Mini-review DOI: 10.1515/aiht-2015-66-2582

Nanoparticle interaction with the immune system

Veno Kononenko[1], Mojca Narat[2], and Damjana Drobne[1]

Department of Biology[1], Department for Animal Science[2], Biotechnical Faculty, University of Ljubljana, Slovenia

[Received in October 2014; CrossChecked in October 2014; Accepted in April 2015]

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

Correspondence to: Veno Kononenko, Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia, E-mail: *veno.kononenko@bf.uni-lj.si*

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 SiO_2, TiO_2, ZrO_2, 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. SiO_2 nanoparticles induced the production of inflammatory cytokines IL-1β 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-1β, 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, CeO_2 and TiO_2 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 Fe_3O_4 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 SiO_2 nanoparticles can cause pulmonary inflammation in mice. Nishimori et al. (28) observed that *i.v.* injected SiO_2 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 Fe_3O_4 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 [$C_{60}(OH)_{20}$] stimulate the production of Th1 cytokines and decrease the production of Th2 cytokines (42). $C_{60}(OH)_{20}$ 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 C_{60} 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 TiO_2 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 Fe_3O_4 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. CeO_2 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 CeO_2 and TiO_2 (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	*In vitro*	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
	In vivo	Fe_3O_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	*In vitro*	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_3O_4 (50, 250, 600 nm)	Human serum	36
Nanoparticles as haptens	*In vivo*	C_{60} fullerene	BALB/c mice	43
Nanoparticles as adjuvants	*In vitro*	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
	In vivo	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	*In vitro*	TiO_2 (60 ± 10 nm)	Rat mast cells (RBL-2H3)	57
	In vivo	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	*In vitro*	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
	In vivo	ZnO (20, 240 nm)	BALB/c mice	118
Suppression of hypersensitivity, reaction to allergens	*In vitro*	C_{60} fullerenes	Human mast cells and peripheral blood basophils	56
	In vivo	C_{60} fullerenes	C57BL/6 mice	56
Suppression of the humoral immune response	*In vivo*	Fe_2O_3	BALB/c mice	65
		MWCNT (10-20 nm × 5-15 μm)	C57BL/6 mice	66
		MWCNT	C57BL/6 mice	67

