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Understanding the immunological interactions of engineered nanomaterials: Role of the bio-corona

Bengt Fadeel

Nanosafety & Nanomedicine Laboratory (NNL), Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Correspondence
Bengt Fadeel, Institute of Environmental Medicine, Nobels väg 13, Karolinska Institutet, 171 77 Stockholm, Sweden.
Email: bengt.fadeel@ki.se

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Abstract

Engineered nanomaterials are a broad class of materials with the potential for breakthrough applications in many sectors of society not least in medicine. Consequently, safety assessment of nanomaterials and nano-enabled products with respect to human health and the environment is of key importance. To this end, the biological interactions of nanoscale materials must be understood. Here, the dual "identities" of nanomaterials, namely, the material-intrinsic properties or synthetic identity and the acquired, context-dependent properties or biological identity, are discussed in relation to nanomaterial interactions with the immune system, our main defense against foreign intrusion. Specifically, we address whether macrophages and other innate immune cells respond to the synthetic identity or the biological identity of nanomaterials, that is, the surface adsorbed proteins and/or other biomolecules known as the bio-corona, or both?

This article is categorized under:

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KEYWORDS

bio-corona, danger, immune system, molecular patterns, nanomaterials

1 | INTRODUCTION

The relationship between humans and technology has been a matter of debate for a long time, and some experts warn that new technologies could have unintended consequences and lead to unanticipated effects (toxicities) that cascade out of control while others have emphasized the considerable benefits of (nano)technology not least in the clinic, with the promise of personalized precision medicine for the imaging and treatment of human disease (Langer & Weissleder, 2015; Toumey, 2009). The term "nanotechnology" was initially applied for the understanding and manipulation of matter at the atomic level, but it is more commonly used as a catch-all phrase for any technology that makes use of materials that have been intentionally manufactured on a nanoscale (commonly understood as 1–100 nm, although this is certainly an arbitrary definition). In fact, it has been pointed out that the strong focus on "nano" may detract from the fact that nanometer as well as micrometer dimensions are relevant when it comes to the interface between materials and biology aka biotechnology (Whitesides, 2003). However, whether a material is defined as a

nanomaterial or not has important regulatory consequences, as discussed by authors from the Joint Research Centre (JRC) of the European Commission (Mech et al., 2020).

How safe are engineered nanomaterials? The answer to this simple question is not a simple one (Valsami-Jones & Lynch, 2015). We know that both chemical and physical properties of a material change as one approaches the nanoscale. Therefore, it is reasonable to assume that the biological effects may also change. However, the novel or unanticipated biological effects of nanoparticles may be due to the fact that these particles escape normal defenses and are able to translocate from their portal of entry (Donaldson et al., 2004; Kreyling et al., 2010). This does not mean a priori that the downstream events or toxicities of nanoparticles are novel (Donaldson & Poland, 2013).

The question is, are we on the right track? (Krug, 2014). Can we draw any general conclusions with regard to the environmental, health, and safety (EHS) implications of nanomaterials? The answer to this question is compounded, in part, by the fact that nanomaterials are bundled into a single material category when this is clearly not the case (Fadeel & Kostarelos, 2020). Furthermore, nanomaterials rapidly adsorb biomolecules upon introduction into a living system which means that the biological responses are dependent both on the synthetic "identity" defined by material-intrinsic properties and the context-dependent biological "identity" of the material. We discussed this previously (Fadeel et al., 2013), and an update is provided in the present review with particular attention to the interactions of nanomaterials with the immune system.

2 | CLOSE ENCOUNTERS OF THE SMALL KIND

Research on ambient ultrafine particles (UFP) and man-made fibers such as asbestos has laid the foundation for the field of nanotoxicology (Oberdörster et al., 2005). However, others have argued that nanotoxicology represents a departure from traditional toxicology and that this field should instead be understood as the investigation of the interference of engineered nanomaterials with the functions of cellular and extracellular nanoscale structures (Shvedova et al., 2010), thus drawing attention to the fact that engineered nanomaterials and biological structures occur on the same size scale. Richard Feynman discussed the design of "infinitesimal machinery" (though he did not seem to care whether there was any sensible use for such machines) (Fadeel et al., 2007). However, it is important to realize that numerous nano-sized protein "machines" already exist in the biological world (Shvedova et al., 2010). Furthermore, viruses are nano-scale objects that interact with host cells. Thus, it stands to reason that there are important lessons to be learned not only from the toxicology of naturally occurring or unintentionally produced particles, but also from adjacent scientific disciplines such as cell biology, microbiology, and immunology. Indeed, if we are to understand and predict the biological effects of engineered nanomaterials, we need to view the human body as something more than a black box. Hence, while nanomaterials may be designed with a view to alter human pathophysiology, it is an inescapable fact that the materials themselves undergo rapid modification through the adsorption of biomolecules as well as long-term biotransformation when introduced into a living system (Malysheva et al., 2021; Matter et al., 2020).

2.1 | The "synthetic" nanomaterial identity

In their recent perspective, Friedersdorf et al. (2019) pointed out that "one thing now clear is that size alone does not define the behavior of a nanomaterial and that not all nanomaterials have the same effect." Indeed, several other parameters need to be considered in addition to size and shape including chemical composition, surface charge and surface chemistry, aggregation, dissolution, chemical reactivity, and so on. However, it is not trivial to assess individual material characteristics and how these properties influence the behavior of a nanomaterial in the environment or in the body (Fadeel et al., 2013). Therefore, toxicological testing for the most part addresses the aggregate outcome of the material properties in toto which may be sufficient if the purpose is to perform hazard ranking of materials, but inadequate if we wish to gain insight into the mechanisms governing nanomaterial interactions with biological systems.

