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Clear and present danger? Engineered nanoparticles and the immune system

Bengt Fadeel[a,b,c]

[a] Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[b] Childhood Cancer Research Unit, Astrid Lindgren's Children's Hospital, Karolinska University Hospital, Stockholm, Sweden
[c] Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA

Summary

The innate immune system is the first line of defense against microbial invasion and involves the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors on the surface of phagocytic cells. The immune system also responds to tissue damage, a process that is triggered by so-called danger- or damage-associated molecular patterns (DAMPs) or "alarmins". How do physico-chemical properties e.g., size, shape, surface charge and solubility affect immune interactions of nanoparticles? Does the adsorption of biomolecules onto the surface of nanoparticles dictate subsequent immune responses? Do engineered nanoparticles *per se* act as "alarmins" or does the bio-corona on nanoparticles convey a new "identity" and allow innocuous nanoparticles to present NAMPs (nanoparticle-associated molecular patterns)? Finally, what are the parameters that determine particle clearance or biodegradation in a living system? Understanding nano-immuno-interactions is critical for the safe application of engineered nanoparticles in medicine.

Key words: *nanoparticles; immune system; danger signal: bio-corona; recognition*

Introduction

Mother Nature has deployed "nanotechnology" for a very long time. Indeed, DNA is a nano-scale structure harbouring the genetic code and the cell operates numerous nano-scale machines such as the proteasome (for degradation of proteins) and the ribosome (for protein synthesis). Viruses are natural nano-scale particles; for example, the human immunodeficiency virus is 120 nm in diameter. Life, as Stephen Mann has pointed out, is a nano-scale phenomenon [1]. Moreover, humankind has been exposed to natural or anthropogenic nanoparticles for millennia [2]. For instance, when you light a candle on a dinner table, over 1.5 million nanodiamond particles form in the flame every second [3]. Therefore, it seems reasonable that our body should have evolved a system to cope with the constant challenge of particles. The innate immune system is the first line of defense against foreign intrusion i.e., microorganisms (and nano-organisms such as viruses) and particles, as well as cellular debris. Here, I will discuss how artificial or engineered nanoparticles interact with the immune system and whether nanoparticles should be considered as "danger" signals.

Abbreviations

DAMPs	damage-associated molecular patterns
DC	dendritic cell
HMGB1	high mobility group protein B1
NAMPs	nanoparticle-associated molecular patterns
NLRP3	nucleotide-binding oligomerisation domain, leucine-rich repeat and pyrin domain containing-3
PAMPs	pathogen-associated molecular patterns
PEG	poly(ethylene glycol)
SWCNT	single-walled carbon nanotubes
TLR	Toll-like receptor.

The danger hypothesis

When I was a medical student, we were told that the immune system distinguishes between "self" and "non-self" (or, "altered" self, such as, for instance, epitopes or structures displayed by apoptotic cells). The inability to discriminate between self and non-self would result in autoimmune disease, in which the immune system attacks its own host [4]. The inability to detect or to combat foreign intrusion would naturally lead to infection. In 1994, Polly Matzinger presented the "danger" hypothesis according to which the immune system is more concerned with entities that do damage than with those that are foreign [5]. This model postulates that the primary driving force is the need to detect and protect against danger and the ultimate control over immune responses is exerted through endogenous, not exogenous signals; they are the alarm signals that emanate from injured tissues. There is evidence to support both models, and more recently, an attempt to reconcile the "stranger" (non-self) model and the "danger" model was presented [6]. According to this proposal, many of the pathogen-associated molecular patterns and tissue-derived alarm signals may belong to an evolutionarily ancient

alert system in which the *hydrophobic portions* of biological molecules act, when exposed, as universal signals of damage to initiate immunity.