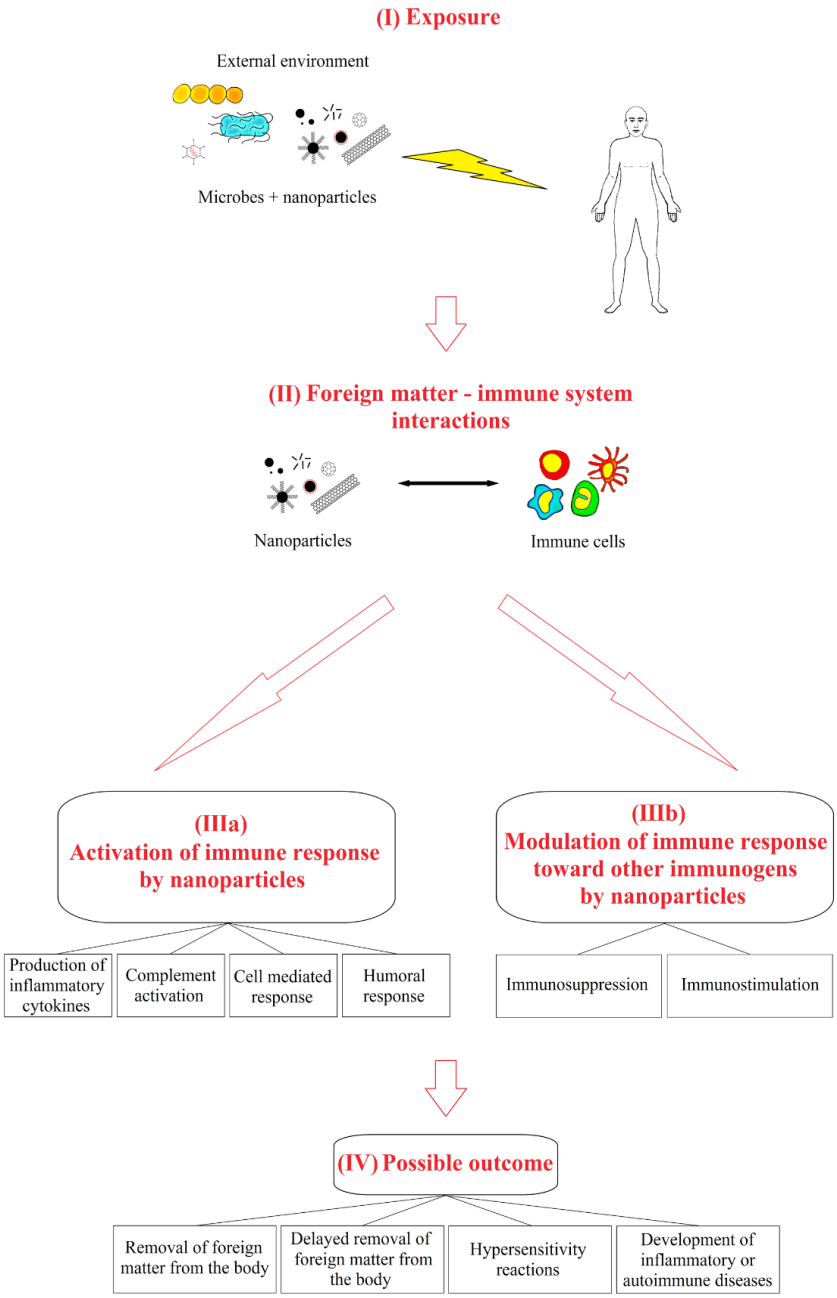

Figure 1 *Nanoparticle interaction with the immune system. The primary function of the immune system is to protect the host from foreign substances. When nanoparticles enter the body (I), they get in contact with different immune cells (II). Nanoparticle interactions with immune cells can activate immune 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.

Acknowledgements

This study is part of Veno Kononenko's PhD dissertation and was supported by the Slovenian Research Agency (ARRS), grant no. 1000-14-0510.

REFERENCES

1. Gartman A, Findlay AJ, Luther GW. Nanoparticulate pyrite and other nanoparticles are a widespread component of hydrothermal vent black smoker emissions. Chem Geol 2014;336:32-41. doi: 10.1016/j.chemgeo.2013.12.013
2. Wise JP, Goodale BC, Wise SS, Craig GA, Pongan AF, Walter RB, Thompson WD, Ng AK, Aboueissa AM, Mitani H, Spalding MJ, Mason MD. Silver nanospheres are cytotoxic and genotoxic to fish cells. Aquat Toxicol 2010;97:34-41. doi: 10.1016/j.aquatox.2009.11.016
3. Ngô C, Van de Voorde MH. Nanotechnology in a Nutshell: From Simple to Complex Systems. Paris: Atlantis Press; 2014.
4. Donaldson K, Poland CA, Schins RPF. Possible genotoxic mechanisms of nanoparticles: Criteria for improved test strategies. Nanotoxicology 2010;4:414-20. doi: 10.3109/17435390.2010.482751
5. Coradeghini R, Gioria S, Garcia CP, Nativo P, Franchini F, Gilliland D, Ponti J, Rossi F. Size-dependent toxicity and cell interaction mechanisms of gold nanoparticles on mouse fibroblasts. Toxicol Lett 2013;217:205-16. doi: 10.1016/j.toxlet.2012.11.022

