

Nanoparticle interaction with the immune system

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When nanoparticles enter the body, their interactions with cells are almost unavoidable. Unintended nanoparticle interaction with immune cells may elicit a molecular response that can have toxic effects and lead to greater susceptibility to infectious diseases, autoimmune disorders, and cancer development. As evidenced by several studies, nanoparticle interactions with biological systems can stimulate inflammatory or allergic reactions and activate the complement system. Nanoparticles can also stimulate immune response by acting as adjuvants or as haptens. Immunosuppressive effects have also been reported. This article gives a brief review of *in vitro* and *in vivo* research evidencing stimulatory or suppressive effects of nanoparticles on the immune system of mammals. In order to ensure safe use of nanosized particles, future research should focus on how their physical and chemical properties influence their behaviour in the biological environment, as they not only greatly affect nanoparticle-immune system interactions but can also interfere with experimental assays.

KEY WORDS: *immune response; immunomodulation; immunotoxicity; nanomaterials; nanoparticle properties; nanosafety*

We are currently witnessing a rapid progress of nanotechnology and an increasing manufacture and use of engineered nanoparticles. Nanoparticles are defined as particles that have at least one dimension smaller than 100 nm (1). Their small size means an increased proportion of surface atoms and therefore changed physicochemical properties (2). These properties can be used beneficially for many applications, from electronics, cosmetics, and textile industry to drug delivery and bioimaging (3). However, the same properties can make nanoparticles more harmful to living organisms due to increased reactivity and easy penetration into organisms and cells (4). Several studies have shown that particles of the same chemical composition but different size pose different risk; smaller particles are more harmful (5-7). Numerous nanotoxicological studies have focused on cytotoxicity (8-10), which occurs at a relatively high nanoparticle concentration/dose. At a lower concentration/dose, the sub-lethal and long-term effects on cells can occur (11-14). Studying the immunomodulatory effects of nanoparticles is particularly important, because immunocompromised organisms are susceptible to infections and cancer development (15). The primary function of the immune system is to detect and recognise foreign substances in order to protect the host. Nanoparticles can interfere with this function or can themselves be recognised as foreign antigens and thus elicit immune response (16). A disturbance in the immune system can

lead to severe medical conditions (17) and understanding how different factors influence the host defence mechanisms is an important part of toxicological research.

Nanoparticles can enter the body unintentionally through the gastrointestinal tract, skin, and airways or can be intentionally administered to the body with biomedical applications (18). Inside the body, there is a high probability that nanoparticles will come into contact with immune cells, which can lead to nanoparticle-immune system interactions (15, 19). These interactions have an immunomodulatory potential, as they can activate or suppress immune function (Figure 1) and lead to inflammation, increased susceptibility to infectious diseases, or even to autoimmune diseases or cancer (15). However, in some biomedical applications, for example in vaccine delivery (19, 20), we can design nanoparticles for targeted modulation of immune response.

Stimulation of immune response

Depending on their physicochemical properties nanoparticles can stimulate innate and adaptive immune response (Table 1). However, it is still unclear how individual nanoparticles affect it.

Activation of innate immune response

When nanoparticles enter the body, they can interact with immune cells and trigger inflammatory response. Inflammatory response is accompanied by the secretion of signalling molecules (cytokines, chemokines) that provide communication between immune cells and coordinate

