenclature. *Nat. Rev. Immunol. 13*, 145−149.
(28) Mao, K., Baptista, A. P., Tamoutounour, S., Zhuang, L., Bouladoux, N., Martins, A. J., Huang, Y., Gerner, M. Y., Belkaid, Y., and Germain, R. N. (2018) Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. *Nature 554*, 255−259.
(29) Gasteiger, G., Fan, X., Dikiy, S., Lee, S. Y., and Rudensky, A. Y. (2015) Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science 350*, 981−985.
(30) Stier, M. T., Zhang, J., Goleniewska, K., Cephus, J. Y., Rusznak, M., Wu, L., Van Kaer, L., Zhou, B., Newcomb, D. C., and Peebles, R. S., Jr. (2018) IL-33 promotes the egress of group 2 innate lymphoid cells from the bone marrow. *J. Exp. Med. 215*, 263−281.
(31) El-Sayed, Y. S., Shimizu, R., Onoda, A., Takeda, K., and Umezawa, M. (2015) Carbon black nanoparticle exposure during middle and late fetal development induces immune activation in male offspring mice. *Toxicology 327*, 53−61.
(32) Licona-Limon, P., Kim, L. K., Palm, N. W., and Flavell, R. A. (2013) TH2, allergy and group 2 innate lymphoid cells. *Nat. Immunol. 14*, 536−542.
(33) Walker, J. A., Barlow, J. L., and McKenzie, A. N. (2013) Innate lymphoid cells–how did we miss them? *Nat. Rev. Immunol. 13*, 75−87.
(34) Gour, N., and Wills-Karp, M. (2015) IL-4 and IL-13 signaling in allergic airway disease. *Cytokine+ 75*, 68−78.
(35) Nygaard, U. C., Hansen, J. S., Samuelsen, M., Alberg, T., Marioara, C. D., and Lovik, M. (2009) Single-walled and multi-walled carbon nanotubes promote allergic immune responses in mice. *Toxicol. Sci. 109*, 113−123.
(36) Brandenberger, C., Rowley, N. L., Jackson-Humbles, D. N., Zhang, Q., Bramble, L. A., Lewandowski, R. P., Wagner, J. G., Chen, W., Kaplan, B. L., Kaminski, N. E., Baker, G. L., Worden, R. M., and Harkema, J. R. (2013) Engineered silica nanoparticles act as adjuvants to enhance allergic airway disease in mice. *Part. Fibre Toxicol. 10*, 26.
(37) Jonasson, S., Gustafsson, A., Koch, B., and Bucht, A. (2013) Inhalation exposure of nano-scaled titanium dioxide (TiO2) particles alters the inflammatory responses in asthmatic mice. *Inhalation Toxicol. 25*, 179−191.
(38) Roy, R., Kumar, S., Verma, A. K., Sharma, A., Chaudhari, B. P., Tripathi, A., Das, M., and Dwivedi, P. D. (2014) Zinc oxide nanoparticles provide an adjuvant effect to ovalbumin via a Th2 response in Balb/c mice. *Int. Immunol. 26*, 159−172.
(39) Beamer, C. A., Girtsman, T. A., Seaver, B. P., Finsaas, K. J., Migliaccio, C. T., Perry, V. K., Rottman, J. B., Smith, D. E., and Holian, A. (2012) IL-33 mediates multi-walled carbon nanotube (MWCNT)-induced airway hyper-reactivity via the mobilization of innate helper cells in the lung. *Nanotoxicology 7*, 1070−1081.
(40) Abukabda, A. B., McBride, C. R., Batchelor, T. P., Goldsmith, W. T., Bowdridge, E. C., Garner, K. L., Friend, S., and Nurkiewicz, T. R. (2018) Group II innate lymphoid cells and microvascular dysfunction from pulmonary titanium dioxide nanoparticle exposure. *Part. Fibre Toxicol. 15*, 43.
(41) Stone, K. D., Prussin, C., and Metcalfe, D. D. (2010) IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol. 125*, S73−80.
(42) Zhang, N., Pan, H. F., and Ye, D. Q. (2011) Th22 in inflammatory and autoimmune disease: prospects for therapeutic intervention. *Mol. Cell. Biochem. 353*, 41−46.
(43) Vely, F., Barlogis, V., Vallentin, B., Neven, B., Piperoglou, C., Ebbo, M., Perchet, T., Petit, M., Yessaad, N., Touzot, F., Bruneau, J., Mahlaoui, N., Zucchini, N., Farnarier, C., Michel, G., Moshous, D., Blanche, S., Dujardin, A., Spits, H., Distler, J. H., Ramming, A., Picard, C., Golub, R., Fischer, A., and Vivier, E. (2016) Evidence of innate lymphoid cell redundancy in humans. *Nat. Immunol. 17*, 1291−1299.
(44) Oh, J. Y., Kim, H. S., Palanikumar, L., Go, E. M., Jana, B., Park, S. A., Kim, H. Y., Kim, K., Seo, J. K., Kwak, S. K., Kim, C., Kang, S., and Ryu, J. H. (2018) Cloaking nanoparticles with protein corona shield for targeted drug delivery. *Nat. Commun. 9*, 4548.
(45) Maiolo, D., Del Pino, P., Metrangolo, P., Parak, W. J., and Bombelli, F. B. (2015) Nanomedicine delivery: does protein corona route to the target or off road? *Nanomedicine 10*, 3231−3247.