6. Shang L, Nienhaus K, Nienhaus GU. Engineered nanoparticles interacting with cells: size matters. J Nanobiotechnol 2014;12:5. doi: 10.1186/1477-3155-12-5
7. Toyooka T, Amano T, Ibuki Y. Titanium dioxide particles phos-phorylate histone H2AX independent of ROS production. Mutat Res 2012;742:84-91. doi: 10.1016/j.mrgentox.2011.12.015
8. Ingle AP, Duran N, Rai M. Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. Appl Microbiol Biotechnol 2014;98:1001-9. doi: 10.1007/s00253-013-5422-8
9. Kroll A, Dierker C, Rommel C, Hahn D, Wohlleben W, Schulze-Isfort C, Göbbert C, Voetz M, Hardinghaus F, Schnekenburger J. Cytotoxicity screening of 23 engineered nanomaterials using a test matrix of ten cell lines and three different assays. Part Fibre Toxicol 2011;8:9. doi: 10.1186/1743-8977-8-9
10. Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanoparticles. Small 2008;4:26-49. doi: 10.1002/smll.200700595
11. Przybytkowski E, Behrendt M, Dubois D, Maysinger D. Nanoparticles can induce changes in the intracellular metabolism of lipids without compromising cellular viability. FEBS J 2009;276:6204-17. doi: 10.1111/j.1742- 4658.2009.07324.x
12. Saptarshi SR, Feltis BN, Wright PF, Lopata AL. Investigating the immunomodulatory nature of zinc oxide nanoparticles at sub-cytotoxic levels *in vitro* and after intranasal instillation *in vivo*. J Nanobiotechnol 2015;13:6. doi:10.1186/s12951-015-0067-7
13. Kawata K, Osawa M, Okabe S. *In vitro* toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. Environ Sci Technol 2009;43:6046-51. doi: 10.1021/es900754q
14. Dworak N, Wnuk M, Zebrowski J, Bartosz G, Lewinska A. Genotoxic and mutagenic activity of diamond nanoparticles in human peripheral lymphocytes *in vitro*. Carbon 2014;68:763-76 doi:10.1016/j.carbon.2013.11.067
15. Zolnik BS, González-Fernández A, Sadrieh N, Dobrovolskaia MA. Nanoparticles and the immune system. Endocrinology 2010;151:458-65. doi: 10.1210/en.2009-1082
16. Klippstein R, Fernandez-Montesinos R, Castillo PM, Zaderenko AP, Pozo D. Silver nanoparticles interactions with the immune system: implications for health and disease. In: Pozo Perez D, editor. Silver nanoparticles. 1st ed. Rijeka: In TechOpen; 2010. p. 309-24.
17. Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol 2005;5:243-51. doi: 10.1038/nri1571
18. Cupaioli FA, Zucca FA, Boraschi D, Zecca L. Engineered nanoparticles. How brain friendly is this new guest? Prog Neuroboil 2014;119-120:20-38. doi: 10.1016/j.pneurobio.2014.05.002
19. Smith MJ, Brown JM, Zamboni WC, Walker NJ. From immunotoxicity to nanotherapy: the effects of nanomaterials on the immune system. Toxicol Sci 2014;138:249-55. doi: 10.1093/toxsci/kfu005
20. Dobrovolskaia MA, McNeil SE. Immunological properties of engineered nanomaterials. Nat Nanotechnol 2007;2:469-78. doi: 10.1038/nnano.2007.223
21. Dwivedi PD, Misra A, Shanker R, Das M. Are nanomaterials a threat to the immune system? Nanotoxicology 2009;3:19-26. doi: 10.1080/17435390802604276
22. Lucarelli M, Gatti AM, Savarino G, Quattroni P, Martinelli L, Monari E, Boraschi D. Innate defence functions of macrophages can be biased by nano-sized ceramic and metallic particles. Eur Cytokine Netw 2004;15:339-46. PMID: 15627643
23. Trickler WJ, Lantz SM, Murdock RC, Schrand AM, Robinson BL, Newport GD, Schlager JJ, Oldenburg SJ, Paule MG, Slikker W Jr, Hussain SM, Ali SF. Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. Toxicol Sci 2010;118:160-70. doi: 10.1093/toxsci/kfq244
24. Chuang HC, Chenc LC, Leic YC, Wuc KY, Fengb PH, Chengc TJ. Surface area as a dose metric for carbon black nanoparticles: A study of oxidative stress, DNA single-strand breakage and inflammation in rats. Atmos Environ 2015;106:329-34. doi:10.1016/j.atmosenv.2015.02.014
25. Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nel AE. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2008;2:2121-34. doi: 10.1021/nn800511k
26. Park EJ, Kim H, Kim Y, Yi J, Choi K, Park K. Inflammatory responses may be induced by a single intratracheal instillation of iron nanoparticles in mice. Toxicology 2010;275:65-71. doi: 10.1016/j.tox.2010.06.002
27. Kaewamatawong T, Shimada A, Okajima M, Inoue H, Morita T, Inoue K, Takano H. Acute and subacute pulmonary toxicity of low dose of ultrafine colloidal silica particles in mice after intratracheal instillation. Toxicol Pathol 2006;34:958-65. doi: 10.1080/01926230601094552
28. Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K. Silica nanoparticles as hepatotoxicants. Eur J Pharm Biopharm 2009;72:496-501. doi: 10.1016/j.ejpb.2009.02.005
29. Cho WS, Kim S, Han BS, Son WC, Jeong J. Comparison of gene expression profiles in mice liver following intravenous injection of 4 and 100 nm-sized PEG-coated gold nanoparticles. Toxicol Lett 2009;191:96-102. doi: 10.1016/j.toxlet.2009.08.010
30. Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S, Schwegler-Berry D, Hubbs AF, Antonini J, Evans DE, Ku BK, Ramsey D, Maynard A, Kagan VE, Castranova V, Baron P. Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. Am J Physiol Lung Cell Mol Physiol 2005;289:L698-708. doi: 10.1152/ajplung.00084.2005
31. Pulskamp K, Diabaté S, Krug HF. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicol Lett 2007;168:58-74. doi: 10.1016/j.toxlet.2006.11.001
32. Shvedova AA, Fabisiak JP, Kisin ER, Murray AR, Roberts JR, Tyurina YY, Antonini JM, Feng WH, Kommineni C, Reynolds J, Barchowsky A, Castranova V, Kagan VE. Sequential exposure to carbon nanotubes and bacteria enhances pulmonary inflammation and infectivity. Am J Respir Cell Mol Biol 2008;38:579-90. doi: 10.1165/rcmb.2007-0255OC
33. Andersen AJ, Robinson JT, Dai HJ, Hunter AC, Andresen TL, Moghimi SM. Single-walled carbon nanotube surface control of complement recognition and activation. ACS Nano 2013;7:1108-19. doi: 10.1021/nn3055175