On the surface of things, this reminds us of engineered nanoparticles i.e. man-made entities that can present vast surface areas to biological systems. Therefore, one may ask whether nanoparticles with hydrophobic surfaces are interpreted as danger signals by the immune system? As a case in point, Moyano et al. [7] provided evidence that nanoparticle hydrophobicity dictates immune responses as determined by gene expression profiling of mouse splenocytes exposed *ex vivo* to gold nanoparticles. However, as discussed by Hubbell et al. [8], it may be difficult to preserve a hydrophobic material surface once exposed as such surfaces are rapidly obscured through protein adsorption. Indeed, cells of the immune system may not "see" the naked nanoparticle surfaces; there is a growing body of evidence that a long-lived protein corona is formed when nanoparticles are introduced into a biological milieu such as plasma [9]. This prompts a further question: do nanoparticles coated with plasma proteins present *nanoparticle-associated molecular patterns* or NAMPs to the immune system? I will attempt to frame this question in the following section.

The bio-corona concept

When nanoparticles are presented to a physiological environment such as human plasma they selectively absorb biomolecules to form a so-called *biomolecular corona* on the surface of the nanoparticle [9]. Needless to say, one should be aware of the fact that nanoparticles – indeed, any material surfaces – may be coated with bacterial endotoxin or lipopolysaccharide (LPS), a membrane component of all Gram-negative bacteria, especially if the nanoparticles are synthesised under non-sterile conditions. This could give rise to interference with the biological assessment of nanomaterials eg. induction of maturation of dendritic cells (DC) [10]. However, of more importance for the present discussion, several studies in recent years have provided evidence for a "hard" (long-lived) corona of human plasma proteins on nanoparticles of different chemical composition [11–14]; the formation of the hard corona was shown to depend on both size and surface properties eg. degree of hydrophobicity [15]. The bio-corona has been reported to impact on cellular uptake [16] and on cell signaling including NF-κB-dependent cytokine production in THP.1 cells [17]. Notably, lipids, not only proteins, may also feature prominently in the bio-corona, as shown recently for single-walled carbon nanotubes (SWCNT) administered into the lungs of mice [18], and the combined lipid-protein corona may play a role in cellular uptake of nanomaterials.

The notion that naked particle surfaces may not exist in nature has profound implications as the bio-corona may convey a new biological "identity" to nanoparticles [19]. This is, in a manner of speaking, a case of the Emperor´s new clothes in reverse; hence, the Emperor is not naked as we thought but fully "clothed" in a layer of biomolecules. In fact, some studies have even provided detailed information on the thickness of this layer, using fluorescence correlation spectroscopy [20]. Moreover, researchers have applied systems approaches to understand or to categorise the components on the bio-corona [21, 22]. However, while common proteins can bind to different nanoparticles, the biological outcome (e.g., cytokine release) may not be the same [23], suggesting that knowledge about the composition of the protein corona may not be sufficient; we need to understand how the proteins bind (i.e., their orientation on the nanoparticle surface) and whether protein unfolding takes place [24]. It is safe to say, however, that the "discovery" of the bio-corona on nanoparticles provides fertile ground for further investigations on bio-nano-interactions, including interactions with cells of the innate and adaptive immune system. Indeed, nanoparticles with an engineered bio-corona of DAMPs or PAMPs could provide researchers with tools ("synthetic pathogens") with which to probe immune responses [8]. The natural extension of the bio-corona concept is that the bio-corona could be exploited through the purposeful design of material surfaces in order to control protein binding. Indeed, a chemical approach to cell-specific targeting based on the induction of protein binding and misfolding on nanoparticle surfaces was reported recently [25].