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molecular events. Positively charged nanoparticles usually possess a higher inflammatory potential than negatively charged or neutral nanoparticles (20). This can be explained by the fact that macrophages have a negatively charged sialic acid on their surface and readily interact with cationic substances (21). Macrophages recognise foreign antigens with their toll-like receptors (TLRs), which bind to corresponding antigens and activate the signal transduction pathway and inflammatory response (21). In their *in vitro* experiment Lucarelli et al. (22), exposed human macrophages to non-toxic concentrations of different SiO₂, TiO₂, ZrO₂, and Co nanoparticles and observed increased expression of TLR receptors and production of inflammatory cytokines. The experiment showed that different nanoparticles triggered inflammatory response in different ways. SiO₂ nanoparticles induced the production of inflammatory cytokines IL-1 β and TNF- α , and Co nanoparticles inhibited anti-inflammatory IL-1RA and induced inflammatory TNF- α (22). Another *in vitro* study (23) showed a cytotoxic and inflammatory effect of Ag nanoparticles on rat brain microvascular endothelial cells (RBMEC), with an increased release of proinflammatory mediators IL-1 β , TNF, and PGE-2. The effect of Ag nanoparticles was significantly stronger with smaller (25 nm) than with larger particles (40 and 80 nm). Chuang et al. (24) recently showed that the intensity of inflammatory response induced by carbon black nanoparticles of different size correlated with their surface area. Xia et al. (25) observed a cytotoxic effect of ZnO nanoparticles and the induction of inflammatory response in RAW 264.7 and BEAS-2B cell lines. In contrast, CeO₂ and TiO₂ nanoparticles did not elicit any such effect.

The results of several *in vivo* studies have also shown how nanoparticles can affect inflammatory response. Park et al. (26) treated mice with Fe₃O₄ nanoparticles by intratracheal instillation and noticed increased production of pro-inflammatory cytokines IL-1, TNF- α , and IL-6. They also reported increased production of Th0-type cytokine IL-2, Th1-type cytokine IL-12, Th2-type cytokines IL-4 and IL-5, TGF- β , and an increased production of IgE. Kaewamatawong et al. (27) have found that intratracheally instilled SiO₂ nanoparticles can cause pulmonary inflammation in mice. Nishimori et al. (28) observed that *i.v.* injected SiO₂ particles in mice had size-dependent hepatotoxic effects. Only smaller particles (<100 nm) caused higher serum markers of liver injury, serum aminotransferase, and inflammatory cytokines IL-6 and TNF- α . Cho et al. (29) noticed a gene expression pattern typical of apoptosis and inflammation in mice liver after *i.v.* administration of Au nanoparticles coated with polyethylene glycol (PEG). Single-walled carbon nanotubes were also hypothesized to cause inflammation (30). Some studies however suggest that the main cause of inflammation are impurities resulting from nanoparticle synthesis (31).

Exposure to nanoparticles can also interfere with response to infection. Mice exposed to carbon nanotubes before infection with *Listeria monocytogenes* had an enhanced acute pulmonary inflammation and delayed bacterial clearance (decreased phagocytosis and nitric oxide production) (32).

Activation of the complement system

The complement system is an important part of the innate immune system that helps antibodies and phagocytic cells to remove pathogens from the host. There are a number of reports claiming that nanoparticles activate the complement system via different pathways (33-40). Furthermore, altering nanoparticle surface properties can increase or decrease complement activation (33, 35, 40). Pondman et al. (38) have shown that complement opsonisation of carbon nanotubes enhances their uptake by U937 cells without inflammatory response. Pedersen et al. (36) have shown that dextran-coated Fe₃O₄ particles can activate the complement system. In another study, Au nanoparticles did not activate the complement system - even though complement proteins prevailed in the corona - nor did they affect complement activation by a known activator (41).

Activation of adaptive immune response

Unlike the innate immune system, the adaptive immune system is antigen-specific, requires some time to achieve its maximum effect, and typically generates an immunological memory. It consists of humoral and cellular antigen-specific responses, and nanoparticles can stimulate both. Liu et al. (42) found that polyhydroxylated fullerenes [C₆₀(OH)₂₀] stimulate the production of Th1 cytokines and decrease the production of Th2 cytokines (42). C₆₀(OH)₂₀ nanoparticles show a low cytotoxic effect on immune cells, but significantly stimulate TNF- α release, which has an important role in the removal of abnormal cells. In addition, they seem to suppress tumours *in vivo*, as they increase the CD4+/CD8+ lymphocyte ratio.