(46) Mortimer, G. M., Butcher, N. J., Musumeci, A. W., Deng, Z. J., Martin, D. J., and Minchin, R. F. (2014) Cryptic epitopes of albumin determine mononuclear phagocyte system clearance of nanomaterials. *ACS Nano 8*, 3357−3366.

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

(47) Tenzer, S., Docter, D., Kuharev, J., Musyanovych, A., Fetz, V., Hecht, R., Schlenk, F., Fischer, D., Kiouptsi, K., Reinhardt, C., Landfester, K., Schild, H., Maskos, M., Knauer, S. K., and Stauber, R. H. (2013) Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat. Nanotechnol. 8*, 772−781.
(48) Hu, W., Peng, C., Lv, M., Li, X., Zhang, Y., Chen, N., Fan, C., and Huang, Q. (2011) Protein corona-mediated mitigation of cytotoxicity of graphene oxide. *ACS Nano 5*, 3693−3700.
(49) Corbo, C., Molinaro, R., Parodi, A., Toledano Furman, N. E., Salvatore, F., and Tasciotti, E. (2016) The impact of nanoparticle protein corona on cytotoxicity, immunotoxicity and target drug delivery. *Nanomedicine (London, U. K.) 11*, 81−100.
(50) Salvati, A., Pitek, A. S., Monopoli, M. P., Prapainop, K., Bombelli, F. B., Hristov, D. R., Kelly, P. M., Aberg, C., Mahon, E., and Dawson, K. A. (2013) Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat. Nanotechnol. 8*, 137−143.
(51) Vu, V. P., Gifford, G. B., Chen, F., Benasutti, H., Wang, G., Groman, E. V., Scheinman, R., Saba, L., Moghimi, S. M., and Simberg, D. (2019) Immunoglobulin deposition on biomolecule corona determines complement opsonization efficiency of preclinical and clinical nanoparticles. *Nat. Nanotechnol. 14*, 260−268.
(52) Ricklin, D., Hajishengallis, G., Yang, K., and Lambris, J. D. (2010) Complement: a key system for immune surveillance and homeostasis. *Nat. Immunol. 11*, 785−797.
(53) Lundqvist, M., Augustsson, C., Lilja, M., Lundkvist, K., Dahlback, B., Linse, S., and Cedervall, T. (2017) The nanoparticle protein corona formed in human blood or human blood fractions. *PLoS One 12*, No. e0175871.
(54) Chen, F., Wang, G., Griffin, J. I., Brenneman, B., Banda, N. K., Holers, V. M., Backos, D. S., Wu, L., Moghimi, S. M., and Simberg, D. (2017) Complement proteins bind to nanoparticle protein corona and undergo dynamic exchange in vivo. *Nat. Nanotechnol. 12*, 387−393.
(55) Flannagan, R. S., Jaumouille, V., and Grinstein, S. (2012) The cell biology of phagocytosis. *Annu. Rev. Pathol.: Mech. Dis. 7*, 61−98.
(56) Helming, L., Winter, J., and Gordon, S. (2009) The scavenger receptor CD36 plays a role in cytokine-induced macrophage fusion. *J. Cell Sci. 122*, 453−459.
(57) Andersson, J., Ekdahl, K. N., Lambris, J. D., and Nilsson, B. (2005) Binding of C3 fragments on top of adsorbed plasma proteins during complement activation on a model biomaterial surface. *Biomaterials 26*, 1477−1485.
(58) Peracchia, M. T., Fattal, E., Desmaele, D., Besnard, M., Noel, J. P., Gomis, J. M., Appel, M., d'Angelo, J., and Couvreur, P. (1999) Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. *J. Controlled Release 60*, 121−128.
(59) Amoozgar, Z., and Yeo, Y. (2012) Recent advances in stealth coating of nanoparticle drug delivery systems. *Wiley Interdiscip Rev. Nanomed Nanobiotechnol 4*, 219−233.
(60) Moghimi, S. M., and Szebeni, J. (2003) Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. *Prog. Lipid Res. 42*, 463−478.
(61) Bazile, D., Prud'homme, C., Bassoullet, M. T., Marlard, M., Spenlehauer, G., and Veillard, M. (1995) Stealth Me.PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. *J. Pharm. Sci. 84*, 493−498.
(62) Schottler, S., Becker, G., Winzen, S., Steinbach, T., Mohr, K., Landfester, K., Mailander, V., and Wurm, F. R. (2016) Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. *Nat. Nanotechnol. 11*, 372−377.
(63) Garcia, K. P., Zarschler, K., Barbaro, L., Barreto, J. A., O'Malley, W., Spiccia, L., Stephan, H., and Graham, B. (2014) Zwitterionic-Coated "Stealth" Nanoparticles for Biomedical Applications: Recent Advances in Countering Biomolecular Corona Formation and Uptake by the Mononuclear Phagocyte System. *Small 10*, 2516−2529.
(64) Ye, L., Zhang, Y., Yang, B., Zhou, X., Li, J., Qin, Z., Dong, D., Cui, Y., and Yao, F. (2016) Zwitterionic-Modified Starch-Based Stealth Micelles for Prolonging Circulation Time and Reducing Macrophage Response. *ACS Appl. Mater. Interfaces 8*, 4385−4398.