For biomedical applications, it is often useful to extend the half-life of the nanoparticles in systemic circulation by preventing the non-specific uptake by cells of the reticulo-endothelial system. Such long-circulating nanoparticles can more effectively deliver their cargo of drugs to the desired tissues. The current gold standard for achieving long-circulating particles involves the grafting of poly(ethylene glycol) (PEG) onto particle surfaces. This surface modification reduces additional biomolecule binding but does not entirely prevent the formation of a bio-corona [26]. In a recent study, Hu et al. [27] developed a new drug delivery platform consisting of biodegradable polymeric nanoparticles camouflaged with membrane lipids and associated membrane proteins derived from erythrocytes. Using these bio-mimetic carriers, the delivery of slow-releasing drug payloads *in vivo* was demonstrated with a carrier circulation half-life beyond what can be achieved by PEG. This study thus provides a new twist on the bio-corona concept whereby the nano-scale carriers are disguised as "self"using membrane constituents derived from red blood cells [28]. Interestingly, spores of the human opportunistic fungal pathogen *Aspergillus fumigatus* are surrounded by a natural protein corona of hydrophobin making them "invisible" to cells of the immune system [29]. In a recent study, hydrophobin-functionalised porous silicon nanoparticles were shown to display a pronounced change in the degree of plasma protein adsorption *in vitro* and altered biodistribution *in vivo* when compared to uncoated nanoparticles [30]. This study provides further evidence that "stealth" properties can be engineered by manipulating the bio-corona on nanoparticles.

The complement system is a group of proteins present in body fluids, which interact to identify and opsonise non-self, altered-self and synthetic materials for phagocytosis by cells of the innate immune system. The complement system plays a major role in innate immune defenses against microorganisms, but exaggerated activation of complement can lead to severe tissue injury. Therefore, the

propensity for complement activation needs to be taken into account for any drug delivery system that comes into contact with the blood, including nano-scale carriers. Indeed, the binding of complement to nanomaterial surfaces represents a special case of undesirable bio-corona formation which may induce clinically significant adverse reactions in susceptible individuals. The interaction between nanomaterials and the complement system is complex and regulated by several factors including size, morphology and surface characteristics [31]. In the case of carbon nanotubes, conflicting data have been reported with respect to binding and/or activation of the complement cascade [32–34]. In a recent report, the structure-activity relationship pertaining to surface-immobilised polyethyleneoxide (PEO) of various configurations on polystyrene nanoparticles and the initiation of the complement cascade was studied [35]. Interestingly, alteration of copolymer architecture on nanospheres from "mushroom" to "brush" configuration not only switched complement activation from the C1q-dependent classical pathway to the so-called lectin pathway but also reduced the level of generated complement activation products. These findings provide a rational basis for improved surface engineering and design of immunologically safer and targetable nanosystems with polymers for use in clinical medicine.

Inflammasome activation

Toll-like receptors (TLRs) are a family of receptors that has evolved to recognise conserved features of microbes and thus provide a broad, first-line defense against microbial pathogens. Activation of the so-called inflammasome complex in the cytoplasm of phagocytic cells occurs via engagement of TLRs leading to subsequent assembly of the NLRP3 (NLR-related protein 3)-containing inflammasome complex and activation of caspase-1 with processing and secretion of the pro-inflammatory cytokine, interleukin (IL)-1β [36]. NLRP3 is also activated in response to host-derived particulate matter precipitates such as uric acid and cholesterol crystals and studies in recent years have shown that exogenous structures including asbestos fibers and crystalline silica also activate the inflammasome [37–40]. In addition, NALP3 is also the molecular target of the immunostimulatory activity of aluminium hydroxide (alum) the most commonly used vaccine adjuvant [41]. Naturally, the observation that this century-old adjuvant acts via inflammasome activation suggests that other agonists of the NALP3 inflammasome should also be considered as vaccine adjuvants. Notably, a recent study reported on a novel class of inflammasome-activating nanomaterials for optimisation of vaccine design [42]. Hence, these authors incorporated LPS onto the surface of nanoparticles constructed of a biocompatible polyester, poly(lactic-co-glycolic acid) (PLGA), loaded with antigen. The LPS-modified particles were preferentially internalised by DC and the system elicited potent humoral and cellular immunity in mice. Wild-type macrophages pulsed with LPS-modified nanoparticles resulted in production of IL-1β consistent with inflammasome activation. Furthermore, when endocytosis and lysosomal destabilisation were inhibited, inflammasome activity was diminished, supporting the notion that nanoparticles may rupture lysosomal compartments and behave as danger signals [42]. The latter study is of considerable interest as it suggests that nanoparticles encapsulating a specific antigen can be engineered to be more effective vaccines by the proper choice of immune potentiators on the particle surface.