34. Dykman LA, Sumaroka MV, Staroverov SA, Zaitseva IS, Bogatyrev VA. Immunogenic properties of the colloidal gold. Biol Bull 2004;31:75-9. doi: 10.1023/B:BIBU.0000014358.98422.9c
35. Hamad I, Christy Hunter A, Rutt KJ, Liu Z, Dai H, Moein Moghimi S. Complement activation by PEGylated single-walled carbon nanotubes is independent of C1q and alternative pathway turnover. Mol Immunol 2008;45:3797-803. doi: 10.1016/j.molimm.2008.05.020
36. Pedersen MB, Zhou X, Larsen EK, Sorensen US, Kjems J, Nygaard JV, Nyengaard JR, Meyer RL, Boesen T, Vorup-Jensen T. Curvature of synthetic and natural surfaces is an important target feature in classical pathway complement activation. J Immunol 2010;184:1931-45. doi: 10.4049/jimmunol.0902214
37. Pham CT, Mitchell LM, Huang JL, Lubniewski CM, Schall OF, Killgore JK, Pan D, Wickline SA, Lanza GM, Hourcade DE. Variable antibody-dependent activation of complement by functionalized phospholipid nanoparticle surfaces. J Biol Chem 2011;286:123-30. doi: 10.1074/jbc.M110.180760
38. Pondman KM, Sobik M, Nayak A, Tsolaki AG, Jäkel A, Flahaut E, Hampel S, Ten Haken B, Sim RB, Kishore U. Complement activation by carbon nanotubes and its influence on the phagocytosis and cytokine response by macrophages. Nanomedicine 2014;10:1287-99. doi: 10.1016/j.nano.2014.02.010
39. Salvador-Morales C, Flahaut E, Sim E, Sloan J, Green ML, Sim RB. Complement activation and protein adsorption by carbon nanotubes. Mol Immunol 2006;43:193-201. doi: 10.1016/j.molimm.2005.02.006
40. Salvador-Morales C, Basiuk EV, Basiuk VA, Green ML, Sim RB. Effects of covalent functionalization on the biocompatibility characteristics of multi-walled carbon nanotubes. J Nanosci Nanotechnol 2008;8:2347-56. doi: 10.1166/jnn.2008.090
41. Dobrovolskaia MA, Neun BW, Man S, Ye X, Hansen M, Patri AK, Crist RM, McNeil SE. Protein corona composition does not accurately predict hematocompatibility of colloidal gold nanoparticles. Nanomedicine 2014;10:1453-63. doi: 10.1016/j.nano.2014.01.009
42. Liu Y, Jiao F, Qiu Y, Li W, Qu Y, Tian C, Li Y, Bai R, Lao F, Zhao Y, Chai Z, Chen C. Immunostimulatory properties and enhanced TNF- alpha mediated cellular immunity for tumor therapy by $C_{60}(OH)_{20}$ nanoparticles. Nanotechnology 2009;20:415102. doi: 10.1088/0957-4484/20/41/415102
43. Chen BX, Wilson SR, Das N, Coughlin DJ, Erlanger BF. Antigenicity of fullerenes: Antibodies specific for fullerenes and their characteristics. Proc Natl Acad Sci USA 1998;95:10809-13. doi: 10.1073/pnas.95.18.10809
44. Andreev SM, Babakhin AA, Petrukhina AO, Romanova VS, Parnes ZN, Petrov RV. Immunogenic and allergenic properties of fulleren conjugates with aminoacids and proteins. Dokl Biochem 2000;370:4-7. PMID: 11977250
45. Castignolles N, Morgeaux S, Gontier-Jallet C, Samain D, Betbeder D, Perrin P. A new family of carriers (biovectors) enhances the immunogenicity of rabies antigens. Vaccine 1996;14:1353-60. doi: 10.1016/S0264-410X(96)00043-6
46. de Haar C, Hassing I, Bol M, Bleumink R, Pieters R. Ultrafine but not fine particulate matter causes airway inflammation and allergic airway sensitization to co-administered antigen in mice. Clin Exp Allergy 2006;36:1469-79. doi: 10.1111/j.1365-2222.2006.02586.x