Some nanoparticles have an epitope structure to which specific antibodies bind. Being small molecules by definition however, most nanoparticles probably act as haptens, which are immunogenic only when attached to a larger carrier molecule. Chen et al. (43) demonstrated that the immune system can generate antibodies specific to nanoparticles. After the immunisation of mice with a C₆₀ fullerene derivate conjugated to bovine thyroglobulin, they produced IgG antibodies specific to fullerenes. Other researchers were not able to detect fullerene-specific antibodies, even when they used a carrier molecule (44). This inconsistency in results could be explained by the use of different fullerene derivatives or differences between the animal models and immunisation protocols employed (20). For some biomedical applications, nanoparticles are functionalised by growth factors,

receptors, and other biomolecules that may induce autoantibodies (20).

Several studies have shown that nanoparticles can also act as adjuvants, i.e. as substances that are added to the antigen in order to stimulate immune response (34, 44-50). Polymethylmethacrylate (PMMA) nanoparticles used as adjuvants for HIV-2 virus vaccine in mice induced up to a 100 times higher antibody response than the conventional adjuvant aluminium hydroxide [Al(OH)₃] (49). How exactly nanoparticles function as adjuvants is poorly understood, but some studies suggest that nanoparticles can promote the uptake of antigens or can stimulate antigen-presenting cells (20). The adjuvant-like properties of nanoparticles depend on their physicochemical properties. Sun et al. (51) found a correlation between the shape and crystallinity of AlOOH nanoparticles and their adjuvant capacity both *in vitro* (activation of dendritic cells) and *in vivo* (production of IgG and IgE against ovalbumin) (51). Li et al. (50) showed that Al(OH)₃ nanoparticles induced a stronger humoral response than microparticles of the same chemical composition. Cao et al. (52) also found that ultra-small graphene oxide-supported gold nanoparticles (usGO-Au) used as an adjuvant stimulated humoral and cellular immune responses.

Some studies have associated exposure to nanoparticles with allergic reactions. Nanoparticles can increase (53-55) or inhibit allergic reactions (56). Chen et al. (57) reported that TiO₂ nanoparticles directly stimulated histamine release from the mast cells. Mast cells can contribute to inflammation and the toxic effect of some nanoparticles (19). There is increasing evidence that mast cells have an important role in the biological events following nanoparticle exposure (58-61).

Suppression of immune response

Nanoparticles can also suppress the immune system (Table 1), which can weaken immune response against infections and cancerous cells. These immunosuppressive properties, on the other hand, can make nanoparticles useful in preventing transplant rejection, in treating inflammatory and autoimmune diseases, and in delivering immunosuppressive drugs (62-64). However, we still do not know which nanoparticle properties are responsible for immunosuppressive effects. While some nanoparticles are used to deliver immunosuppressive drugs, others have their own immunosuppressive properties. Shen et al. (65) have shown that Fe₃O₄ nanoparticles weaken the antigen-specific humoral response and T cell cytokine expression in ovalbumin-challenged mice. Mitchell et al. (66, 67) reported that multi-walled carbon nanotubes (MWCNTs) suppressed systemic humoral immunity in mice. Some nanoparticles have been shown to possess anti-inflammatory properties. CeO₂ nanoparticles were reported to reduce ROS and the level of inflammatory cytokines IL-6 and TNF- α in murine macrophages (68). Shaunak et al.

(69) reported that polyvalent dendrimer glucosamine conjugates inhibited the induction of inflammatory cytokines and chemokines in human macrophages and dendritic cells exposed to bacterial endotoxin. John et al. (70) have designed polymerised lipid nanoparticles that bind to specific selectins on inflammation-activated lung endothelial cells and reduce inflammation in the allergic airway disease. Ryan et al. (56) report that fullerene inhibits hypersensitivity reaction to allergens *in vitro* and *in vivo*.