Grouping of nanomaterials with respect to their hazard could facilitate decision making for regulatory purposes. The European Chemicals Agency (ECHA) describes grouping as the process of uniting substances into a common group "*if they are structurally similar with physicochemical*, *toxicological*, *ecotoxicological*, *and/or environmental fate properties that are likely to be similar or to follow a regular pattern*." One illustrative example put forward by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) is the so-called decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping) (Arts et al., 2015, 2016). The approach is based on the identification

of four main groups of nanomaterials, and it is instructive that the concept is based on "functionalities" of nanomaterials instead of structural similarities, thereby circumventing the problem of nailing down distinct material-intrinsic properties (although the functional outcomes must also be connected to or dictated by the material-intrinsic or physicochemical properties of the materials). Hence, the four main groups according to the DF4nanoGrouping approach are: (1) soluble nanomaterials, (2) high aspect ratio nanomaterials, (3) "passive" nanomaterials, and (4) "active" nanomaterials (Arts et al., 2015). On the basis of subsequent case studies, the DF4nanoGrouping was shown to be an efficient means of identifying nanomaterials that may undergo hazard assessment without further testing. As noted by Arts et al. (2016), these were the soluble nanomaterials, where hazard assessment may rely on information available on the dissolved species, the high aspect ratio nanomaterials, which may be considered akin to asbestos-like fibers, and the so-called passive nanomaterials for which a general dust threshold may be applied. Thus, the approach allowed the investigators to single out "active" nanomaterials for further in-depth investigations (Arts et al., 2016). On a more general level, these efforts also show that the term "nanomaterial" is not a practical one as it is too broad (Feliu & Fadeel, 2010). However, while grouping approaches may facilitate decision making for regulatory purposes, thereby preventing regulatory paralysis, they do not advance our understanding of the mechanisms underpinning the biological effects of nanomaterials. Indeed, unexpected biological effects may occur that are not foreseen by grouping approaches which, by default, are based on existing knowledge (from studies of other particles or chemicals) (Fadeel et al., 2013). However, systems biology approaches, taking into account the whole gamut of cellular perturbations evoked by nanomaterials, could also be used to support grouping (Riebeling et al., 2017).

The combination of all material-intrinsic or physicochemical properties can be viewed as the synthetic "identity" of a nanomaterial (Fadeel et al., 2013). This is what the nanomaterial looks like in its virgin state before it encounters a biological system. The biological "identity" of a nanomaterial is a function of its synthetic identity (size, shape, surface chemistry, and so on) and the physiological environment (Walkey & Chan, 2014). The biological identity, and the bio-corona concept, is discussed in the section below.

2.2 | The "biological" nanomaterial identity

Nanomaterials adopt a new "identity" in the body through the adsorption of proteins and other biomolecules (Nel et al., 2009). This phenomenon, known as bio-corona formation, is dependent upon intrinsic properties of the nanomaterials including size or surface curvature and surface chemistry (Cedervall et al., 2007; Lundqvist et al., 2008; Tenzer et al., 2011) and on the physiological environment (i.e., the extracellular or intracellular milieu). Thus, the biological identity of nanomaterials is context dependent (Nyström & Fadeel, 2012). Importantly, while most publications are focused on the protein corona, one should not ignore other biomolecules including lipids, as lipid-binding proteins are commonly found in the bio-corona of nanoparticles (Hellstrand et al., 2009).

The environmental dimensions of the protein corona (often referred to as the "eco-corona") have been expertly covered (Wheeler et al., 2021) and will not be discussed here.

In our previous survey of bio-corona research, it was noted that we and others had deployed proteomics approaches to catalogue the proteins present on the surface of a wide range of nanomaterials including spherical particles such as superparamagnetic iron oxide nanoparticles (SPIONs) or polystyrene (PS) particles and fiber-like materials including single-walled carbon nanotubes (SWCNTs) (Fadeel et al., 2013). The majority of these studies were carried out using human plasma or fetal bovine serum (FBS), and in many cases, several hundred proteins were identified in the bio-corona (Tenzer et al., 2013). However, these results prompt several questions: first, why do so many different proteins congregate on the surface of nanoparticles? Indeed, as previously pointed out, it seems counterintuitive that "proteins in the blood that evolved not to bind nonspecifically to each other would do so around nanoparticles" (Lundqvist & Cedervall, 2020). This could potentially be explained by the fact that centrifugation is commonly used to separate the nanoparticle–protein complexes from unbound proteins, and as recently highlighted by others, this approach may give rise to the false detection of proteins "trapped" in the corona (Lundqvist & Cedervall, 2020). Related to this is the fact that the protein corona is commonly depicted or envisioned as a smooth "shell" covering the entire surface of the nanoparticle, but this is in all likelihood incorrect. In fact, Simberg et al. (2009) reported that both the dextran coating and the iron oxide core of dextran-coated SPIONs remained accessible to specific probes following incubation in mouse plasma. Moreover, the authors concluded that plasma protein opsonization was not responsible for the uptake of SPIONs by the liver and spleen. Instead, they favored a model whereby a direct interaction between SPIONs and cellular receptors is responsible for cellular recognition in vivo (Simberg et al., 2009). Indeed, using transmission electron

microscopy (TEM) to study the morphology of the protein corona, it was shown that the corona on PS particles is not a dense shell surrounding the particle but a loose network of proteins (Kokkinopoulou et al., 2017). Moreover, using TEM under cryogenic conditions, the protein corona on silica nanoparticles was examined to obtain information on the morphology and thickness of the unstained protein corona in simple and complex media (i.e., FBS) (Galdino et al., 2021). Using super-resolution optical microscopy (STORM), Feiner-Gracia et al. (2017) were able to visualize the protein corona formed on mesoporous silica nanoparticles with single protein sensitivity and found a significant heterogeneity in protein absorption within the same nanoparticle population. Moreover, the authors demonstrated that changes in the corona composition occurred over time due to the chemistry and degradability of the particles, thus adding a further level of complexity to our understanding of the biological identity of nanomaterials (Feiner-Gracia et al., 2017).

The second, non-technical (and non-trivial) question refers to the biological "meaning," if any, of all these proteins. It is well understood that the presence or absence of proteins, regardless of their identity, may influence the degree of agglomeration of nanoparticles. The question is whether there is a role for *specific* proteins in the bio-corona? Tenzer et al. (2013) performed comprehensive studies using a panel of PS nanoparticles and silica nanoparticles of various sizes displaying different surface charges and could show that the rapid formation of a plasma protein corona could modulate distinct pathophysiological effects as evidenced by the ex vivo analysis of platelet activation/aggregation and red blood cell lysis (i.e., hemolysis). However, the complexity of the protein corona precluded the assignment of specific proteins to account for these effects (and perhaps the effects were related to a shielding of the particle surface, thereby passivating the nanoparticles) (see, e.g., Hu et al., 2011, Lesniak et al., 2012). Indeed, we have shown that the cytotoxicity of silica nanoparticles is mitigated in the presence of serum whereas ZnO nanoparticles that undergo rapid dissolution are cytotoxic both in the absence and presence of serum (Shi et al., 2012). However, in another comprehensive in vitro study, Walkey and Chan (2014) applied proteomics based "fingerprinting" of the corona of a library of 105 surface-modified gold nanoparticles and could show that the so-called protein corona fingerprint predicted cell association 50% more accurately than a model based on parameters describing material-intrinsic properties such as size, aggregation, and surface charge. The authors also speculated that a set of hyaluronan-binding proteins served as mediators of nanoparticle-cell interactions though this was not formally proven (Walkey and Chan (2014)). It is noted that cell association was evaluated by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using the A549 human lung carcinoma cell line (i.e., a non-phagocytic cell model). The authors also found that the model derived using gold nanoparticles could not be applied to predict the cell association of silver nanoparticles. However, the study suggests that the protein corona may harbor predictive information at least with regard to cellular uptake, if not toxicity. Other investigators have presented evidence that specific proteins including clusterins may act as dysopsonins that allow nanoparticles to evade cellular uptake (Fedeli et al., 2015; Schöttler, Becker, et al., 2016). This may have considerable implications for medical applications of nanoparticles for instance as drug carriers (Digiacomo et al., 2020).