Exposure of macrophages to carbon black nanoparticles was shown to result in caspase-1-dependent, inflammasome-mediated cell death [43], and Tschopp and co-workers reported that nano-TiO_2 and nano-SiO_2, but not nano-ZnO, activate the NLRP3 inflammasome, leading to IL-1β release and in addition, induce the release of IL-1α [44]. Furthermore, inhalation of TiO_2 nanoparticles provoked lung inflammation which was suppressed in IL-1R- and IL-1α-deficient mice. Thus, the inflammation caused by these nanoparticles *in vivo* is largely caused by the biological effect of IL-1α. Lunov et al. [45] found that amino-functionalised polystyrene nanoparticles (PS-NH_2), but not carboxyl- or non-functionalised particles, triggered NLRP3 inflammasome activation and release of IL-1β by human macrophages. Of note, the macrophage activation could be antagonised by the radical scavenger, N-acetyl-cysteine.

Recent studies have shown that "needle-like" MWCNT can activate the NLRP3 inflammasome in LPS-primed human macrophages [46]. Moreover, one of my post docs has shown that hollow carbon spheres i.e., non-fiber-like structures can also trigger inflammasome-dependent secretion of IL-1β in primary human macrophages (unpublished observations). Taken together, these studies point towards similarities in terms of immune sensing of nanoparticles, environmental agents and microorganisms. However, very recent work suggests that there are also differences between bacterial cell wall components and crystalline (solid) agents such as alum [47].

Recognition: role of size and shape

Mechnikov received the Nobel Prize in Physiology or Medicine more than a century ago for his work on phagocytosis and the key role of macrophages ("gravediggers") in preserving the integrity, and, in some cases, defining the "identity" of the organism [48]. Macrophages engulf microbes and apoptotic debris, but the question is: are nanoparticles recognised by phagocytes or do such particles fly under the radar and escape immune recognition? An intriguing example of nanoparticles that possess intrinsic membrane-penetrating abilities based on the spatial distributions of hydrophobicity and charge on the surface has been reported [49]. However, it appears that most if not all nanoparticles are internalised by cells through an active (endocytic) mechanism [see, for instance, 50, 51]; primary macrophages may not behave in the same manner as macrophage-like cell lines [52] and nanoparticles that are taken up by cells through endocytosis may also exit the cell through the back door [53, 54]. Nevertheless, the immune system is capable of also recognising nano-scale particles.

Size is an important determinant of nanoparticle uptake by immune-competent cells. Manolova et al. [55] reported that nanoparticles target distinct DC populations *in vivo* in a size-dependent manner. In addition, Rettig et al. [56] have provided evidence for a new dimension in danger sig-

nalling insofar as they were able to show how size quantitatively affects innate immune responses. Hence, nano-sized particles made from single-stranded RNA (ssRNA) mixed with protamine induced production of interferon-α, whereas microparticles mainly induced production of tumour necrosis factor-α (TNF-α) in human immune cells. The authors found that nanoparticles but not micro-sized particles were selectively phagocytosed by plasmacytoid DC, which produce interferon-α [56]. Thus, at the same time as sensing PAMPs such as ssRNA, the immune system apparently distinguishes the size of the associated structure in such a way as to trigger antivirus (interferon-α) or antibacterial/antifungal (TNF-α) immune responses.