Nanoparticle physicochemical properties affecting immune response

The effect of nanoparticles on the immune system is determined by their physicochemical properties (15, 20). For a proper interpretation of the biological effects of nanoparticles it is therefore important to know their physicochemical properties (21, 71). Warheit (72) suggests that a nanotoxicological experiment should be preceded by the characterisation of at least the following nanoparticle properties: size, size distribution, surface area and reactivity, crystallinity, aggregation in relevant medium, composition and surface coating, method of synthesis, and impurities. The effect of nanoparticles can also depend on surface ion dissolution (73); more soluble particles such as ZnO and FeO are more toxic than the less soluble ones such as CeO₂ and TiO₂ (74). Therefore, it is advisable to check their solubility in relevant media before testing. Nevertheless, some studies have shown that nanoparticle effects on the immune system are different from the effects of their ions (75-77).

Several studies have demonstrated that size significantly determines nanoparticle biological effects (5-7, 78-83). The smaller the size, the higher the relative surface area, and therefore the higher the dissolution of toxic ions and reactive oxygen species (ROS) production (71). Nanoparticle shape is also important for biological effects (84). For example, fullerenes and carbon nanotubes have the same chemical composition, but different shape, which influences their toxicological properties (85). The surface properties of nanoparticles affect their behaviour in suspensions and interactions with cell membranes. The surface charge correlates with nanoparticle aggregation/agglomeration in media and with the ability to cross biological barriers (86). Sonication, which is often used to disperse nanoparticle aggregates/agglomerates in suspension, can accelerate ion dissolution and ROS production on the surface of nanoparticles (87) and increase cytotoxicity.

Biological effects can also be altered by impurities, generated as by-products in nanoparticle synthesis (31, 88), or by endotoxins (89). We also have to take into consideration that the properties of nanoparticles can change in biological environments such as cell culture media *in vitro* or bloodstream *in vivo*, which can influence biological response to nanoparticle exposure. Several studies have

Table 1 Stimulation and suppression of immune response by nanoparticles

Immunostimulatory effects of nanoparticles					
Observed immune effect	Study design	Tested particles (size)	Test system	Reference	
Pro-inflammatory effects	<i>In vitro</i>	SiO ₂ (4-40 nm), TiO ₂ (20-160 nm), ZrO ₂ (5-30 nm), Co (50-200 nm)	Human myelomonocytes (U-937)	22	
		Ag (25, 40, 80 nm)	Rat brain microvessel endothelial cells (RBMEC)	23	
		SiO ₂ (10, 100 nm)	Human peripheral blood mononuclear cells (PBMC)	108	
		ZnO (13 nm)	Murine macrophages (RAW 264.7) and human bronchial epithelial cells (BEAS-2B)	25	
		NiO (<50 nm)	Human bronchial epithelial cells (BEAS-2B) and human lung carcinoma cells (A549)	109	
		Au (3, 6, 40 nm)	Murine macrophages (J774 A1)	110	
		<i>In vivo</i>	Fe ₃ O ₄ (~5 nm)	ICR mice	26
			SiO ₂ (14 nm)	ICR mice	27
			SiO ₂ (70 nm)	BALB/c mice	28
			SiO ₂ (50, 500 nm)	Tuck-Ordinary mice	111
			Au coated with PEG (4, 100 nm)	BALB/c mice	29
			SWCNT (1-4 nm × 1-3 μm)	C57BL/6 mice	32
			SWCNT (1-2 nm × 10 nm to several μm)	ICR mice	112
			TiO ₂ nanorods (diameter of 4-6 nm)	Wistar rats	113
Latex nanomaterial (25, 50, 100 nm)	ICR mice		80		
Ag (20 nm)	Brown Norway rats		114		
Ag (15 nm)	Fischer rats	115			
Carbon black (15, 51, 95 nm)	SH rats	24			
Activation of the complement system	<i>In vitro</i>	SWCNT (different sizes)	Human serum	33	
		SWCNT coated with PEG (1-5 nm × 50-300 nm)	Human serum	35	
		CNTs (different sizes)	Human serum	38	