Several groups have endeavored to map the cellular uptake pathways of nanoparticles using pharmacological inhibitors and/or genetic approaches to probe these pathways (Rennick et al., 2021). Francia et al. (2019) found that silica nanoparticles dispersed in cell medium with different amounts of serum, yielding protein coronas of varying composition, were internalized through different pathways. The latter studies were performed using human cancer cell lines (HeLa and A549) as well as primary human umbilical vein endothelial cells (HUVEC). It is pertinent to note that while the presence or absence of serum is important, the choice of cell model will also play a significant role in determining the uptake pathways of nanoparticles (Lunov et al., 2011). In particular, the activation status of professional phagocytes such as primary human monocyte-derived macrophages (HMDM) will affect the repertoire of phagocytosis receptors including scavenger receptors (Gallud et al., 2017). Similarly, Kupffer cells, the liver-resident macrophages, were shown to display a varying degree of uptake of nanoparticles depending on their activation status (MacParland et al., 2017). In addition to addressing which receptors are involved, and whether or not the presence of serum influences cellular uptake (see, e.g., Shannahan et al., 2015; Gallud et al., 2017), it is reasonable to ask whether corona proteins are correctly presented such that they might interact with the corresponding receptor(s) on the cell surface. To begin to address this, O'Connell et al. (2015) performed "interactome" profiling for PS nanoparticles incubated in varying concentrations of plasma (1%–55%) using a human protein microarray containing 9483 full-length, purified human proteins. The authors found that a "fully formed" and biologically meaningful identity of the nanoparticles only emerged at higher plasma concentrations, which is relevant in terms of understanding differences between in vitro and in vivo studies (O'Connell et al., 2015). It is notable that the extracellular environment is dynamic, and that so-called cell conditioning may also influence the nanoparticle corona (Albanese et al., 2014; Dai et al., 2017). This shows how important it is to understand not only the material but also the model, as the cell type and its activation state or phenotype may come into play.

2.3 | Decoding the nanomaterial bio-corona

Two important developments have occurred in the bio-corona research field in recent years: first, investigators have shifted their attention from in vitro to in vivo studies, and second, studies on the intracellular corona now complement previous studies on the extracellular formation of a bio-corona. Here, a few illustrative examples are provided. It goes without saying that the experimental conditions are important (Lundqvist & Cedervall, 2020). Hence, the choice of anticoagulant will affect the composition of the protein corona (Schöttler, Klein, et al., 2016). However, ex vivo studies have also been performed using non-anticoagulated human blood (Ekstrand-Hammarström et al., 2015). Overall, careful characterization of the experimental conditions is required for a full understanding of nanomaterial interactions with biological systems (Mahmoudi, 2021).

Hadjidemetriou et al. (2015) addressed the protein corona formed in vivo in mice following the intravenous injection of PEGylated liposomes. The corona formation was determined after the recovery of the liposomes from the blood of CD-1 mice 10 min post-injection. The authors also performed in vitro studies in which liposomes were incubated in CD-1 mouse plasma. The authors found that the molecular complexity of the in vivo corona could not be accurately predicted on the basis of the in vitro formed corona. In a subsequent study, the authors performed a clinical study in which the protein corona formed on PEGylated doxorubicin-encapsulated liposomes was analyzed following the recovery of the liposomes from the blood of six ovarian carcinoma patients immediately upon completion of their first cycle of treatment (Hadjidemetriou et al., 2019). In parallel, ex vivo incubations of liposomes with plasma samples obtained from the same patients were performed. In agreement with the previous investigation in mice, the in vivo corona was found to show a greater molecular complexity in comparison to its ex vivo counterpart (Hadjidemetriou et al., 2019).

Bertrand et al. (2017) performed comprehensive studies using a library of polymeric nanoparticles and different knockout mouse models to examine the role of the in vivo protein adsorption for the clearance of nanoparticles from the bloodstream. They could show that the adsorption of apolipoprotein E (ApoE) following intravenous injection of the nanoparticles was dependent on poly(ethylene glycol) (PEG) density. Hence, while ApoE appeared to rescue nanoparticles with low PEG coverage from rapid clearance, it also seemed to act as a potential ligand for low-density-lipoprotein (LDL) receptor on all nanoparticles irrespective of PEG density (Bertrand et al., 2017). The LDL receptor is normally responsible for receptor-mediated endocytosis of lipoprotein particles decorated with apolipoproteins. Moreover, studies using complement-deficient mice suggested that complement activation cannot be the sole predictor of nanoparticle residency in the bloodstream in mice since the biological fate of both short-circulating and long-circulating nanoparticles was unaffected by the absence of complement protein 3 (C3) (Bertrand et al., 2017). It is noteworthy that patisiran (Onpattro™), a lipid nanoparticle formulation of small interfering RNA for the treatment of hereditary transthyretin (TTR)-mediated amyloidosis, appears to be targeted to hepatocytes through the adsorption of ApoE on the surface of the nanoparticles following i.v. administration (Akinc et al., 2019). Thus, while targeting of nanomedicines may be achieved by the incorporation of specific ligands, it seems that the adsorption of ApoE enables patisiran to exploit the body's own targeting system for lipid-laden particles.

In another recent study, Abbina et al. (2020) studied a set of polymeric nanomaterials with varying circulation times in mice and used proteomics protocols to probe the protein corona. This in vivo "fingerprinting" study showed that the protein corona changed dynamically over time. Hence, coagulation and complement proteins were decreased and immunoglobulins, acute phase reactants, and lipoproteins were increased in abundance over time. The authors found that nanoparticles that circulated longer were apparently able to shed some of the initial surface bound common opsonins, and they speculated that this continuous remodeling of surface bound proteins may be an important step in dictating the in vivo fate of soft nanomaterials in mice.