47. Niikura K, Matsunaga T, Suzuki T, Kobayashi S, Yamaguchi H, Orba Y, Kawaguchi A, Haegawa H, Kajino K, Ninomiya T, Ijiro K, Sawa H. Gold nanoparticles as a vaccine platform: Influence of size and shape on immunological responses *in vitro* and *in vivo*. ACS Nano 2013;7:3926-38. doi: 10.1021/nn3057005
48. Rajananthanan P, Attard GS, Sheikh NA, Morrow WJ. Evaluation of novel aggregate structures as adjuvants: composition, toxicity studies and humoral responses. Vaccine 1999;17:715-30. doi: 10.1016/S0264-410X(98)00256-4
49. Stieneker F, Kreuter J, Löwer J. 1991. High antibody titres in mice with polymethylmethacrylate nanoparticles as adjuvant for HIV vaccines. AIDS 1991;5:431-5. doi: 10.1097/00002030-199104000-00012
50. Li X, Aldayel AM, Cui Z. Aluminum hydroxide nanoparticles show a stronger vaccine adjuvant activity than traditional aluminum hydroxide microparticles. J Control Release 2014;173:148-57. doi: 10.1016/j.jconrel.2013.10.032
51. Sun B, Ji Z, Liao YP, Wang M, Wang X, Dong J, Chang CH, Li R, Zhang H, Nel AE, Xia T. Engineering an effective immune adjuvant by designed control of shape and crystallinity of aluminum oxyhydroxide nanoparticles. ACS Nano 2013;7:10834-49. doi: 10.1021/nn404211j
52. Cao Y, Ma Y, Zhang M, Wang H, Tu X, Shen H, Dai J, Guo H, Zhang Z. Ultrasmall graphene oxide supported gold nanoparticles as adjuvants improve humoral and cellular immunity in mice. Adv Funct Mater 2014;24:6963-71. doi: 10.1002/adfm.201401358
53. Al-Humadi NH, Siegel PD, Lewis DM, Barger MW, Ma JY, Weissman DN, Ma JK. The effect of diesel exhaust particles (DEP) and carbon black (CB) on thiol changes in pulmonary ovalbumin allergic sensitized Brown Norway rats. Exp Lung Res 2002;28:333-49. doi: 10.1080/01902140290091976
54. Nel AE, Diaz-Sanchez D, Ng D, Hiura T, Saxon A. Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system. J Allergy Clin Immunol 1998; 102:539-54. doi: 10.1016/S0091-6749(98)70269-6
55. Nygaard UC, Hansen JS, Samuelsen M, Alberg T, Marioara CD, Løvik M. Single-walled and multi-walled carbon nanotubes promote allergic immune responses in mice. Toxicol Sci 2009;109:113-23. doi: 10.1093/toxsci/kfp057
56. Ryan JJ, Bateman HR, Stover A, Gomez G, Norton SK, Zhao W, Schwartz LB, Lenk R, Kepley CL. Fullerene nanomaterials inhibit the allergic response. J Immunol 2007;179:665-72. PMID: 17579089
57. Chen EY, Garnica M, Wang YC, Mintz AJ, Chen CS, Chin WC. A mixture of anatase and rutile TiO_2 nanoparticles induces histamine secretion in mast cells. Part Fibre Toxicol 2012;9:2. doi: 10.1186/1743-8977-9-2
58. Katwa P, Wang X, Urankar RN, Podila R, Hilderbrand SC, Fick RB, Rao AM, Ke PC, Wingard CJ, Brown JM. A carbon nanotube toxicity paradigm driven by mast cells and the IL-33/ST2 axis. Small 2012;8:2904-12. doi: 10.1002/smll.201200873
59. Murray AR, Kisin E, Leonard SS, Young SH, Kommineni C, Kagan VE, Castranova V, Shvedova AA. Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. Toxicology 2009;257:161-71. doi: 10.1016/j.tox.2008.12.023
60. Shannahan JH, Kodavanti UP, Brown JM. Manufactured and airborne nanoparticle cardiopulmonary interactions: A