Shape is also important for cellular (macrophage) recognition. Mitragotri and co-workers have shown that particles possessing the longest dimension in the range of 2–3 microns exhibited highest attachment to macrophages [57]. This also happens to be the size range of most commonly found bacteria in nature. The authors speculated that surface features of macrophages, in particular the membrane ruffles, might play an important role in this geometry-based target recognition by macrophages. Understanding the mechanism by which cells are capable of sensing aspect ratio differences in nanoparticles could be exploited to achieve more efficient drug delivery [58]. Using highly stable, polymer micelle assemblies designated as filomicelles to compare the transport and trafficking of flexible filaments with spheres of similar chemistry, Geng et al. were able to show that filomicelles persisted in the circulation up to one week after intravenous injection [59]. This is about ten times longer than their spherical counterparts and thus demonstrates further that shape is important.

TLR signalling in macrophages links the autophagy pathway to phagocytosis (of microorganisms) [60] while inflammasome activation is negatively regulated by autophagy [61]. This is potentially relevant for the understanding of nanoparticle-induced effects as nanoparticles are frequently of the same shape and size as microorganisms suggesting that the immune system may recognize these materials through conserved pathways [62]. Indeed, as pointed out by Szebeni et al., clinically used liposomes bear a remarkable resemblance in terms of size and shape to many pathogenic human viruses [63]. In fact, several studies have suggested that nanoparticles may activate an autophagic response [64–66]. In contrast, Ma et al. [67] provided evidence that autophagosome accumulation following exposure to gold nanoparticles results from blockade of autophagy flux, rather than induction of autophagy. Notwithstanding, it is important to note the distinction between autophagy (a survival pathway) and autophagic cell death (a form of programmed cell death) [68]. It will be of interest to understand the molecular events that determine whether the uptake of nanoparticles by phagocytes will trigger or dampen inflammasome activation.

Requiem for the dying cell

Considerable efforts have been made during the past decades to elucidate the molecular mechanisms that govern apoptotic cells death; however, the subsequent recognition and removal of apoptotic corpses by neighbouring phagocytes has received less attention. Nevertheless, macrophage engulfment of apoptotic cells is known to be important in the remodelling of tissues, and contributes to the resolution of inflammation through the removal of effete cells prior to the release of noxious cellular constituents [69]. Moreover, apoptotic cells are a potential source of self-antigens, and clearance of cell corpses is thought to preclude the induction of autoimmune responses [70]. The view is thus emerging that tissue homeostasis is dependent not only on the balance between cell division and cell death, but also on the rate of cell death versus that of cell clearance. I refer to the disposal of apoptotic cells by professional phagocytes and other neighbouring cells as *programmed cell clearance* to underscore that this is a genetically regulated process much like apoptosis [71].

Several studies have shown that serum proteins may serve to facilitate interactions between the phagocyte and its apoptotic prey [72]. For instance, the glycoprotein, MGF-E8 binds to the anionic phospholipid, phosphatidylserine or PS on the surface of apoptotic cells and to integrin receptors on the surface of macrophages and promotes programmed cell clearance [73]. These observations are relevant for the understanding of nanoparticle interactions with phagocytes because nanoparticles are rapidly covered with a bio-corona of plasma proteins when they are introduced into the bloodstream and this may affect cellular uptake of nanoparticles, as discussed above. The immune system may not really care whether the target is a bacterium, a gold nanoparticle, or an apoptotic cell corpse, as long as the relevant “eat-me” signals are displayed on the surface; whether or not the engulfment of nanoparticles by macrophages will lead to “indigestion” in phagocytic cells is another matter.

In fact, some studies have assessed whether the interaction of nanomaterials with macrophages may impede the normal process of programmed cell clearance. Witasp et al. [50] reported that macrophage uptake of mesoporous silica particles of different sizes does not affect subsequent macrophage engulfment of apoptotic cells, whereas pre-incubation of primary human macrophages with SWCNT negatively affected uptake of apoptotic cells [74]. The recent demonstration by Holt et al. [75] that SWCNT may impact on the actin cytoskeleton provides a plausible mechanism for the impaired phagocytosis as cytoskeletal reorganisation is required for programmed cell clearance.