PEGylation remains a common strategy for the preparation of long-circulating nanoparticles, although, as we have seen, this surface modification does not prevent protein binding altogether. Hacene et al. (2021) developed an ingenious immunoprecipitation method using anti-PEG antibodies cross-linked to magnetic beads to extract different PEGylated particles including polymeric nanoparticles and liposomes. Using Balb/c mice, the authors injected PEGylated polymeric nanoparticles intravenously, and blood was collected 15 min after dosing. After separation of red blood cells, the nanoparticles were quantitatively recovered from plasma (thus suggesting that the epitope recognized by the anti-PEG antibody remains available on the surface of the nanoparticles even in the presence of a protein corona). The authors determined the composition of the surface adsorbed proteins and found that apolipoproteins were enriched on the surface of the nanoparticles (Hacene et al., 2021).

Once inside the cell, it is presumed that the protein corona is removed in endolysosomal compartments through cleavage by cellular cathepsins (Bertoli et al., 2016; Sée et al., 2009), thereby causing the true (synthetic) "identity" of

the nanoparticles to be unveiled (Fadeel et al., 2013). One may ask whether nanoparticles could acquire a new bio-corona inside the cell, for instance, following their escape from the lysosomal compartment? Sund et al. (2011) reported that metal oxide nanoparticles bound several ribosomal and cytoskeletal proteins upon incubation with cytoplasmic extracts of human macrophages, and the binding was more effective for the nano-sized TiO_2 nanoparticles when compared with the coarse form (5 μm) of TiO_2. In a similar study, Klein et al. (2016) identified RNA-binding proteins as the major cellular targets of silica nanoparticles in cellular extracts derived from human lung adenocarcinoma cells (the interaction was not mediated through the binding of RNA). We could show, using a set of gold (Au) nanoparticles with varying surface chemistries, that is, alkyl ammonium bromide, alkyl sodium carboxylate, or PEG-terminated Au-nanoparticles (Gallud et al., 2019), that the positively charged Au particles interacted with RNA and DNA extracted from human THP-1 cells (Gallud et al., 2020). These studies were performed using cell lysates or nucleic acids isolated from cells. However, we recently provided evidence, using thermal proteome profiling (TPP), that the ammonium-modified Au nanoparticles interacted with tumor necrosis factor (TNF) alpha-induced protein 8-like protein 2 (TIPE2) in intact THP-1 cells (Tarasova et al., 2017). TIPE2 is a negative regulator of NF-κB and is required for maintaining immune homeostasis (Sun et al., 2008). Moreover, we could show that the same Au nanoparticles caused activation of NF-κB in cells, possibly through the inactivation of TIPE2.

In a recent study, Qin et al. (2020) characterized the intracellular protein corona on Au nanoparticles dispersed in bovine serum albumin (BSA) using the human colorectal adenocarcinoma cell line Caco-2. The authors collected Au nanoparticles following cellular transport across the epithelial monolayer through transcytosis or exocytosis, respectively, and conducted a proteomic analysis of the surface adsorbed proteins. Based on the identification of these proteins, the authors then deduced the subcellular location and transport pathways deployed by the Au nanoparticles and suggested the existence of a novel pathway of nanoparticle transport from endosomes to secretory vesicles which was dominant during transcytosis. It is possible that the protein corona evolves when nanoparticles are transferred from one biological environment to another (Lundqvist et al., 2011), though it is not entirely clear how this back-tracking could be used to ascertain transport pathways. Nevertheless, the study shows that a protein corona comprised of intracellular proteins is formed as nanoparticles cross an epithelial barrier.

Several studies have shown that proteins in the bio-corona are unfolded and/or denatured. Mortimer et al. (2014) provided evidence for albumin-directed macrophage uptake of layered silicate nanoparticles (LSN) pointing toward a role for the unfolding of albumin due to nanoparticle binding. The authors suggested the existence of cryptic epitopes buried within the protein that may act as a ligand for scavenger receptors. Yan et al. (2013) found that albumin was the most abundant component of the protein corona formed on polymeric particles (500 nm) in cell culture medium containing 10% FBS. Upon adsorption onto the particles, BSA was found to undergo conformational changes, and this was coupled with a decreased uptake by monocyte-like THP-1 cells. In contrast, the unfolded BSA on the nanoparticles triggered scavenger receptor-mediated phagocytosis in differentiated macrophage-like cells THP-1 cells (Yan et al., 2013). Chaperones can bind misfolded proteins and a recent study has shown that nanoparticle-triggered unfolding of albumin resulted in the recruitment of the heat shock protein Hsp90ab1 in the corona of iron-cobalt-nickel alloy nanoparticles (FeCoNi) and iron oxide nanoparticles (Fe_3O_4) (Liu et al., 2020). In a follow-up study, the authors demonstrated that the denatured corona proteins recruited heat shock proteins intracellularly leading to the induction of the unfolded protein response (UPR) (Liu et al., 2021). The authors argued that the "stubborn" retention of denatured proteins in the corona might disturb the conformation of Hsp90ab1, which might affect its function. The authors also performed experiments in mice to examine the role of Hsp90ab1. To this end, animals were exposed to Fe_3O_4 nanoparticles through intratracheal instillation in the presence or absence of the Hsp90ab1 inhibitor geldanamycin (GA), and GA was shown to reverse several indices of pulmonary inflammation (Liu et al., 2021). Furthermore, the inflammation induced by PEGylated Fe_3O_4 nanoparticles was significantly lower, which might be due to the fact that PEGylation not only reduces the amount of protein adsorbed on the nanoparticles but might also decrease the degree of denaturation of bound corona proteins (Liu et al., 2021).

3 | TANGLED UP IN THE IMMUNE SYSTEM

The overarching function of the immune system is to prevent or limit infection, and many of the major paradigms in immunology are based on studies using animal models (refer to: Pulendran & Davis, 2020, for an excellent overview). However, the study of human immunology is uniquely important as humans are not a model but the "real deal." As we address the potential impact of engineered nanomaterials on the immune system, and the exploitation of nanomaterials

to modulate immune responses, it is relevant not only to consider the choice of model system, but also to reflect on the fact that our conceptualization and understanding of the human immune system is constantly changing—from the classical model of self versus non-self discrimination (Medzhitov & Janeway, 2002) to the so-called danger model (Matzinger, 2002) and beyond.