(65) Zhang, P., Sun, F., Tsao, C., Liu, S., Jain, P., Sinclair, A., Hung, H. C., Bai, T., Wu, K., and Jiang, S. (2015) Zwitterionic gel encapsulation promotes protein stability, enhances pharmacokinetics, and reduces immunogenicity. *Proc. Natl. Acad. Sci. U. S. A. 112*, 12046−12051.
(66) Dobrovolskaia, M. A., Aggarwal, P., Hall, J. B., and McNeil, S. E. (2008) Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. *Mol. Pharmaceutics 5*, 487−495.
(67) Unfried, K., Albrecht, C., Klotz, L. O., Von Mikecz, A., Grether-Beck, S., and Schins, R. P. F. (2007) Cellular responses to nanoparticles: Target structures and mechanisms. *Nanotoxicology 1*, 52−71.
(68) Qie, Y., Yuan, H., von Roemeling, C. A., Chen, Y., Liu, X., Shih, K. D., Knight, J. A., Tun, H. W., Wharen, R. E., Jiang, W., and Kim, B. Y. (2016) Surface modification of nanoparticles enables selective evasion of phagocytic clearance by distinct macrophage phenotypes. *Sci. Rep. 6*, 26269.
(69) Rattan, R., Bhattacharjee, S., Zong, H., Swain, C., Siddiqui, M. A., Visovatti, S. H., Kanthi, Y., Desai, S., Pinsky, D. J., and Goonewardena, S. N. (2017) Nanoparticle-macrophage interactions: A balance between clearance and cell-specific targeting. *Bioorg. Med. Chem. 25*, 4487−4496.
(70) Oberdorster, G., Oberdorster, E., and Oberdorster, J. (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect. 113*, 823−839.
(71) Shang, L., Nienhaus, K., and Nienhaus, G. U. (2014) Engineered nanoparticles interacting with cells: size matters. *J. Nanobiotechnol. 12*, 5.
(72) Champion, J. A., and Mitragotri, S. (2006) Role of target geometry in phagocytosis. *Proc. Natl. Acad. Sci. U. S. A. 103*, 4930−4934.
(73) Richards, D. M., and Endres, R. G. (2016) Target shape dependence in a simple model of receptor-mediated endocytosis and phagocytosis. *Proc. Natl. Acad. Sci. U. S. A. 113*, 6113−6118.
(74) Zheng, M., and Yu, J. (2016) The effect of particle shape and size on cellular uptake. *Drug Delivery Transl. Res. 6*, 67−72.
(75) Agudo-Canalejo, J., and Lipowsky, R. (2015) Critical Particle Sizes for the Engulfment of Nanoparticles by Membranes and Vesicles with Bilayer Asymmetry. *ACS Nano 9*, 3704−3720.
(76) Yang, K., and Ma, Y. Q. (2010) Computer simulation of the translocation of nanoparticles with different shapes across a lipid bilayer. *Nat. Nanotechnol. 5*, 579−583.
(77) Poland, C. A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W. A. H., Seaton, A., Stone, V., Brown, S., MacNee, W., and Donaldson, K. (2008) Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat. Nanotechnol. 3*, 423−428.
(78) Bisso, P. W., Gaglione, S., Guimaraes, P. P. G., Mitchell, M. J., and Langer, R. (2018) Nanomaterial Interactions with Human Neutrophils. *ACS Biomater. Sci. Eng. 4*, 4255−4265.
(79) Goncalves, D. M., Chiasson, S., and Girard, D. (2010) Activation of human neutrophils by titanium dioxide (TiO2) nanoparticles. *Toxicol. In Vitro 24*, 1002−1008.
(80) Bonner, J. C., Silva, R. M., Taylor, A. J., Brown, J. M., Hilderbrand, S. C., Castranova, V., Porter, D., Elder, A., Oberdorster, G., Harkema, J. R., Bramble, L. A., Kavanagh, T. J., Botta, D., Nel, A., and Pinkerton, K. E. (2013) Interlaboratory Evaluation of Rodent Pulmonary Responses to Engineered Nanomaterials: The NIEHS Nano GO Consortium. *Environ. Health Perspect. 121*, 676−682.
(81) Noel, A., Charbonneau, M., Cloutier, Y., Tardif, R., and Truchon, G. (2013) Rat pulmonary responses to inhaled nano-TiO2: effect of primary particle size and agglomeration state. *Part. Fibre Toxicol. 10*, 48.

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

(82) Li, B., Ze, Y. G., Sun, Q. Q., Zhang, T., Sang, X. Z., Cui, Y. L., Wang, X. C., Gui, S. X., Tan, D. L., Zhu, M., Zhao, X. Y., Sheng, L., Wang, L., Hong, F. S., and Tang, M. (2013) Molecular Mechanisms of Nanosized Titanium Dioxide-Induced Pulmonary Injury in Mice. *PLoS One 8*, e55563.
(83) Fuchs, T. A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., Weinrauch, Y., Brinkmann, V., and Zychlinsky, A. (2007) Novel cell death program leads to neutrophil extracellular traps. *J. Cell Biol. 176*, 231−241.
(84) von Kockritz-Blickwede, M., Goldmann, O., Thulin, P., Heinemann, K., Norrby-Teglund, A., Rohde, M., and Medina, E. (2008) Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. *Blood 111*, 3070−3080.