Table 1 continued

		CNTs (different sizes)	Human serum	39
		Functionalized MWCNT (10-20 nm × 10-50 nm)	Human serum	40
		Dextran-coated Fe ₃ O ₄ (50, 250, 600 nm)	Human serum	36
Nanoparticles as haptens	<i>In vivo</i>	C ₆₀ fullerene	BALB/c mice	43
Nanoparticles as adjuvants	<i>In vitro</i>	AlOOH (different shapes and sizes)	Human leukemic monocyte (THP-1) and murine bone marrow derived dendritic cells (BMDCs)	51
		usGO-Au (5-10 nm)	Murine macrophages (RAW 264.7)	52
	<i>In vivo</i>	AlOOH (different shapes and sizes)	C57BL/6 mice	51
		usGO-Au (5-10 nm)	C57BL/6 mice	52
		Al(OH) ₃ (112 nm)	BALB/c mice	50
Stimulation of allergic reactions	<i>In vitro</i>	TiO ₂ (60 ± 10 nm)	Rat mast cells (RBL-2H3)	57
	<i>In vivo</i>	TiO ₂ (15, 50, 100 nm)	NC/Nga mice	116
		Ag (10 nm)	BALB/c mice	117
		ZnO (20, 240 nm)	BALB/c mice	118
Immunosuppressive effects of nanoparticles				
Observed immune effect	Study design	Tested particles (size)	Test system	Reference
Anti-inflammatory effect	<i>In vitro</i>	CeO ₂	Murine macrophages (J774)	68
		Polyvalent dendrimer glucosamine conjugates	Peripheral blood mononuclear cells (PBMCs)	69
		SWCNT (0,8-1,2 nm × 800 nm)	Human lung carcinoma cells (A549) and human bronchial epithelial cells (NHBE)	119
	<i>In vivo</i>	ZnO (20, 240 nm)	BALB/c mice	118
Suppression of hypersensitivity, reaction to allergens	<i>In vitro</i>	C ₆₀ fullerenes	Human mast cells and peripheral blood basophils	56
	<i>In vivo</i>	C ₆₀ fullerenes	C57BL/6 mice	56
Suppression of the humoral immune response	<i>In vivo</i>	Fe ₂ O ₃	BALB/c mice	65
		MWCNT (10-20 nm × 5-15 µm)	C57BL/6 mice	66
		MWCNT	C57BL/6 mice	67