3.1 | Conceptualizing the immune system

Here, two aspects are highlighted that distinguish the "old" immunology from the "new." First, according to the "old" view, the immune system appeared to operate more or less like a medieval marketplace in which peasants would harvest their crops far afield, then travel to the market town to display and sell their goods. Similarly, it was believed that antigen recognition and processing took place in peripheral tissues whereas the actual immune response occurred in the lymph nodes. There was thus a clear distinction between the periphery and the so-called central immune organs. However, we now know that tissue-resident immune cells including tissue-resident memory T cells, a T cell population that is obviously overlooked in studies using peripheral blood mononuclear cells (PBMCs), are critical mediators of immunity (Schenkel & Masopust, 2014). Indeed, it has been argued that residence, rather than renewal or recirculation, defines the immune surveillance program in non-lymphoid tissues (Wijeyesinghe et al., 2021). Furthermore, the size of the tissue-resident immune system is adaptable and this phenomenon of "expansible residence" has been observed not only for T cells but for most populations of immune cells (Wijeyesinghe et al., 2021). Thus, according to the latter authors, "it may be reasonable to conceptualize the immune system as its own organ system, albeit one that consists of a diverse network of motile sensory cells that are durably integrated throughout the entire body." Furthermore, recent work has enforced the notion that structural cells such as fibroblasts, and endothelial and epithelial cells, are key regulators of organ-specific immune responses (Gomes & Teichmann, 2020). Hence, Krausgruber et al. (2020) used multi-omics profiling and integrative bioinformatics analysis of structural cells from 12 mouse organs to create a high-resolution "atlas" or repository of immune-related gene activity. The authors found that structural cells have an epigenetically primed immune potential, with both organ-specific and cell type-specific features. Hence, structural cells do not merely fulfill a barrier function, but seem poised for defense, possibly through the cross-talk with tissue-resident hematopoietic immune cells.

Second, according to the (old) textbook version, the immune system consists of two arms namely the innate immune system and the adaptive immune system. According to this convenient dichotomy, the innate immune cells including macrophages and neutrophils are tasked with menial duties such as garbage disposal (i.e., phagocytosis of microorganisms and cell debris) whereas the adaptive immune system (T cells and B cells) is endowed with higher functions including immunological memory (defined as the ability to respond more rapidly and effectively to pathogens that have been encountered previously). However, the boundaries between the two branches of the immune system are dissolving and we now find that the "primitive" innate immune system may not be so primitive after all. Indeed, "the traditional view of neutrophils as short-lived effector cells with limited functional capacity is incomplete" (Scapini & Cassatella, 2014). Moreover, innate lymphoid cells (ILCs) are an emerging family of immune cells with phenotypes that mirror those of T cells (Eberl et al., 2015). Natural killer (NK) cells may be viewed as the innate counterparts of $CD8^+$ cytotoxic T cells, whereas ILC1, ILC2, and ILC3 are thought to represent the innate counterparts of $CD4^+$ T helper 1 (T_H1), T_H2, and T_H17 cells. However, in contrast to T cells, ILCs do not express antigen receptors or undergo clonal expansion when stimulated (Eberl et al., 2015). Additionally, a program of "trained immunity" whereby macrophages, monocytes, ILCs, and NK cells show enhanced responsiveness to infectious agents has been proposed and experimentally proven in recent years (Netea et al., 2011, 2016). It has also been suggested that inflammatory "memory" of environmental exposures (such as allergens, microorganisms, and other noxious agents) is encoded in barrier tissues present throughout the body (Ordovas-Montanes et al., 2020). It makes sense that the immune system is "everywhere" as infection or danger (defined as tissue damage) may be lurking in every organ of the body. This certainly does not preclude a role for the central lymphoid organs and hematopoiesis, but these new findings add a further level of complexity to our understanding of the immune system.

In addition to these important discoveries, we now know that the gut microbiome (our "forgotten organ") plays a crucial role in the development and fine-tuning of the host immune system (Honda & Littman, 2016; Thaiss et al., 2016). Therefore, the potential impact of engineered nanomaterials on the host microbiome should be considered following oral exposure as this could lead to indirect effects on the immune system.

3.2 | Corona-dependent immune responses

Inflammasomes are cytosolic, multiprotein signaling platforms that control the inflammatory response (Broz & Dixit, 2016). Each distinct inflammasome complex is assembled in the cytosol of the cell in response to pathogen-derived molecular patterns or endogenous danger signals (discussed below). The most well-studied inflammasome is the nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LRR)-containing protein (NLR) family member, NLRP3 (also known as cryopyrin). NLRP3 is activated by a diverse set of stimuli including crystalline and particulate matter such as uric acid crystals, silica (quartz), asbestos, and alum, as well as extracellular ATP, pore-forming toxins, and several microbial pathogens (Broz & Dixit, 2016). The question is whether these ligands share any common features or whether they trigger a common cellular event, or a perturbation of cellular homeostasis, that is sensed by the inflammasome (Liston & Masters, 2017). Muñoz-Planillo et al. (2013) could show that K^+ depletion is necessary and sufficient for inflammasome activation by pore-forming toxins and particulate matter including silica and calcium pyrophosphate crystals. During the past decade, numerous studies have shown that various nanomaterials also trigger the NLRP3 inflammasome (Farrera & Fadeel, 2015).

Does the protein corona play a role for inflammasome activation? Using gold nanorods as a model nanoparticle, Cai et al. (2020) evaluated the effect of surface ligand composition on the human plasma protein corona, and the cellular pathways involved in internalization of the particles. The authors used PMA-differentiated THP-1 cells as a macrophage model and incubations with the gold nanorods were performed in serum-free cell medium. There was a strong induction of IL-1β release for all surface-modified nanoparticles displaying a protein corona as compared with the pristine nanoparticles. Interestingly, when the interactions between macrophages and nanoparticles were blocked using specific antibodies against CD35 (also known as complement receptor type I or CR1), *IL1B*, and *CASP1* mRNA expression was downregulated, providing evidence that the protein corona effects occurred, at least in part, through proteins involved in the complement cascade. However, there was no direct evidence for NLRP3 inflammasome activation. It will be interesting to study this further, using relevant cell models (Andón et al., 2017), though it may be difficult to disentangle the effects of the protein corona on cellular uptake versus intracellular signaling events. Furthermore, the experimental conditions matter. Giulimondi et al. (2019) found that liposomes are readily taken up by THP-1 cells when incubated in low plasma concentrations (5%) whereas at high plasma concentrations (50%), cellular uptake was generally low irrespective of the synthetic identity (lipid composition) of the liposomes. Ju et al. (2020) incubated nanoparticles of varying sizes and surface chemistries with plasma obtained from two dozen human donors who were expected to exhibit variations in the plasma proteome and studied the role of the "personalized" bio-coronas for subsequent interactions with PBMCs. The authors could show that the enrichment of specific proteins in the bio-corona was linked to donor-dependent nanoparticle association with monocytes and B cells (professional antigen-presenting cells). This could have important implications for the use of nanoparticles for drug delivery.