(85) Kagan, V. E., Konduru, N. V., Feng, W., Allen, B. L., Conroy, J., Volkov, Y., Vlasova, II, Belikova, N. A., Yanamala, N., Kapralov, A., Tyurina, Y. Y., Shi, J., Kisin, E. R., Murray, A. R., Franks, J., Stolz, D., Gou, P., Klein-Seetharaman, J., Fadeel, B., Star, A., and Shvedova, A. A. (2010) Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. *Nat. Nanotechnol. 5*, 354−359.
(86) Bartneck, M., Keul, H. A., Zwadlo-Klarwasser, G., and Groll, J. (2010) Phagocytosis Independent Extracellular Nanoparticle Clearance by Human Immune Cells. *Nano Lett. 10*, 59−63.
(87) Rizzi, M., Carniato, F., Tonello, S., Migliario, M., Invernizzi, M., Rocchetti, V., Marchese, L., and Reno, F. (2018) Charged molecular silica trigger in vitro NETosis in human granulocytes via both oxidative and autophagic pathways. *Eur. Rev. Med. Pharmaco 22*, 7058−7068.
(88) Liz, R., Simard, J. C., Leonardi, L. B. A., and Girard, D. (2015) Silver nanoparticles rapidly induce atypical human neutrophil cell death by a process involving inflammatory caspases and reactive oxygen species and induce neutrophil extracellular traps release upon cell adhesion. *Int. Immunopharmacol. 28*, 616−625.
(89) Hwang, T. L., Aljuffali, I. A., Hung, C. F., Chen, C. H., and Fang, J. Y. (2015) The impact of cationic solid lipid nanoparticles on human neutrophil activation and formation of neutrophil extracellular traps (NETs). *Chem.-Biol. Interact. 235*, 106−114.
(90) Schauer, C., Janko, C., Munoz, L. E., Zhao, Y., Kienhofer, D., Frey, B., Lell, M., Manger, B., Rech, J., Naschberger, E., Holmdahl, R., Krenn, V., Harrer, T., Jeremic, I., Bilyy, R., Schett, G., Hoffmann, M., and Herrmann, M. (2014) Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. *Nat. Med. 20*, 515−521.
(91) Biermann, M. H. C., Podolska, M. J., Knopf, J., Reinwald, C., Weidner, D., Maueroder, C., Hahn, J., Kienhofer, D., Barras, A., Boukherroub, R., Szunerits, S., Bilyy, R., Hoffmann, M., Zhao, Y., Schett, G., Herrmann, M., and Munoz, L. E. (2016) Oxidative Burst-Dependent NETosisis Is Implicated in the Resolution of Necrosis-Associated Sterile Inflammation. *Front. Immunol. 7*, 557.
(92) Petersen, L. K., Ramer-Tait, A. E., Broderick, S. R., Kong, C. S., Ulery, B. D., Rajan, K., Wannemuehler, M. J., and Narasimhan, B. (2011) Activation of innate immune responses in a pathogen-mimicking manner by amphiphilic polyanhydride nanoparticle adjuvants. *Biomaterials 32*, 6815−6822.
(93) Turner, J. R. (2009) Intestinal mucosal barrier function in health and disease. *Nat. Rev. Immunol. 9*, 799−809.
(94) Wlodarska, M., Thaiss, C. A., Nowarski, R., Henao-Mejia, J., Zhang, J. P., Brown, E. M., Frankel, G., Levy, M., Katz, M. N., Philbrick, W. M., Elinav, E., Finlay, B. B., and Flavell, R. A. (2014) NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell 156*, 1045−1059.
(95) Shirasuna, K., Karasawa, T., and Takahashi, M. (2019) Exogenous nanoparticles and endogenous crystalline molecules as danger signals for the NLRP3 inflammasomes. *J. Cell. Physiol. 234*, 5436−5450.
(96) Jo, E. K., Kim, J. K., Shin, D. M., and Sasakawa, C. (2016) Molecular mechanisms regulating NLRP3 inflammasome activation. *Cell. Mol. Immunol. 13*, 148−159.
(97) Bastus, N. G., Sanchez-Tillo, E., Pujals, S., Farrera, C., Kogan, M. J., Giralt, E., Celada, A., Lloberas, J., and Puntes, V. (2009) Peptides conjugated to gold nanoparticles induce macrophage activation. *Mol. Immunol. 46*, 743−748.
(98) Qu, G., Liu, S., Zhang, S., Wang, L., Wang, X., Sun, B., Yin, N., Gao, X., Xia, T., Chen, J. J., and Jiang, G. B. (2013) Graphene oxide induces toll-like receptor 4 (TLR4)-dependent necrosis in macrophages. *ACS Nano 7*, 5732−5745.
(99) Tsai, C. Y., Lu, S. L., Hu, C. W., Yeh, C. S., Lee, G. B., and Lei, H. Y. (2012) Size-dependent attenuation of TLR9 signaling by gold nanoparticles in macrophages. *J. Immunol. 188*, 68−76.
(100) Konduru, N. V., Tyurina, Y. Y., Feng, W., Basova, L. V., Belikova, N. A., Bayir, H., Clark, K., Rubin, M., Stolz, D., Vallhov, H., Scheynius, A., Witasp, E., Fadeel, B., Kichambare, P. D., Star, A., Kisin, E. R., Murray, A. R., Shvedova, A. A., and Kagan, V. E. (2009) Phosphatidylserine targets single-walled carbon nanotubes to professional phagocytes in vitro and in vivo. *PLoS One 4*, No. e4398.