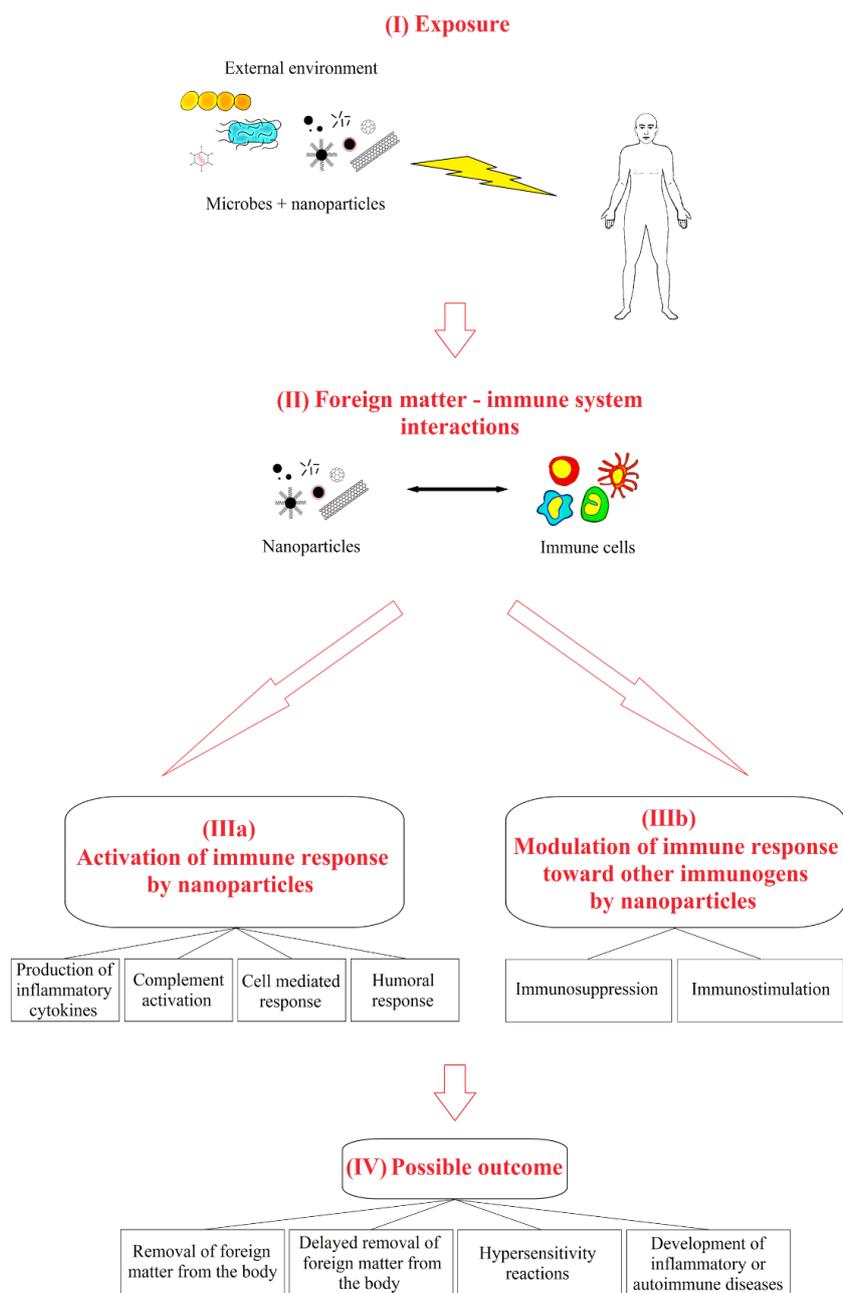


Figure 1 Nanoparticle interaction with the immune system. The primary function of the immune system is to protect the host from foreign substances. When nanoparticles enter the body (I), they get in contact with different immune cells (II). Nanoparticle interactions with immune cells can activate immune response (IIIa). Nanoparticles can also interfere with the immune system's recognition of other immunogenic substances and can stimulate or suppress immune response (IIIb). Normally, immune response gradually leads to the removal of foreign matter from the body, but nanoparticle interaction with immune response can have toxic consequences (IV)

evaluated how biomolecules bound to nanoparticle surface (the so-called biomolecular corona) affect nanoparticle effects on cells (90-92). Nanoparticles that enter the bloodstream can bind with opsonins, which makes them more visible to phagocytic cells, which in turn remove them from the circulation (93). But, even phagocytes can be affected by nanoparticle toxicity (94). Therefore, the surface of nanoparticles that need to enter the bloodstream should be modified to avoid the opsonin binding.

Adjustment and validation of standard methods for testing nanoparticle interaction with the immune system

In vitro evaluation of nanoparticle effects on immune cells and the immune system is essential for comprehensive understanding of nanoparticle effects on living organisms in order to make their use safe. Although common cytotoxicity tests may be useful in identifying acute toxicity risks for host cells, including the immune cells, they do not detect the sublethal effects and the dysregulation of the immune system function. Therefore, researchers studying immunotoxicity have established a set of methods for testing immune function (95-99).