The possibility that proteins in the bio-corona are unfolded and/or denatured was discussed in a previous section. Does this impact on immune cell recognition or signaling? Deng et al. (2011) showed that polymer-coated Au nanoparticles triggered the unfolding of fibrinogen, a major coagulation factor in plasma, which promoted binding to the integrin receptor, Mac-1 (CD11b/CD18) on human monocyte-like THP-1 cells. It is notable that fibrinogen itself is a large molecule with a length of 45 nm and diameter of 5 nm (i.e., larger than the nanoparticles). The authors could show that the activation of the Mac-1 receptor increased NF-κB activity in cells, resulting in the release of pro-inflammatory cytokines. Fibrinogen bound to the 5 nm Au nanoparticles increased nuclear NF-κB. Fibrinogen and Au nanoparticles alone also increased NF-κB in the nucleus, albeit to a lesser extent. Park et al. (2021) formed a corona around CNTs using four different plasma proteins, α_1 acid-glycoprotein (AGP), immunoglobulin G (IgG), fibronectin, and vitronectin. The authors found that the secondary structure of AGP and IgG was significantly altered whereas fibronectin and vitronectin exhibited negligible conformational changes when conjugated to the CNTs. The unfolded corona structures on CNTs prompted significant release of proinflammatory cytokines in an in vitro model. In contrast, such cytokine expression was not induced by the normally structured fibronectin or vitronectin corona. These differences were recapitulated in vivo in mice following repeated i.v. injections of CNTs with an unfolded or non-deformed protein corona, as evidenced by the selective activation of immune responses in the spleen by CNTs displaying AGP or IgG (Park et al., 2021). However, the administration of CNTs alone or the individual proteins (AGP or IgG) alone did not result in the expression of any pro-inflammatory cytokines in the spleen. Using a panel of polymeric nanoparticles, Vincent et al. (2021) could show that the surface chemistry of nanoparticles can be engineered to stabilize or denature the conformation of adsorbed albumin, to prevent or promote macrophage uptake via SR-A1.

3.3 | Nanomaterials as novel danger signals

The innate immune system can detect microorganisms through the recognition of pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) (Takeuchi & Akira, 2010). There are several classes of PRRs including transmembrane receptors such as the Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), as well as cytoplasmic proteins such as the retinoic acid-inducible gene (RIG)-like receptors (RLRs), and the NOD-like receptors (NLRs). Additionally, several DNA sensors have been identified (refer to: Gong et al., 2020, for an excellent overview). LPS (lipopolysaccharide, a component of the outer membrane of gram-negative bacteria) is a prototypic PAMP, and the crystal structure of LPS and its corresponding receptors (i.e., TLR4 together with MD2) has been solved (Park et al., 2009).[1]

The "pattern recognition" theory was originally proposed in 1989 by Charles Janeway, who theorized that the innate immune system is activated by conserved "non-self" motifs expressed by bacteria and other microorganisms (for an historical perspective, see: Medzhitov, 2009). Five years later, Polly Matzinger presented the blueprint for her controversial "danger" theory as a rival theory to the long-standing self/non-self theory, in which she elaborated on (and departed from) Janeway's pattern recognition theory. Hence, Matzinger (1994) discussed "the possibility that the immune system does not care about self and non-self, that its primary driving force is the need to detect and protect against danger, and that it does not do the job alone, but receives positive and negative communications from an extended network of other bodily tissues." According to the danger model, dying cells release endogenous factors that have been labeled as damage-associated molecular patterns (DAMPs), in analogy with PAMPs. However, as pointed out by others, it is not actually clear whether endogenous danger signals display distinctive molecular patterns (Kono & Rock, 2008). The current catalogue of DAMPs contains a diverse range of molecules including proteins such as high-mobility group box 1 protein (HMGB1) (Scaffidi et al., 2002), as well as uric acid (Shi et al., 2003), ATP (Iyer et al., 2009), and heme (Dutra et al., 2014). In addition, fragments of extracellular matrix proteins are perceived as danger signals (Heil & Land, 2014), and heat shock proteins have also been suggested to act as danger signals, as these proteins are upregulated during stressful cellular conditions, though this has been called into question by several authors (Bausinger et al., 2002; Wallin et al., 2002). Do these diverse DAMPs share any molecular features? Seong and Matzinger (2004) proposed that one common feature is the fact that *hydrophobic* portions of molecules that are normally hidden from the immune system are released into the extracellular environment and recognized by innate immune cells. Others have argued that the danger model contradicts Janeway's theory as an essential feature of the latter model is that PRRs are not activated by endogenous ligands (Józefowski, 2016). But perhaps this conundrum can be partially resolved if one factors in that the endogenous molecules and/or epitopes expressed by such molecules are normally hidden from the immune system and it is the "sudden" exposure of these cryptic epitopes that serves as a danger signal whereas other, pedestrian epitopes that are continuously exposed to the immune system fail to trigger an immune response. Immunological "danger" is thus context dependent (Matzinger, 2002). Similarly, the biological identity of a nanomaterial is context dependent (see above), and the proteins that form the corona on nanoparticles could be either of extracellular or intracellular origin. Can nanomaterials act as danger signals, in analogy with PAMPs and DAMPs? The term "nanomaterial-associated molecular patterns" (NAMPs) was coined 10 years ago as a potentially useful way of (re)framing the interactions between engineered nanomaterials and the immune system (Alsaleh & Brown, 2018; Fadeel, 2012; Neagu et al., 2017; Pradeu & Cooper, 2012). Obviously, nanomaterials could trigger cell death leading to the release of conventional danger signals (see, e.g., Bi et al., 2021). It is also trivial that nanomaterials that are contaminated with endotoxin (LPS) could trigger a response in immune-competent cells such as dendritic cells (DCs) that express receptors for LPS (Vallhov et al., 2006). However, the question is whether the immune system recognizes nanomaterials per se as danger signals? Furthermore, do innate immune cells respond to the synthetic identity or the biological identity (in other words, the adsorbed proteins and other biomolecules) of nanomaterials, or perhaps to both?