(101) Kostarelos, K., Lacerda, L., Pastorin, G., Wu, W., Wieckowski, S., Luangsivilay, J., Godefroy, S., Pantarotto, D., Briand, J. P., Muller, S., Prato, M., and Bianco, A. (2007) Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. *Nat. Nanotechnol. 2*, 108−113.
(102) Chen, S., Xiong, C., Liu, H., Wan, Q., Hou, J., He, Q., Badu-Tawiah, A., and Nie, Z. (2015) Mass spectrometry imaging reveals the sub-organ distribution of carbon nanomaterials. *Nat. Nanotechnol. 10*, 176−182.
(103) Turabekova, M., Rasulev, B., Theodore, M., Jackman, J., Leszczynska, D., and Leszczynski, J. (2014) Immunotoxicity of nanoparticles: a computational study suggests that CNTs and C-60 fullerenes might be recognized as pathogens by Toll-like receptors. *Nanoscale 6*, 3488−3495.
(104) Shoenfelt, J., Mitkus, R. J., Zeisler, R., Spatz, R. O., Powell, J., Fenton, M. J., Squibb, K. A., and Medvedev, A. E. (2009) Involvement of TLR2 and TLR4 in inflammatory immune responses induced by fine and coarse ambient air particulate matter. *J. Leukocyte Biol. 86*, 303−312.
(105) Zhao, C., Liao, J., Chu, W., Wang, S., Yang, T., Tao, Y., and Wang, G. (2012) Involvement of TLR2 and TLR4 and Th1/Th2 shift in inflammatory responses induced by fine ambient particulate matter in mice. *Inhalation Toxicol. 24*, 918−927.
(106) Lee, C. W., Chi, M. C., Hsu, L. F., Yang, C. M., Hsu, T. H., Chuang, C. C., Lin, W. N., Chu, P. M., and Lee, I. T. (2019) Carbon monoxide releasing molecule-2 protects against particulate matter-induced lung inflammation by inhibiting TLR2 and 4/ROS/NLRP3 inflammasome activation. *Mol. Immunol. 112*, 163−174.
(107) Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science 320*, 674−677.
(108) Morishige, T., Yoshioka, Y., Inakura, H., Tanabe, A., Yao, X., Narimatsu, S., Monobe, Y., Imazawa, T., Tsunoda, S., Tsutsumi, Y., Mukai, Y., Okada, N., and Nakagawa, S. (2010) The effect of surface modification of amorphous silica particles on NLRP3 inflammasome mediated IL-1beta production, ROS production and endosomal rupture. *Biomaterials 31*, 6833−6842.
(109) Lunov, O., Syrovets, T., Loos, C., Nienhaus, G. U., Mailander, V., Landfester, K., Rouis, M., and Simmet, T. (2011) Amino-Functionalized Polystyrene Nanoparticles Activate the NLRP3 Inflammasome in Human Macrophages. *ACS Nano 5*, 9648−9657.
(110) Wang, X., Xia, T., Duch, M. C., Ji, Z., Zhang, H., Li, R., Sun, B., Lin, S., Meng, H., Liao, Y. P., Wang, M., Song, T. B., Yang, Y., Hersam, M. C., and Nel, A. E. (2012) Pluronic F108 coating decreases the lung fibrosis potential of multiwall carbon nanotubes by reducing lysosomal injury. *Nano Lett. 12*, 3050−3061.
(111) Yang, M., Flavin, K., Kopf, I., Radics, G., Hearnden, C. H., McManus, G. J., Moran, B., Villalta-Cerdas, A., Echegoyen, L. A., Giordani, S., and Lavelle, E. C. (2013) Functionalization of carbon nanoparticles modulates inflammatory cell recruitment and NLRP3 inflammasome activation. *Small 9*, 4194−4206.

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

(112) Apopa, P. L., Qian, Y., Shao, R., Guo, N. L., Schwegler-Berry, D., Pacurari, M., Porter, D., Shi, X. L., Vallyathan, V., Castranova, V., and Flynn, D. C. (2009) Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling. *Part. Fibre Toxicol. 6*, 1.
(113) Simard, J. C., Vallieres, F., de Liz, R., Lavastre, V., and Girard, D. (2015) Silver nanoparticles induce degradation of the endoplasmic reticulum stress sensor activating transcription factor-6 leading to activation of the NLRP-3 inflammasome. *J. Biol. Chem. 290*, 5926−5939.
(114) Ruiz, P. A., Moron, B., Becker, H. M., Lang, S., Atrott, K., Spalinger, M. R., Scharl, M., Wojtal, K. A., Fischbeck-Terhalle, A., Frey-Wagner, I., Hausmann, M., Kraemer, T., and Rogler, G. (2017) Titanium dioxide nanoparticles exacerbate DSS-induced colitis: role of the NLRP3 inflammasome. *Gut 66*, 1216−1224.
(115) Tsugita, M., Morimoto, N., and Nakayama, M. (2017) SiO2 and TiO2 nanoparticles synergistically trigger macrophage inflammatory responses. *Part. Fibre Toxicol. 14*, 11.