Dead cells must be buried but the mode of cell death determines how cell death is decoded by the immune system [69]. Hence, apoptotic cell death normally leads to the suppression of pro-inflammatory cytokine secretion and the promotion of anti-inflammatory cytokine production and induces immunological tolerance [70]. In contrast, necrosis leads to the release of alarmins such as high mobility group protein B1 (HMGB1) and consequent activation of inflammation and immune responses [76]. Upon release of intracellular constituents, an inflammatory response is rapidly mobilised and serves as an initial defence and also attempts to clear and repair damage. In parallel, DC are stimulated to mature and induce adaptive immune responses if immunogenic antigen are detected (see [77] for an excellent overview). The litmus test for cell death is thus to understand how cell death is decoded by cells of the innate and ad-

aptive immune system. It follows from this argument that it is essential to understand how nanoparticles affect the specific mode of cell death [68]. In other words, do cytotoxic nanoparticles trigger immunogenic or tolerogenic cell death?

Hasta la vista, carbon nanotubes!

Non-degradable nanomaterials can accumulate in cells and tissues where they may exert detrimental effects. Indeed, it has been shown that intravenously injected, pristine (non-functionalised) SWCNT are highly enriched in liver, lungs, and spleen in mice and remain in the body over an extended period of time [78]. Of note, enzymatic degradation of SWCNT was recently demonstrated by incubating SWCNT in a cell-free system with horseradish peroxidase (HRP) and low amounts of hydrogen peroxide [79]. Moreover, the complete biodegradation of SWCNT by human myeloperoxidase (MPO) was recently reported [80]. Biodegradation was shown in a cell-free system but evidence was also presented for MPO-driven biodegradation in neutrophils isolated from normal healthy donors. It is noted that the carbon nanotubes were coated with a corona of immunoglobulins in these studies in order to promote efficient neutrophil uptake, presumably via Fc receptors [80]. Macrophages were found to be less proficient at digesting SWCNT, in line with the fact that these cells express much lower amounts of MPO when compared to neutrophils. SWCNT fully biodegraded by MPO *in vitro* did not elicit typical inflammatory and oxidative stress responses characteristic of CNT after pharyngeal aspiration in mice [80]. More recently, *in vivo* biodegradation was demonstrated in a mouse model of pharyngeal aspiration of SWCNT [81]. In this study, oxidation and clearance of SWCNT from the lungs of MPO-deficient mice was markedly less effective whereas the inflammatory response was more robust as compared to wild-type C57Bl/6 mice. Collectively, these reports suggest new ways to control the biopersistence of carbon nanotubes through genetic or pharmacological manipulations. It should be noted that the immune system utilises the same enzymatic pathways for degradation of microorganisms.

If properly modified, CNTs may also be excreted from the body. Hence, Kostarelos and co-workers have reported that surface-functionalised, water-dispersible SWCNT (average diameter 1 nm; average length 300–1000 nm) were capable of rapid and effective renal clearance and urinary excretion with a blood circulation half-life of a few hours [82]. This occurs only if adequate individualisation of carbon nanotubes is achieved *in vivo*; if the injected nanomaterial is in aggregates or bundles, the latter will not be able to cross the glomerular filter and will accumulate in the liver, spleen, or lungs [83].

Concluding remarks

I have attempted to discuss how engineered nanomaterials interact with immune-competent cells as well as the crucial role of components of the innate immune system e.g. TLRs and complement. Detailed information on the molecular mechanisms underlying such interactions are emerging and we now have evidence that nanomaterials can activate the so-called inflammasome in macrophages leading to the production of pro-inflammatory cytokines. This knowledge may also prove beneficial as novel classes of immunostimulatory agents (adjuvants) are being produced [8]. Recent studies also suggest that cells of the innate immune system can enzymatically digest carbon-based nanomaterials, thus demonstrating that these materials are not necessarily biopersistent as asbestos fibers; these studies also underscore the amazing versatility of our immune system, our primary defense against foreign intrusion, and suggest that carbon nanotubes may be considered as tools for delivery of therapeutic agents if used at appropriate and readily degradable concentrations. In addition, I have highlighted the emerging realisation that the bio-corona on nanoparticle surfaces may dictate biological responses. The recognition versus non-recognition of nanomaterials and its importance for biodistribution of nanomaterials *in vivo* has been discussed and the importance of a proper diagnosis of cell death i.e. apoptosis, necrosis, etc. in order to predict immunological outcomes of nanoparticle-induced cytotoxicity has been highlighted; see [68] for a detailed discussion.