TLRs are important PRRs and TLR4, in particular, has been shown to bind a wide range of ligands. Using knockout mice, Danielsen et al. (2021) demonstrated that TLR2 and TLR4 contributed to the acute inflammatory response in

[1]Pattern recognition in immunology refers to the recognition of conserved molecular motifs or "patterns" that are present in pathogens but not in the host. In other words, it is a mechanism whereby the immune system discriminates between infectious "non-self" and non-infectious "self" (Janeway, 1992). These molecular patterns correspond to a broad array of microbial molecules such as bacterial lipopolysaccharide or LPS, nucleic acids including bacterial as well as viral DNA or RNA, bacterial cell wall peptidoglycans, fungal cell wall glucans, and several others. These molecules bind to their cognate receptors on immune-competent cells. This is in distinction to pattern recognition in the computational sciences which refers to the automated recognition of patterns or regularities in (large) data sets. For instance, machine learning, which is a branch of artificial intelligence premised on the idea that computer algorithms can learn from data, identify "patterns," and make decisions with minimal human intervention. The (superficial) similarity between the two is the fact that a "system" has evolved whether through evolution or by design that is capable of learning from past experiences (of data).

mice caused by graphene oxide (GO) and MWCNTs. Evidence for a direct interaction between nanomaterials and TLRs could not be obtained. However, Turabekova et al. (2014) previously predicted that the hydrophobic pockets of some TLRs might be capable of binding pristine (non-coronated) SWCNTs and fullerenes. Moreover, using both experimental (in vitro) and computational modeling approaches, we provided evidence that SWCNTs bind to and signal through TLRs leading to the activation of NF-κB and the secretion of multiple chemokines (Mukherjee et al., 2018). GO, on the other hand, did not signal via TLR2/4. We also performed computational studies to elucidate how SWCNTs may interact with TLR4 in the absence of a bio-corona. Interestingly, the binding of SWCNTs and TLR4 was reminiscent of the experimentally resolved structure of double-stranded (viral) RNA complexed with the endosomal receptor, TLR3 (Keshavan et al., 2019). Using a proteomics approach, He et al. (2018) provided evidence that glycoprotein non-metastatic melanoma protein B (GPNMB) (also known as osteoactivin), a glycosylated transmembrane protein, served as a cellular receptor and mediator of toxicity for carbon-based nanomaterials. Silencing of GPNMB expression in a macrophage-like cell line reduced the cytotoxicity of single- and multiwalled CNTs. On the basis of a targeted screening for phagocyte receptors, Omori et al. (2021) identified T cell immunoglobulin mucin 4 (Tim4) as a receptor for MWCNTs. Hence, Tim4 was found to play a role for the recognition of MWCNTs by mouse peritoneal macrophages and shown to promote granuloma development in mice following i.p. injection of MWCNTs. TIM-4 is known as a phagocyte receptor for phosphatidylserine (PS) exposed on the surface of apoptotic cells (Fadeel & Xue, 2009). Omori et al. (2021) provided evidence for stable interactions between MWCNTs and aromatic residues in the extracellular domain of Tim4. However, they could not show any binding of MWCNTs with other PRRs including CLRs, Fc receptors, complement receptors, or class B scavenger receptors (SR), and only weak binding to the scavenger receptor SR-A1, when these were expressed on the surface of the mouse fibroblast cell line, NIH 3T3. The study shows that Tim4 is a receptor for unopsonized MWCNTs. This does not, however, rule out that opsonization could direct MWCNTs to other cellular receptors. Taken together, it appears that CNTs are "sensed" as pathogens by innate immune cells, supporting the notion of so-called NAMPs.

Scavenger receptors are a family of receptors that recognize a diverse range of ligands in addition to modified lipoproteins, including several microbial structures (Canton et al., 2013). Indeed, to rationalize the broad range of endogenous and microbial ligands, it has been suggested that epitopes generated by the peroxidation of endogenous proteins or lipoproteins may mimic microbial structures (reviewed in: Canton et al., 2013). The class A scavenger receptor, MARCO (macrophage receptor with collagenous structure) is the major receptor mediating the binding of unopsonized bacteria and particles on alveolar macrophages (Arredouani et al., 2005; Palecanda et al., 1999). Chao et al. (2013) showed that 10 kDa dextran-coated superparamagnetic iron oxide nanoparticles (SPIONs) were efficiently recognized and taken up by SR-A1-transfected cells, whereas 20 kDa dextran and hydrogel coating blocked the binding and uptake via SR-AI. Computational modeling revealed a strong complementarity between the surface Fe–OH groups of the magnetite crystal and the charged lysines of the scavenger receptor, suggesting a specific mode of recognition of these particles. In sum, the scavenger receptor SR-AI appears to recognize the charged crystalline core of SPIONs, while polymer coating sterically prevented the interaction (Chao et al., 2013). In a more recent study, Tsugita et al. (2017) performed non-biased functional expression screening using a macrophage cDNA library to identify mouse and human scavenger receptor SR-BI as a cell surface receptor for amorphous and crystalline silica. Notably, SR-B1 did not bind TiO_2 nanoparticles or monosodium urate crystals, although these ligands all exhibited negative surface potentials. The latter finding suggested that SR-B1 interacts with specific molecular determinants or molecular patterns within silica. The authors also showed that SR-B1-mediated recognition of silica leads to inflammasome activation (Tsugita et al., 2017). Thus, scavenger receptors recognize not only endogenous molecules and pathogens, but particles as well.

To address whether nanomaterials act as danger signals, receptor binding is not sufficient, even though cellular recognition and uptake may represent a critical first step. It will also be important to evaluate whether nanomaterials trigger DC maturation and T cell activation, as shown, for instance, for silica nanoparticles (Feray et al., 2021). In another fascinating example, Xu et al. (2022) reported that achiral and left- and right-handed gold nanoparticles differed in terms of their ability to activate immune responses. Both types of chiral particles engaged with the adhesion receptors CD97 and EMR1 (EGF-like module-containing, mucin-like hormone receptor-1), but the left-handed particles did so with higher affinity owing to the interactions between the chiral extracellular domains of the receptors and the chiral nanoparticle surfaces (Xu et al., 2022). The left-handed particles were also found to elicit a more potent adjuvant effect in mice when combined with a vaccine against the H9N2 influenza virus (Xu et al., 2022). The latter study focused on bone marrow-derived macrophages and DCs, but CD97 is also highly expressed in neutrophils and plays a role in neutrophil migration (Leemans et al., 2004; Veninga et al., 2008). Thus, it will be of interest to address whether neutrophil migration or function (such as, in the context of inflammation) is affected by the chiral nanoparticles devised by Xu et al. (2022).

Enantioselective nanoparticles can be achieved through the functionalization of particles with chiral molecules (Wang et al., 2017; Yeom et al., 2020). However, the exciting recent study by Xu et al. (2022) provides an exemplary illustration of the strong link between the synthetic "identity" of nanoparticles and their biological (receptor-mediated) responses.