review of mechanisms and the possible contribution of mast cells. Inhal Toxicol 2012;24:320-39. doi: 10.3109/08958378.2012.668229
61. Wang X, Katwa P, Podila R, Chen P, Ke PC, Rao AM, Walters DM, Wingard CJ, Brown JM. Multi-walled carbon nanotube instillation impairs pulmonary function in C57BL/6 mice. Part Fibre Toxicol 2011;8:24. doi: 10.1186/1743-8977-8-24
62. Azzi J, Tang L, Moore R, Tong R, Haddad NE, Akiyoshi T, Mfarrej B, Yang S, Jurewicz M, Ichimura T, Lindeman N, Cheng J, Abdi R. Polylactide-cyclosporin A nanoparticles for targeted immunosuppression. FASEB J 2010;24:3927-38. doi: 10.1096/fj.10-154690
63. Higaki M, Ishihara T, Izumo N, Takatsu M, Mizushima Y. Treatment of experimental arthritis with poly(D, L-lactic/glycolic acid) nanoparticles encapsulating betamethasone sodium phosphate. Ann Rheum Dis 2005;64:1132-6. doi: 10.1136/ard.2004.030759
64. Xu W, Ling P, Zhang T. Toward immunosuppressive effects on liver transplantation in rat model: tacrolimus loaded poly(ethylene glycol)-poly(D,L-lactide) nanoparticle with longer survival time. Int J Pharm 2014;460:173-80. doi: 10.1016/j.ijpharm.2013.10.035
65. Shen CC, Wang CC, Liao MH, Jan TR. A single exposure to iron oxide nanoparticles attenuates antigen-specific antibody production and T-cell reactivity in ovalbumin-sensitized BALB/c mice. Int J Nanomedicine 2011;6:1229-35. doi: 10.2147/IJN.S21019
66. Mitchell LA, Gao J, Wal RV, Gigliotti A, Burchiel SW, McDonald JD. Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. Toxicol Sci 2007;100:203-14. doi: 10.1093/toxsci/kfm196
67. Mitchell LA, Lauer FT, Burchiel SW, McDonald JD. Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice. Nat Nanotechnol 2009;4:451-6. doi: 10.1038/NNANO.2009.151
68. Hirst SM, Peairs AD, Gogal R, Seal S, Reilly CM. Cerium oxide nanoparticles decrease inflammation in J774 cells. FASEB J 2008;22(Meeting Abstracts):758.2.
69. Shaunak S, Thomas S, Gianasi E, Godwin A, Jones E, Teo I, Mireskandari K, Luthert P, Duncan R, Patterson S, Khaw P, Brocchini S. Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. Nat Biotechnol 2004;22:977-84. doi: 10.1038/nbt995
70. John AE, Lukacs NW, Berlin AA, Palecanda A, Bargatze RF, Stoolman LM, Nagy JO. Discovery of a potent nanoparticle P-selectin antagonist with anti-inflammatory effects in allergic airway disease. FASEB J 2003;17:2296-8. doi: 10.1096/fj.03-0166fje
71. Magdolenova Z, Collins A, Kumar A, Dhawan A, Stone V, Dusinska M. Mechanisms of genotoxicity. A review of *in vitro* and *in vivo* studies with engineered nanoparticles. Nanotoxicology 2014;8:233-78. doi: 10.3109/17435390.2013.773464
72. Warheit DB. How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? Toxicol Sci 2008:101;183-5. doi: 10.1093/toxsci/kfm279
73. Brun NR, Lenz M, Wehrli B, Fent K. Comparative effects of zinc oxide nanoparticles and dissolved zinc on zebrafish embryos and eleuthero-embryos: importance of zinc ions. Sci Total Environ 2014;476-477:657-66. doi: 10.1016/j.scitotenv.2014.01.053
74. Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Bruinink A, Stark WJ. *In vitro* cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 2006;40:4374-81. doi: 10.1021/es052069i
75. Lin W, Xu Y, Huang CC, Ma Y, Shannon KB, Chen DR, Huang YW. Toxicity of nano- and micro-sized ZnO particles in human lung epithelial cells. J Nanopart Res 2009;11:25-39. doi: 10.1007/s11051-008-9419-7
76. Petrarca C, Perrone A, Verna N, Verginelli F, Ponti J, Sabbioni E, Di Giampaolo L, Dadorante V, Schiavone C, Boscolo P, Mariani Costantini R, Di Gioacchino M. Cobalt nanoparticles modulate cytokine *in vitro* release by human mononuclear cells mimicking autoimmune disease. Int J Immunopathol Pharmacol 2006;19(4 Suppl):11-4. PMID: 17291400
77. Reale M, Vianale G, Lotti LV, Mariani-Costantini R, Perconti S, Cristaudo A, Leopold K, Antonucci A, Di Giampaolo L, Iavicoli I, Di Gioacchino M, Boscolo P. Effects of palladium nanoparticles on the cytokine release from peripheral blood mononuclear cells of palladium-sensitized women. J Occup Environ Med 2011;53:1054-60. doi: 10.1097/JOM.0b013e318228115e
78. Ariano P, Zamburlin P, Gilardino A, Mortera R, Onida B, Tomatis M, Ghiazza M, Fubini B, Lovisolo D. Interaction of spherical silica nanoparticles with neuronal cells: size-dependent toxicity and perturbation of calcium homeostasis. Small 2011;7:766-74. doi: 10.1002/smll.201002287
79. Fifis T, Gamvrellis A, Crimeen-Irwin B, Pietersz GA, Li J, Mottram PL, McKenzie IF, Plebanski M. Size-dependent immunogenicity: therapeutic and protective properties of nano-vaccines against tumors. J Immunol 2004;173:3148-54. doi: 10.4049/jimmunol.173.5.3148
80. Inoue K, Takano H, Yanagisawa R, Koike E, Shimada A. Size effects of latex nanomaterials on lung inflammation in mice. Toxicol Appl Pharmacol 2009;234:68-76. doi: 10.1016/j.taap.2008.09.012
81. Mottram PL, Leong D, Crimeen-Irwin B, Gloster S, Xiang SD, Meanger J, Ghildyal R, Vardaxis N, Plebanski M. Type 1 and 2 immunity following vaccination is influenced by nanoparticle size: formulation of a model vaccine for respiratory syncytial virus. Mol Pharm 2007;4:73-84. doi: 10.1021/mp060096p
82. Pan Y, Neuss S, Leifert A, Fischler M, Wen F, Simon U, Schmid G, Brandau W, Jahnen-Dechent W. Size-dependent cytotoxicity of gold nanoparticles. Small 2007;3:1941-9. doi: 10.1002/smll.200700378
83. Schöler N, Hahn H, Müller RH, Liesenfeld O. Effect of lipid matrix and size of solid lipid nanoparticles (SLN) on the viability and cytokine production of macrophages. Int J Pharm 2002;231:167-76. doi: 10.1016/S0378-5173(01)00882-1
84. Yang H, Liu C, Yang D, Zhang H, Xi Z. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. J Appl Toxicol 2009;29:69-78. doi: 10.1002/jat.1385
85. Radomski A, Jurasz P, Alonso-Escolano D, Drews M, Morandi M, Malinski T, Radomski MW. Nanoparticle-induced platelet aggregation and vascular thrombosis. Br J Pharmacol 2005;146:882-93. doi: 10.1038/sj.bjp.0706386