Careful assessment of the interactions of nanoparticles with the immune system is of the utmost importance for the safe development of the nanotechnologies, not least for biomedical use [84]. Important lessons in this regard can be learned from immunology [62]. Indeed, common principles have been derived for immune recognition of pathogens (non-self) and we now know that a relatively small number of immune receptors operate to detect microbial molecules

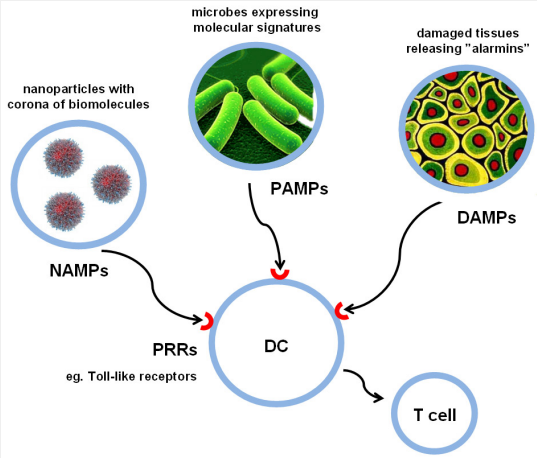

Figure 1

Do nanoparticles act as danger signals and/or display such signals on their surface? In this hypothetical diagram, the analogy is made between pathogens (microorganisms) that display pathogen-associated molecular patterns (PAMPs) and damaged or stressed tissues that release damage-associated molecular patterns (DAMPs), such as HMGB1, a non-histone nuclear protein that acts as a secreted alarmin, and engineered nanoparticles that are coated with a bio-corona of proteins, possibly unfolded, thereby revealing hidden epitopes, that may act as nanomaterial-associated molecular patterns (NAMPs). These molecular signatures are recognised by so-called pattern recognition receptors or PRRs – including the Toll-like receptors – on the surface of innate immune cells or expressed within such cells. The activation of PRRs triggers inflammation and alerts the adaptive immune system to impending danger [85].

or PAMPs that herald infection [77]. Thus, we may anticipate that general principles of interactions between artificial nanoparticles and biological systems can be deduced as well and that common principles for the understanding of nanomaterial interactions with cells and tissues can be derived [62]. To this end, we need to decipher the bio-corona on nanoparticles as this layer of biomolecules may bestow upon nanoparticles their true "identity". In so doing, we may begin to understand the basic principles that will allow for the rational design of safe (and useful) nanomaterials. Do engineered nanoparticles act as danger signals and/or display biomolecules that elicit immune responses (fig. 1)? I hope that the present discourse may stimulate further studies on these topics.

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Correspondence: *Bengt Fadeel, MD, PhD, Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Nobels väg 13, SE-171 77 Stockholm, Sweden, bengt.fadeel[at]ki.se*

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Figures (large format)