(116) Winkler, H. C., Kornprobst, J., Wick, P., von Moos, L. M., Trantakis, I., Schraner, E. M., Bathke, B., Hochrein, H., Suter, M., and Naegeli, H. (2017) MyD88-dependent pro-interleukin-1beta induction in dendritic cells exposed to food-grade synthetic amorphous silica. *Part. Fibre Toxicol. 14*, 21.
(117) Yazdi, A. S., Guarda, G., Riteau, N., Drexler, S. K., Tardivel, A., Couillin, I., and Tschopp, J. (2010) Nanoparticles activate the NLR pyrin domain containing 3 (Nlrp3) inflammasome and cause pulmonary inflammation through release of IL-1alpha and IL-1beta. *Proc. Natl. Acad. Sci. U. S. A. 107*, 19449−19454.
(118) Cruz, C. M., Rinna, A., Forman, H. J., Ventura, A. L., Persechini, P. M., and Ojcius, D. M. (2007) ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. *J. Biol. Chem. 282*, 2871−2879.
(119) Chen, Y., Zhou, Z., and Min, W. (2018) Mitochondria, Oxidative Stress and Innate Immunity. *Front. Physiol. 9*, 1487.
(120) Wu, J. B., Yan, Z. B., Schwartz, D. E., Yu, J. G., Malik, A. B., and Hu, G. C. (2013) Activation of NLRP3 Inflammasome in Alveolar Macrophages Contributes to Mechanical Stretch-Induced Lung Inflammation and Injury. *J. Immunol. 190*, 3590−3599.
(121) Wang, X., Chang, C. H., Jiang, J. H., Liu, Q., Liao, Y. P., Lu, J. Q., Li, L. J., Liu, X. S., Kim, J., Ahmed, A., Nel, A. E., and Xia, T. (2019) The Crystallinity and Aspect Ratio of Cellulose Nanomaterials Determine Their Pro-Inflammatory and Immune Adjuvant Effects In Vitro and In Vivo. *Small 15*, 1901642.
(122) Menas, A. L., Yanamala, N., Farcas, M. T., Russo, M., Friend, S., Fournier, P. M., Star, A., Iavicoli, I., Shurin, G. V., Vogel, U. B., Fadeel, B., Beezhold, D., Kisin, E. R., and Shvedova, A. A. (2017) Fibrillar vs crystalline nanocellulose pulmonary epithelial cell responses: Cytotoxicity or inflammation? *Chemosphere 171*, 671−680.
(123) Migliore, L., Saracino, D., Bonelli, A., Colognato, R., D'Errico, M. R., Magrini, A., Bergamaschi, A., and Bergamaschi, E. (2010) Carbon nanotubes induce oxidative DNA damage in RAW 264.7 cells. *Environ. Mol. Mutagen. 51*, 294−303.
(124) Palomaki, J., Valimaki, E., Sund, J., Vippola, M., Clausen, P. A., Jensen, K. A., Savolainen, K., Matikainen, S., and Alenius, H. (2011) Long, Needle-like Carbon Nanotubes and Asbestos Activate the NLRP3 Inflammasome through a Similar Mechanism. *ACS Nano 5*, 6861−6870.
(125) Sun, B., Wang, X., Ji, Z., Wang, M., Liao, Y. P., Chang, C. H., Li, R., Zhang, H., Nel, A. E., and Xia, T. (2015) NADPH Oxidase-Dependent NLRP3 Inflammasome Activation and its Important Role in Lung Fibrosis by Multiwalled Carbon Nanotubes. *Small 11*, 2087−2097.
(126) Shvedova, A. A., Kisin, E. R., Murray, A. R., Kommineni, C., Castranova, V., Fadeel, B., and Kagan, V. E. (2008) Increased accumulation of neutrophils and decreased fibrosis in the lung of NADPH oxidase-deficient C57BL/6 mice exposed to carbon nanotubes. *Toxicol. Appl. Pharmacol. 231*, 235−240.
(127) Hamilton, R. F., Buford, M., Xiang, C. C., Wu, N. Q., and Holian, A. (2012) NLRP3 inflammasome activation in murine alveolar macrophages and related lung pathology is associated with MWCNT nickel contamination. *Inhalation Toxicol. 24*, 995−1008.
(128) Jessop, F., and Holian, A. (2015) Extracellular HMGB1 regulates multi-walled carbon nanotube-induced inflammation in vivo. *Nanotoxicology 9*, 365−372.
(129) Cui, X., Wan, B., Yang, Y., Xin, Y., Xie, Y. C., Guo, L. H., and Mantell, L. L. (2019) Carbon Nanomaterials Stimulate HMGB1 Release From Macrophages and Induce Cell Migration and Invasion. *Toxicol. Sci. 172*, 398−410.
(130) Wynn, T. A., Chawla, A., and Pollard, J. W. (2013) Macrophage biology in development, homeostasis and disease. *Nature 496*, 445−455.
(131) Orecchioni, M., Ghosheh, Y., Pramod, A. B., and Ley, K. (2019) Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front. Immunol. 10*, 1084.
(132) Ginhoux, F., and Jung, S. (2014) Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat. Rev. Immunol. 14*, 392−404.
(133) Mosser, D. M., and Edwards, J. P. (2008) Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol. 8*, 958−969.
(134) Dale, D. C., Boxer, L., and Liles, W. C. (2008) The phagocytes: neutrophils and monocytes. *Blood 112*, 935−945.
