Seminars in Immunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Immunology

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

Review

Effects of engineered nanoparticles on the innate immune system

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ARTICLE INFO

Keywords:
Immune response
Engineered nanoparticle
Physicochemical properties
Immunomodulation
Immunotherapy

ABSTRACT

Engineered nanoparticles (NPs) have broad applications in industry and nanomedicine. When NPs enter the body, interactions with the immune system are unavoidable. The innate immune system, a non-specific first line of defense against potential threats to the host, immediately interacts with introduced NPs and generates complicated immune responses. Depending on their physicochemical properties, NPs can interact with cells and proteins to stimulate or suppress the innate immune response, and similarly activate or avoid the complement system. NPs size, shape, hydrophobicity and surface modification are the main factors that influence the interactions between NPs and the innate immune system. In this review, we will focus on recent reports about the relationship between the physicochemical properties of NPs and their innate immune response, and their applications in immunotherapy.

1. Introduction

Due to their unique physical and chemical properties, Nanoparticles (NPs) are widely used in electronics, cosmetics, textiles and nanomedicine [1–5]. At present, human exposure to engineered NPs is widespread, through environmental routes (inhalation, ingestion, dermal contact and parenteral) or deliberate administration [6,7]. Interactions between nanoparticles(NPs) and the immune system have become important, and there are foundational questions about the safety of these special materials. NPs can communicate with various biological components (cells, receptors, proteins *etc*.) of the immune system, trigger cell signaling cascades, and consequently cause unpredictable immune responses (activation or suppression) and even harmful outcomes (autoimmune diseases or cancer) [5,7,8]. There is also evidence that NPs can alter the development of immune systems *in utero* in mouse models. [9]. Therefore, understanding how NPs influence or tune the immune system is critical to better knowing the potential risks in developing new nanomaterials.

The basic concept of the immune system is a biological network that reacts to foreign threats (*i.e*. antigens) to protect the host and maintain homeostasis [5]. The overall system is divided into two subsystems: innate immunity and adaptive immunity. Innate immunity is the first line of defense, generating a non-specific inflammatory response upon the detection of conserved biological motifs, often associated with bacteria and viruses. The adaptive immune system is a more nuanced defense mechanism that involves the development of antibodies highly-specific to detected antigens, followed by the generation of memory cells for future immunological protection [10]. Components of the innate immune system recognize pathogens mainly *via* pattern-recognition receptors (PRPs), while antigen presenting cells (APCs) present acquired antigens to T cells for the activation of acquired immune system. When NPs enter the body, they have a high probability of interacting with the innate immune system first, generating an immunomodulatory response based on their physicochemical properties [8,11]. Hence, understanding how NPs interact with the innate immune system is particularly important, and would provide insight into designing immune-compatible NP technologies.

Engineered NPs can be designed to either specifically interact with or avoid recognition by the immune system. Synthetic NPs have been utilized frequently to generate novel immunotherapy strategies. Immunotherapy involves intentional modulation of the immune system as a therapeutic strategy. One of the primary strengths of immunotherapy is that there can be less negative side effects than those associated with traditional therapies [12,13]. A frequent use for NPs in immunotherapy contexts has been for developing new vaccines, which has been previously discussed [14–18]. Here, we will focus on understanding the interactions between the innate immune system and engineered NPs for other immunomodulatory purposes. First, we will discuss how physicochemical properties of NPs affect the contact of NPs with the innate immune system and the resulting immune response. Then, we will demonstrate how to take advantage of NPs immunomodulatory properties for biological applications. At last, we will discuss remaining challenges that need to be considered for NP applications.

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http://dx.doi.org/10.1016/j.smim.2017.09.011
Received 11 July 2017; Received in revised form 26 September 2017; Accepted 27 September 2017

2. Innate immune system

The innate immune system is a broad, less-specific defense mechanism, which includes molecular (complement system, cytokines) and cellular (phagocytes and leukocytes) components that recognize classes of molecules particular to frequently encountered pathogens. Most components of the innate immune system are present before the onset of the infection and rapidly respond to invasion within minutes. In conjunction with this system is the highly organized complement system, which involves a set of serum proteins that circulate in an inactive state. Those proteins are converted into an active state through three pathways (classical, lection and alternative pathway) to damage and clear pathogenic organisms [19]. Activation of the complement system leads to the formation of the potent anaphylatoxins C3a and C5a. These proteins elicit physiological responses such as chemoattraction (attract phagocytes to sites of injury or inflammation) and enhanced vascular permeability [20]. The innate immune system includes several circulating and tissue-specific cell types, such as natural killer cells, granulocytes (neutrophils, basophils, eosinophils, mast cells) and antigen-presenting cells (macrophage and dendritic cells (DC)). APCs and neutrophils are responsible for recognizing pathogens *via* PRRs, which identify pathogen-associated molecular patterns (PAMPs). Following identification, the cells uptake and digest the pathogen, generating an inflammatory response [19,21]. APCs are also activated by damage-associated molecular pattern molecules (DAMPs) (such as ATP, uric acid, heparin sulfate) from stressed or damaged tissues or microbes [22]. These cells usually produce higher levels of reactive oxygen species (ROS), causing an accumulation of oxidative glutathione (GSSG). These changes further elicit inflammatory responses through distinct signaling pathways, such as nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and NACHT-LRR and PYD domain-containing proteins 3 (NLRP3). These changes can also cause cytokine secretion (*e.g.* interleukins (ILs), tumor necrosis factor (TNF-α)) [21].