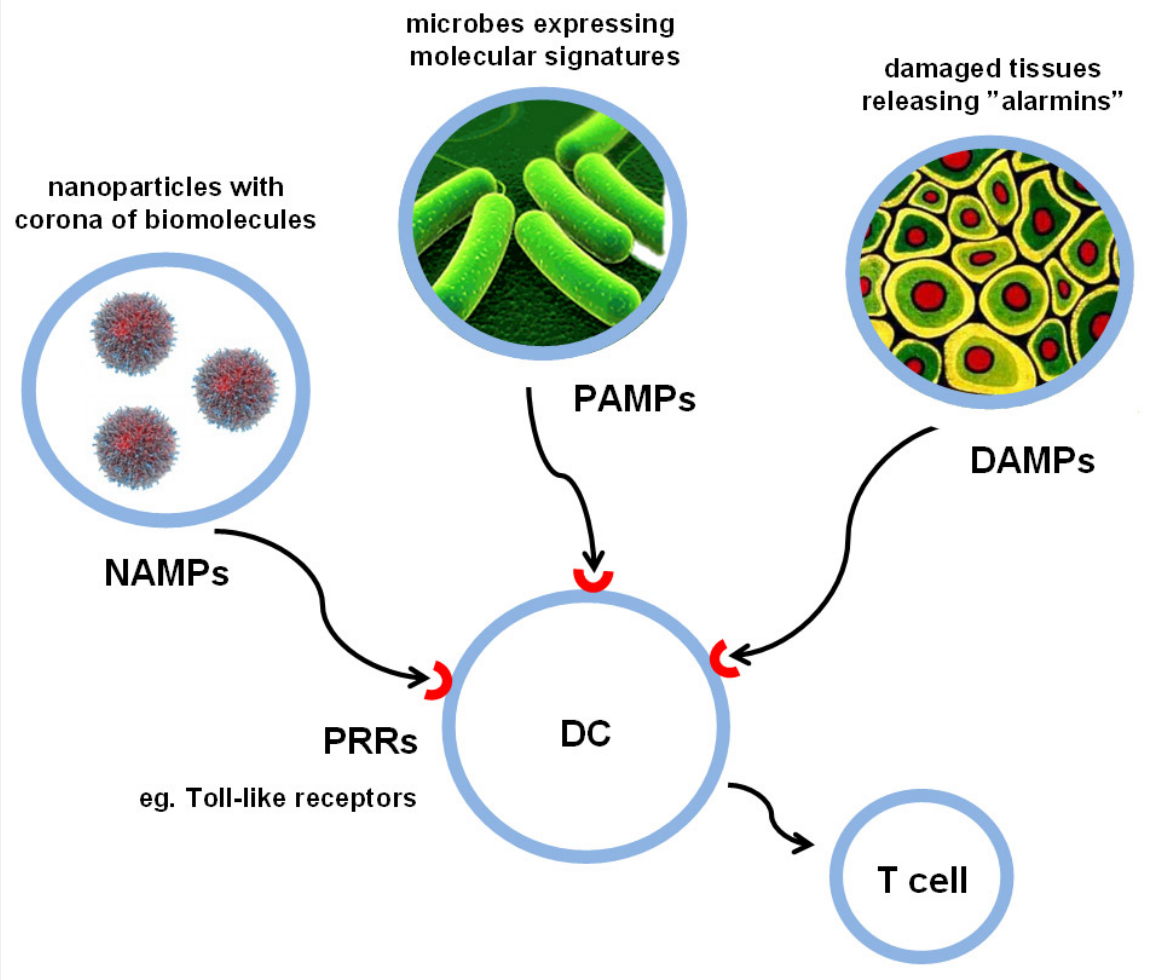

Figure 1

Do nanoparticles act as danger signals and/or display such signals on their surface? In this hypothetical diagram, the analogy is made between pathogens (microorganisms) that display pathogen-associated molecular patterns (PAMPs) and damaged or stressed tissues that release damage-associated molecular patterns (DAMPs), such as HMGB1, a non-histone nuclear protein that acts as a secreted alarmin, and engineered nanoparticles that are coated with a bio-corona of proteins, possibly unfolded, thereby revealing hidden epitopes, that may act as nanomaterial-associated molecular patterns (NAMPs). These molecular signatures are recognised by so-called pattern recognition receptors or PRRs – including the Toll-like receptors – on the surface of innate immune cells or expressed within such cells. The activation of PRRs triggers inflammation and alerts the adaptive immune system to impending danger [85].