(135) O'Shea, J. J., and Murray, P. J. (2008) Cytokine signaling modules in inflammatory responses. *Immunity 28*, 477−487.
(136) Jin, W., and Dong, C. (2013) IL-17 cytokines in immunity and inflammation. *Emerging Microbes Infect. 2*, No. e60.
(137) Hesse, M., Modolell, M., La Flamme, A. C., Schito, M., Fuentes, J. M., Cheever, A. W., Pearce, E. J., and Wynn, T. A. (2001) Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: Granulomatous pathology is shaped by the pattern of L-arginine metabolism. *J. Immunol. 167*, 6533−6544.
(138) Munitz, A., Brandt, E. B., Mingler, M., Finkelman, F. D., and Rothenberg, M. E. (2008) Distinct roles for IL-13 and IL-4 via IL-13 receptor alpha1 and the type II IL-4 receptor in asthma pathogenesis. *Proc. Natl. Acad. Sci. U. S. A. 105*, 7240−7245.
(139) Lunov, O., Syrovets, T., Loos, C., Beil, J., Delacher, M., Tron, K., Nienhaus, G. U., Musyanovych, A., Mailander, V., Landfester, K., and Simmet, T. (2011) Differential uptake of functionalized polystyrene nanoparticles by human macrophages and a monocytic cell line. *ACS Nano 5*, 1657−1669.
(140) Hoppstadter, J., Seif, M., Dembek, A., Cavelius, C., Huwer, H., Kraegeloh, A., and Kiemer, A. K. (2015) M2 polarization enhances silica nanoparticle uptake by macrophages. *Front. Pharmacol. 6*, 55.
(141) Binnemars-Postma, K. A., ten Hoopen, H. W. M., Storm, G., and Prakash, J. (2016) Differential uptake of nanoparticles by human M1 and M2 polarized macrophages: protein corona as a critical determinant. *Nanomedicine 11*, 2889−2902.
(142) Kumar, S., Meena, R., and Paulraj, R. (2016) Role of Macrophage (M1 and M2) in Titanium-Dioxide Nanoparticle-Induced Oxidative Stress and Inflammatory Response in Rat. *Appl. Biochem. Biotechnol. 180*, 1257−1275.
(143) Fuchs, A. K., Syrovets, T., Haas, K. A., Loos, C., Musyanovych, A., Mailander, V., Landfester, K., and Simmet, T. (2016) Carboxyl- and amino-functionalized polystyrene nanoparticles differentially affect the polarization profile of M1 and M2 macrophage subsets. *Biomaterials 85*, 78−87.
(144) Gordon, S., and Pluddemann, A. (2017) Tissue macrophages: heterogeneity and functions. *BMC Biol. 15*, 53.
(145) Ngobili, T. A., and Daniele, M. A. (2016) Nanoparticles and direct immunosuppression. *Exp. Biol. Med. (London, U. K.) 241*, 1064−1073.
(146) Wilson, N. S., El-Sukkari, D., Belz, G. T., Smith, C. M., Steptoe, R. J., Heath, W. R., Shortman, K., and Villadangos, J. A. (2003) Most lymphoid organ dendritic cell types are phenotypically and functionally immature. *Blood 102*, 2187−2194.

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073

(147) Almeida, J. P. M., Lin, A. Y., Langsner, R. J., Eckels, P., Foster, A. E., and Drezek, R. A. (2014) In Vivo Immune Cell Distribution of Gold Nanoparticles in Naive and Tumor Bearing Mice. *Small 10*, 812−819.
(148) Fytianos, K., Rodriguez-Lorenzo, L., Clift, M. J., Blank, F., Vanhecke, D., von Garnier, C., Petri-Fink, A., and Rothen-Rutishauser, B. (2015) Uptake efficiency of surface modified gold nanoparticles does not correlate with functional changes and cytokine secretion in human dendritic cells in vitro. *Nanomedicine 11*, 633−644.
(149) Kang, K., Jung, H., and Lim, J. S. (2012) Cell Death by Polyvinylpyrrolidine-Coated Silver Nanoparticles is Mediated by ROS-Dependent Signaling. *Biomol. Ther. 20*, 399−405.
(150) Zhang, T. G., Zhang, Y. L., Zhou, Q. Q., Wang, X. H., and Zhan, L. S. (2020) Impairment of mitochondrial dynamics involved in iron oxide nanoparticle-induced dysfunction of dendritic cells was alleviated by autophagy inhibitor 3-methyladenine. *J. Appl. Toxicol. 40*, 631.
(151) Pavot, V., Rochereau, N., Primard, C., Genin, C., Perouzel, E., Lioux, T., Paul, S., and Verrier, B. (2013) Encapsulation of Nod1 and Nod2 receptor ligands into poly(lactic acid) nanoparticles potentiates their immune properties. *J. Controlled Release 167*, 60−67.
(152) Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., Yokoyama, W. M., and Ugolini, S. (2011) Innate or adaptive immunity? The example of natural killer cells. *Science 331*, 44−49.
(153) Moretta, A., Marcenaro, E., Parolini, S., Ferlazzo, G., and Moretta, L. (2008) NK cells at the interface between innate and adaptive immunity. *Cell Death Differ. 15*, 226−233.