Due to their specific physicochemical properties nanoparticles can interfere with the established tests, which were originally developed for testing the biological effects of conventional chemicals. Interactions between nanoparticles and the test method can lead to false positive or false negative results (100-104). Because of that and because of different mechanisms through which nanoparticles can interact with the immune system, it is necessary to use a battery of broad-range methods. There are several *in vitro* and *in vivo* assays for testing nanoparticle effects on the immune system, which have been reviewed elsewhere (105-107). Their protocols have to be properly adjusted and validated.

When studying the effects of nanoparticles on the immune system, we should also consider the type of the selected biological system as well as time and route of exposure. Different immune cells have different functions in immune response, as they have different receptors and uptake mechanisms.

Furthermore, when testing the long-term and chronic effects of nanoparticles we have to avoid the use of high nanoparticle concentrations that can result in acute toxicity and cell death.

CONCLUSIONS

Studies that have been done to date have shown that nanoparticles can interact with different components of the immune system. These interactions are diverse, complex, and not well understood, yet. They may result in unforeseen changes in the functioning of different immune

cells, leading to unpredictable outcomes. The diversity and specific properties of nanoparticles make their risk assessment difficult. To date, the correlation between the properties of nanoparticles and their biological effects, including the effect on the immune system, are poorly understood. Since nanoparticles can interfere with the traditional testing methods developed for testing the biological effects of chemicals, additional attention should be given to the selection of appropriate methods.

Identifying the effects of nanoparticles on the immune system is crucial for their safe use. Nanoparticles for biomedical applications can be designed to interact with the immune system in an intended way or not to react at all. However, we are still a long way from being able to design nanoparticles that will have only a desirable biological effect.

Future research should focus on which nanoparticle property contributes to which effect. This means more *in vitro* and *in vivo* studies with detailed nanoparticle characterisation. More attention should also be given to determining the mechanisms of interaction between nanoparticles and different components of the immune system to understand why the same nanoparticles stimulate certain immune functions and suppress others.

With new findings about the interactions between nanoparticles and the immune system we will be able to make better and safer nanotechnological products.

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Interakcije nanodelcev z imunskim sistemom

Ko nanodelci vstopijo v organizem, pridejo v kontakt s celicami imunskega sistema. Nezaželene interakcije nanodelcev z imunskim sistemom lahko sprožijo molekularni odziv, ki lahko pripelje do toksičnih učinkov in povečane dovzetnosti organizma za okužbe, avtoimunska obolenja ter razvoj raka. Dosedanje raziskave so pokazale, da nanodelci lahko sprožijo vnetne in alergijske reakcije, lahko pa tudi aktivirajo sistem komplementa. Nanodelci lahko delujejo kot adjuvansi ali kot hapteni. Obstajajo pa tudi poročila, ki kažejo na sposobnost nanodelcev, da zavrejo imunski odziv. V članku bomo povzeli ugotovitve dosedanjih raziskav *in vitro* ter *in vivo*, ki so bile narejene na področju proučevanja vplivov nanodelcev na stimulacijo ali supresijo imunskega sistema sesalcev. Za zagotovitev varne uporabe nanodelcev moramo razumeti kako fizikalno-kemijske lastnosti nanodelcev vplivajo na njihovo obnašanje v biološkem okolju. Lastnosti nanodelcev moramo upoštevati tudi ob izvajanju poskusov, da se izognemo lažnim rezultatom zaradi potencialne interference nanodelcev z dejavniki v eksperimentalnem okolju. Čeprav je bilo do sedaj narejenih že več nanotoksikoloških raziskav, je vpliv nanodelcev na imunski sistem še vedno slabo razumljen. Sposobnost nanodelcev za modulacijo imunskega odziva narekuje potrebo po nadaljnjih raziskavah interakcij nanodelcev z imunskim sistemom.

KLJUČNE BESEDE: imunomodulacija; imunitoksičnost; imunski odziv; lastnosti nanodelcev; nanomateriali; nanovarnost