Activation of PRRs is an essential part of the inflammatory immune response that direct the host cell to distinguish “self” from “non-self”. PRRs are expressed on either the cell membrane (such as Toll-like receptors (TLRs) and C-type lectin receptors (CLRs)) or in the cytosol (such as NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs)) [22]. Based on their function, PRRs are divided into signaling PRRs and endocytic PRRs. Signaling PRRs (TLRs and NLRs) have a variety of functions in the regulation of inflammation and apoptotic response. For example, TLR signaling results in the activation of NF-κB, mitogen-activated protein kinase, and interferon-regulatory factors (IRFs). Those signal pathways ultimately result in gene expression and secretion of cytokines, chemokines, cell adhesion molecules, and immunoreceptors [22,23]. Endocytic PRRs can promote the attachment, engulfment and destruction of microorganisms and foreign entities by phagocytes. Each PRR family member binds a specific molecular pattern. For example, TLR4 recognizes extracellular bacterial lipopolysaccharide (LPS), and the NLR/inflammasome recognizes cytosolic bacterial DNA or peptidoglycan molecules [23,24]. By controlling the interaction with these specific receptors, the overall immunological response can be regulated.

3. Physicochemical properties of nanoparticles modulate innate immune response

NPs have been prepared with a variety of controlled structures and functionalities for delivery, therapeutic, and diagnostic purposes. Once inside the body, engineered NPs as foreign substance are immediately encounter the innate immune system and generate specific immune response based on their properties. The physicochemical properties (*e.g.* size, charge, shape, hydrophobicity, and stiffness) of NPs determine their interactions with soluble proteins, APCs and neutrophils, in particular effect the downstream signaling that are used to detect pathogens or other immunoregulatory events [7,11,24,25].

3.1. Size and shape

The size of NPs has a significant impact on the uptake of these materials by cells (particularly those of the innate immune system), the initiation of innate immune response, and their overall bio-distribution in vivo [7,10]. With an increase in size, the surface to volume ratio of NPs is decreased, which affects their interactions with the innate immune system. There are four endocytotic NPs uptake mechanisms: pinocytosis, macropinocytosis, phagocytosis and clathrin/caveolar mediated endocytosis. Pinocytosis and micropinocytosis, both commonly involved in NP uptake by the immune system, are non-specific processes to internalize NPs and fluids together into cells [26]. By using fluorescent PEGylated NPs, Kruth et al. reported the visualization of receptor-independent fluid-phase pinocytosis by macrophages *in vitro* and in a mouse atherosclerotic lesion model [27]. The uptake of NPs usually occurs with the cooperation of several endocytic uptake mechanisms. Through endocytic inhibitor analysis, Gu et al. reported that superparamagnetic iron oxide NPs (~10 nm) were internalized into Raw 264.7 macrophage cells using clathrin-dependent endocytosis (inhibited with chlorpromazine), caveolae-dependent endocytosis (inhibited with β-cyclodextrin), and macropinocytosis (inhibited with amiloride) [28]. Rothen et al. showed that 600 nm polystyrene NPs (PS) were engulfed by phagocytosis/macropinocytosis, while 40 nm PS were internalized by both clathrin-mediated endocytosis as well as phagocytosis or macropinocytosis by macrophages (J774.1A) [29]. Čolić et al. also reported that gold NPs of 10 nm and 50 nm were internalized predominantly *via* a clathrin/dynamin-dependent mechanism by DCs, and a significantly higher number of 10 nm AuNPs per cell were uptaken than 50 nm AuNPs. It was demonstrated that smaller AuNPs inhibit the maturation of LPS induced DCs more strongly than larger ones [30]. Similarly, silica-titania hollow nanoparticles with 50 nm diameter, compared with up to 125 nm, generated more ROS (Fig. 1a), and exhibited the highest induction of inflammatory cytokines (IL-1, IL-6 and TNF-α) with mouse alveolar macrophage (J774.1) *in vitro* [31]. However, the relationship between size of NPs and innate immune response is not linear. Jang et al. synthesized five different diameters (20 nm, 40 nm, 60 nm, 80 nm and 100 nm) of monodispersed polypyrrole (PPy) nanoparticles. Mid-sized 60 nm PPy NPs evoked the highest level of ROS and increased the expression level of costimulatory markers (CD40 and CD80) in macrophages [32]. For the bio-distribution, several studies demonstrated that smaller NPs had higher retention compared to larger NPs, and that most NPs accumulate in the liver (Kupffer cells), the amount increasing with the size of NPs [33,34]. Additionally, small NPs (20 nm–200 nm) rapidly drained to the lymph nodes (LN), where they were taken up by resident DC. Large NPs (500 nm, 1000 nm) depended on cellular transport by DC, immigrating from the injection site (skin) to LN *in vivo* [35]. These data suggested that larger NPs prefer interacting with tissue-resident APCs, while smaller NPs (< 200 nm) could circulate through vein and lymphatic drainage, providing better antigen presentation.

The shape of NPs has also been demonstrated to affect interactions with the innate immune system [21,25,36]. Groll et al. found that the uptake of nanorods by macrophages was more efficient than the uptake of nanospheres. This is a result of the generation of > 1 μm vesicles, allowing the nanorods to enter easily through macropinocytosis. It was also observed that neutrophil granulocytes did not fully internalize the particles but trapped them in their extracellular structures [37]. Within various aluminum oxyhydroxide nanomaterials (nanorod, nanoplate, and nanopolyhedra), nanorods are the most redox active materials, capable of NLRP3 inflammasome activation and stimulating IL-1β production in human THP-1 cells and bone marrow-derived DCs (BMDCs) (Fig. 1b) [38]. Moreover, higher aspect ratios of nanorods minimized phagocytosis and enhanced cytokine secretion (IL-6, and IFN-γ) [39]. Similarly, Moghimi et al. compared complement activation using spherical, prolate ellipsoidal (rods) and oblate ellipsoidal (disks) polystyrene NPs with equivalent surface area in pig and human blood.