(154) Culley, F. J. (2009) Natural killer cells in infection and inflammation of the lung. *Immunology 128*, 151−163.
(155) Horvath, K. M., Herbst, M., Zhou, H. B., Zhang, H. T., Noah, T. L., and Jaspers, I. (2011) Nasal lavage natural killer cell function is suppressed in smokers after live attenuated influenza virus. *Respir. Res. 12*, 102.
(156) Vivier, E., Tomasello, E., Baratin, M., Walzer, T., and Ugolini, S. (2008) Functions of natural killer cells. *Nat. Immunol. 9*, 503−510.
(157) Cooper, M. A., Fehniger, T. A., Fuchs, A., Colonna, M., and Caligiuri, M. A. (2004) NK cell and DC interactions. *Trends Immunol. 25*, 47−52.
(158) Striz, I., Mio, T., Adachi, Y., Heires, P., Robbins, R. A., Spurzem, J. R., Illig, M. J., Romberger, D. J., and Rennard, S. I. (1999) IL-4 induces ICAM-1 expression in human bronchial epithelial cells and potentiates TNF-alpha. *Am. J. Physiol. 277*, L58−64.
(159) De Maria, A., Bozzano, F., Cantoni, C., and Moretta, L. (2011) Revisiting human natural killer cell subset function revealed cytolytic CD56(dim)CD16(+) NK cells as rapid producers of abundant IFN-gamma on activation. *Proc. Natl. Acad. Sci. U. S. A. 108*, 728−732.
(160) Muller, L., Steiner, S. K., Rodriguez-Lorenzo, L., Petri-Fink, A., Rothen-Rutishauser, B., and Latzin, P. (2018) Exposure to silver nanoparticles affects viability and function of natural killer cells, mostly via the release of ions. *Cell Biol. Toxicol. 34*, 167−176.
(161) Hanley, C., Thurber, A., Hanna, C., Punnoose, A., Zhang, J. H., and Wingett, D. G. (2009) The Influences of Cell Type and ZnO Nanoparticle Size on Immune Cell Cytotoxicity and Cytokine Induction. *Nanoscale Res. Lett. 4*, 1409−1420.
(162) Andersson-Willman, B., Gehrmann, U., Cansu, Z., Buerki-Thurnherr, T., Krug, H. F., Gabrielsson, S., and Scheynius, A. (2012) Effects of subtoxic concentrations of TiO2 and ZnO nanoparticles on human lymphocytes, dendritic cells and exosome production. *Toxicol. Appl. Pharmacol. 264*, 94−103.
(163) De Jong, W. H., Van Der Ven, L. T. M., Sleijffers, A., Park, M. V. D. Z., Jansen, E. H. J. M., Van Loveren, H., and Vandebriel, R. J. (2013) Systemic and immunotoxicity of silver nanoparticles in an intravenous 28 days repeated dose toxicity study in rats. *Biomaterials 34*, 8333−8343.
(164) van der Zande, M., Vandebriel, R. J., Van Doren, E., Kramer, E., Rivera, Z. H., Serrano-Rojero, C. S., Gremmer, E. R., Mast, J., Peters, R. J. B., Hollman, P. C. H., Hendriksen, P. J. M., Marvin, H. J. P., Peijnenburg, A. A. C. M., and Bouwmeester, H. (2012) Distribution, Elimination, and Toxicity of Silver Nanoparticles and Silver Ions in Rats after 28-Day Oral Exposure. *ACS Nano 6*, 7427−7442.
(165) Herzog, F., Clift, M. J. D., Piccapietra, F., Behra, R., Schmid, O., Petri-Fink, A., and Rothen-Rutishauser, B. (2013) Exposure of silver-nanoparticles and silver-ions to lung cells in vitro at the air-liquid interface. *Part. Fibre Toxicol. 10*, 11.
(166) Gustafsson, A., Lindstedt, E., Elfsmark, L. S., and Bucht, A. (2011) Lung exposure of titanium dioxide nanoparticles induces innate immune activation and long-lasting lymphocyte response in the Dark Agouti rat. *J. Immunotoxicol. 8*, 111−121.
(167) Fisichella, M., Berenguer, F., Steinmetz, G., Auffan, M., Rose, J., and Prat, O. (2012) Intestinal toxicity evaluation of TiO2 degraded surface-treated nanoparticles: a combined physico-chemical and toxicogenomics approach in caco-2 cells. *Part. Fibre Toxicol. 9*, 18.
(168) Gaiser, B. K., Hirn, S., Kermanizadeh, A., Kanase, N., Fytianos, K., Wenk, A., Haberl, N., Brunelli, A., Kreyling, W. G., and Stone, V. (2013) Effects of silver nanoparticles on the liver and hepatocytes in vitro. *Toxicol. Sci. 131*, 537−547.
(169) Hansmann, J., Egger, D., and Kasper, C. (2018) Advanced Dynamic Cell and Tissue Culture. *Bioengineering 5*, 65.
(170) Schlachet, I., and Sosnik, A. (2019) Mixed Mucoadhesive Amphiphilic Polymeric Nanoparticles Cross a Model of Nasal Septum Epithelium in Vitro. *ACS Appl. Mater. Interfaces 11*, 21360−21371.

https://dx.doi.org/10.1021/acs.chemrestox.0c00051
Chem. Res. Toxicol. 2020, 33, 1061−1073