86. Ahamed M, Karns M, Goodson M, Rowe J, Hussain SM, Schlager JJ, Hong Y. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol 2008;233:404-10. doi: 10.1016/j.taap.2008.09.015
87. Lee WM, An YJ,Yoon H, Kweon HS. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles. Environ Toxicol Chem 2008;27:1915-21. doi: 10.1897/07-481.1
88. Schöler N, Olbrich C, Tabatt K, Müller RH, Hahn H, Liesenfeld O. Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages. Int J Pharm 2001;221:57-67. PMID: 11397567
89. Vallhov H, Qin J, Johansson SM, Ahlborg N, Muhammed MA, Scheynius A, Gabrielsson S. The importance of an endotoxin-free environment during the production of nanoparticles used in medical applications. Nano Lett 2006;6:1682-6. doi: 10.1021/nl060860z
90. Fedeli C, Segat D, Tavano R, De Franceschi G, de Laureto PP, Lubian E, Selvestrel F, Mancin F, Papini E. Variations of the corona HDL:albumin ratio determine distinct effects of amorphous SiO_2 nanoparticles on monocytes and macrophages in serum. Nanomedicine (Lond) 2014; 9:2481-97. PMID: 24661258
91. Tenzer S, Docter D, Kuharev J, Musyanovych A, Fetz V, Hecht R, Schlenk F, Fischer D, Kiouptsi K, Reinhardt C, Landfester K, Schild H, Maskos M, Knauer SK, Stauber RH. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. Nat Nanotechnol 2013;8:772-81. doi: 10.1038/nnano.2013.181
92. Wang F, Yu L, Monopoli MP, Sandin P, Mahon E, Salvati A, Dawson KA. The biomolecular corona is retained during nanoparticle uptake and protects the cells from the damage induced by cationic nanoparticles until degraded in the lysosomes. Nanomedicine 2013;9:1159-68. doi: 10.1016/j.nano.2013.04.010
93. Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. Biodegradable long-circulating polymeric nanospheres. Science 1994;263:1600-3. doi: 10.1126/science.8128245
94. Costantini LM, Gilberti RM, Knecht DA. The phagocytosis and toxicity of amorphous silica. PLoS One 2011;6:e14647. doi: 10.1371/journal.pone.0014647
95. Dujmović IH. Comparison of two guidelines on immunotoxicity testing of medicinal products. Arh Hig Rada Toksikol 2005;56:265-8. PMID: 16180612
96. Kawabata TT, Evans EW. Development of immunotoxicity testing strategies for immunomodulatory drugs. Toxicol Pathol 2012;40:288-93. doi: 10.1177/0192623311430238
97. Lankveld DP, Van Loveren H, Baken KA, Vandebriel RJ. *In vitro* testing for direct immunotoxicity: state of the art. Methods Mol Biol 2010;598:401-23. doi: 10.1007/978-1-60761-401-2_26
98. Luebke R. Immunotoxicant screening and prioritization in the twenty-first century. Toxicol Pathol 2012;40:294-9. doi: 10.1177/0192623311427572
99. Luster MI, Portier C, Pait DG, White KL Jr, Gennings C, Munson AE, Rosenthal GJ. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. Fundam Appl Toxicol 1992;18:200-10. doi: 10.1016/0272-0590(92)90047-L
100. Ong KJ, MacCormack TJ, Clark RJ, Ede JD, Ortega VA, Felix LC, Dang MKM, Ma G, Fenniri H, Veinot JGC, Goss GG. Widespread nanoparticle-assay interference: implications for nanotoxicity testing. PLoS ONE 2014;9:e90650. doi: 10.1371/journal.pone.0090650
101. Wörle-Knirsch JM, Pulskamp K, Krug HF. Oops they did it again! Carbon nanotubes hoax scientists in viability assays. Nano Lett 2006;6:1261-8. doi: 10.1021/nl060177c
102. Oostingh GJ, Casals E, Italiani P, Colognato R, Stritzinger R, Ponti J, Pfaller T, Kohl Y, Ooms D, Favilli F, Leppens H, Lucchesi D, Rossi F, Nelissen I, Thielecke H, Puntes VF, Duschl A, Boraschi D. Problems and challenges in the development and validation of human cell-based assays to determine nanoparticle-induced immunomodulatory effects. Part Fibre Toxicol 2011;8:8. doi: 10.1186/1743-8977-8-8
103. Kroll A, Pillukat MH, Hahn D, Schnekenburger J. Interference of engineered nanoparticles with *in vitro* toxicity assays. Arch Toxicol 2012;86:1123-36. doi: 10.1007/s00204-012-0837-z
104. Rajapakse K, Drobne D, Kastelec D, Marinsek-Logar R. Experimental evidence of false-positive Comet test results due to TiO_2 particle-assay interactions. Nanotoxicology 2013;7:1043-51. doi: 10.3109/17435390.2012.696735
105. Dobrovolskaia MA, Aggarwal P, Hall JB, McNeil SE. Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. Mol Pharm 2008;5:487-95. doi: 10.1021/mp800032f
106. Dobrovolskaia MA, Germolec DR, Weaver JL. Evaluation of nanoparticle immunotoxicity. Nat Nanotechnol 2009;4:411-4. doi: 10.1038/nnano.2009.175
107. Dobrovolskaia MA, McNeil SE. Understanding the correlation between *in vitro* and *in vivo* immunotoxicity tests for nanomedicines. J Control Release 2013;172:456-66. doi: 10.1016/j.jconrel.2013.05.025
108. Mendoza A, Torres-Hernandez JA, Ault JG, Pedersen-Lane JH, Gao D, Lawrence DA. Silica nanoparticles induce oxidative stress and inflammation of human peripheral blood mononuclear cells. Cell Stress Chaperones 2014;19:777-90. doi: 10.1007/s12192-014-0502-y
109. Capasso L, Camatini M, Gualtieri M. Nickel oxide nanoparticles induce inflammation and genotoxic effect in lung epithelial cells. Toxicol Lett 2014;226:28-34. doi: 10.1016/j.toxlet.2014.01.040
110. Yen HJ, Hsu SH, Tsai CL. Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes. Small 2009;5:1553-61. doi: 10.1002/smll.200900126
111. Nemmar A, Albarwani S, Beegam S, Yuvaraju P, Yasin J, Attoub S, Ali BH. Amorphous silica nanoparticles impair vascular homeostasis and induce systemic inflammation. Int J Nanomedicine 2014;9:2779-89. doi: 10.2147/IJN.S52818
112. Chou CC, Hsiao HY, Hong QS, Chen CH, Peng YW, Chen HW, Yang PC. Single-walled carbon nanotubes can induce pulmonary injury in mouse model. Nano Lett 2008;8:437-45. doi: 10.1021/nl0723634
113. Nemmar A, Melghit K, Ali BH. The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO_2 nanorods in rats. Exp Biol Med (Maywood) 2008;233:610-9. doi: 10.3181/0706-RM-165