As discussed above, the synthetic identity defined by particle size, shape, surface properties, and so on, drives the biological behavior of nanoparticles (Kim et al., 2013; Zhu et al., 2013) (as well as dictating the bio-corona formation). However, it is challenging to experimentally disentangle these properties. Moyano et al. (2012) were able to show that hydrophobicity of a set of gold nanoparticles scales with cytokine responses in mouse splenocytes (a mixed population of B cells, T cells, and monocytes). Moreover, using a panel of carefully designed polymeric nanoparticles, Nandi et al. (2021) could show that core hydrophobicity positively correlated with NLRP3 inflammasome assembly in contrast to surface charge, core rigidity, and surface hydrophobicity. The authors used immortalized bone marrow-derived macrophages as a model, and the cells were maintained in cell medium supplemented with FBS and primed with LPS. Mechanistically, they could show that high core hydrophobicity triggered lysosomal disruption with the release of cathepsin B, resulting in NLRP3 activation (Nandi et al., 2021). This is a very exciting study as it provides direct evidence that hydrophobicity (a material-intrinsic property) may function as a NAMP, with the triggering of relevant signaling events in macrophages. Other authors have referred to the positive or negative impact of engineered nanomaterials on biological systems as "ancillary" effects while drug delivery was perceived as the main effect, at least from a nanomedicine point of view (Stater et al., 2021). However, it may be the other way around.

Myerson et al. (2022) recently reported that the presence of agglutinated proteins (agglutination = protein clumping) is predictive of the neutrophil-selective uptake of nanoparticles with homing of the cells to inflamed lungs. In contrast, nanoparticles with a symmetrical protein arrangement (e.g., viral capsids) had no selectivity for inflamed lungs. The authors also found that the nanoparticles displaying agglutinated proteins (NAPs) were opsonized with complement, and this was necessary for the observed neutrophil tropism (Myerson et al., 2022). In brief, the study shows that the protein arrangement determines whether the particles are "targeted" toward inflammatory cells.

To conclude, it is entirely plausible that the protein corona is denatured and recognized by receptors such as Mac-1 (also referred to as CR3) consisting of CD11b (integrin α_M) and CD18 (integrin β_2) that are known to act as receptors for denatured proteins. In fact, the apparent promiscuity of these PRRs (Lamers et al., 2021) is reminiscent of the broad binding specificity of the heat shock proteins. Furthermore, it has been proposed that the redox state of the injured tissues could play a critical role for immune sensing of danger signals (Rubartelli & Lotze, 2007). Consequently, oxidized and/or denatured corona proteins could serve as bona fide NAMPs regardless of the specific identity of these proteins. Moreover, the hydrophobic regions of proteins that are normally hidden from the immune system may be exposed in proteins that undergo misfolding on the surface of nanoparticles, in line with the original "danger" model proposed by Matzinger (discussed above). It is also possible that other biomolecules in the corona apart from proteins may act as danger signals. Indeed, it is important to note that the scavenger receptor superfamily is defined by its ability to recognize and bind a broad range of common ligands including oxidized low-density lipoprotein (LDL). Thus, it is also plausible that oxidized corona lipids with or without associated proteins could act as NAMPs. Indeed, it is possible that nanoparticles cloaked in oxidized lipids (and proteins) are perceived as pathogens by the immune system (Zhivaki & Kagan, 2021). At any rate, the take-home message is that the misfolded and/or oxidized corona may be a common and sufficient danger signal. This does not mean that individual corona proteins cannot play specific roles, under certain circumstances, or that the nanoparticle corona cannot be tailored to achieve targeting, and so on. However, in terms of understanding innate immune responses toward nanomaterials, it may be useful to ask whether proteins are denatured as opposed to asking whether specific epitopes are exposed. It is noteworthy that ligand-receptor binding can be more or less "specific." Hence, B cell and T cell receptors display exquisite antigen specificity, whereas PRRs expressed by innate immune cells show a broad binding specificity precisely because these cells should act as a first line of cellular defense. Having a missile defense system that can intercept different kinds of missiles at an early stage is better than intercepting only one type of missile at a later stage. Finally, the nanomaterial itself could act as a "missile" in the sense that the material-intrinsic properties may be sensed as a danger signal by the immune system, and this has been shown for both organic and inorganic materials, as discussed above.

4 | CLOSING REMARKS

Nanomaterials may either stimulate or suppress the immune system, and the surface chemistry of the materials is believed to be an important determinant of these responses (Dobrovolskaia & McNeil, 2007). However, there is also a wealth of data to suggest that the adsorption of proteins or other biomolecules onto nanomaterials (referred to as the

bio-corona) is linked to the biological outcomes (Monopoli et al., 2012). Here, we revisited the concept of "nanomaterial-associated molecular patterns" as a potentially useful way of framing the interactions between nanomaterials and the immune system, especially cells of the innate or "primitive" arm of the immune system. We discussed the synthetic (material-intrinsic) "identity" and the biological (context-dependent) "identity" of engineered nanomaterials, largely defined by the adsorption of biomolecules, and the fact that these shifting identities will ultimately impact on cellular recognition and other biological responses. Hence, engineered nanomaterials are dynamic entities and should be studied (and regulated) as such (Fadeel et al., 2018).

From a more general point of view, the quality of "bio-nano" studies should be critically assessed. The need for a reporting checklist aimed at improving reproducibility and consistency in experiments using nanomaterials for biological applications has recently been advocated (Faria et al., 2018). This may serve to improve communication provided that such standards are endorsed by the scientific community and by scientific journals (Leong et al., 2019). Building on these guidelines, other investigators recently proposed a checklist for experimental design and reporting guidelines for bio-corona experiments (Chetwynd et al., 2019). In other words, we should be careful when we design and report our research not only with respect to the characterization of the synthetic and biological "identities" of the test materials but also with respect to the test systems. It should be noted that cell lines are only a model of primary cells, and "mice are not men" (Lin et al., 2014; Warren et al., 2015). Indeed, "studying the immune system in humans, who are genetically diverse and afflicted by a multitude of diseases" (Pulendran & Davis, 2020) may be the key to clinical translation in medicine as well as new discoveries in (human) biology and such studies may also serve to inform toxicology.

Safety is of paramount importance in the development and deployment of any new technology. Indeed, it is axiomatic that the "development of novel nanoparticles must proceed in tandem with assessment of any toxicological and environmental effects" (Seaton & Donaldson, 2005). This is currently referred to as the safe-and-sustainable-by-design approach. Understanding the interface between engineered nanomaterials and biological systems (especially the immune system), and the role of adsorbed proteins and other biomolecules aka the bio-corona, is a key part of this endeavor.

CONFLICT OF INTEREST

The author has declared no conflict of interest for this article.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable for this review article.

ORCID

Bengt Fadeel https://orcid.org/0000-0001-5559-8482

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