114. Seiffert J, Hussain F, Guo C, Chang Y, Zhang J, Smith R, Tetley T, Chung F. Inhaled silver nanoparticles induce pulmonary oxidative injury and inflammation: Differential effects between rat strains. Eur Respir J 2014;44(Suppl 58):P3939.
115. Braakhuis HM, Gosens I, Krystek P, Boere J, Cassee FR, Fokkens P, Post J, van Loveren H, Park M. Particle size dependent deposition and pulmonary inflammation after short-term inhalation of silver nanoparticles. Part Fibre Toxicol 2014;11:49. doi: 10.1186/s12989-014-0049-1
116. Yanagisawa R, Takano H, Inoue K, Koike E, Kamachi T, Sadakane K, Ichinose T. Titanium dioxide nanoparticles aggravate atopic dermatitis-like skin lesions in NC/Nga mice. Exp Biol Med (Maywood) 2009;234:314-22. doi: 10.3181/0810-RM-304
117. Hirai T, Yoshioka Y, Ichihashi K, Mori T, Nishijima N, Handa T, Takahashi H, Tsunoda S, Higashisaka K, Tsutsumi Y. Silver nanoparticles induce silver nanoparticle-specific allergic responses. J Immunol 2014;192(Suppl 1):118.19.
118. Ilves M, Palomäki J, Vippola M, Lehto M, Savolainen K, Savinko T, Alenius H. Topically applied ZnO nanoparticles suppress allergen induced skin inflammation but induce vigorous IgE production in the atopic dermatitis mouse model. Part Fibre Toxicol 2014;11:38. doi: 10.1186/s12989-014-0038-4
119. Herzog E, Byrne HJ, Casey A, Davoren M, Lenz AG, Maier KL, Duschl A, Oostingh GJ. SWCNT suppress inflammatory mediator responses in human lung epithelium *in vitro*. Toxicol Appl Pharmacol 2009;234:378-90. doi: 10.1016/j.taap.2008.10.015

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; imunotoksičnost; imunski odziv; lastnosti nanodelcev; nanomateriali; nanovarnost*