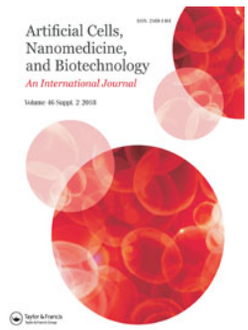

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# Human macrophage responses to metal-oxide nanoparticles: a review

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**ABSTRACT**

Nanomaterials have been widely used in our daily lives in medicine, cosmetics, paints, textiles and food products. Many studies aim to determine their biological effects in different types of cells. The interaction of these materials with the immune system leads to reactions by modifying the susceptibility or resistance of the host body which could induce adverse health effects. Macrophages, as specific cells of the innate immune response, play a crucial role in the human defence system to foreign agents. They can be used as a reliable test object for the investigation of immune responses under nanomaterials exposure displayed by expression of a variety of receptors and active secretion of key signalling substances for these processes. This report covers studies of human macrophage behaviours upon exposure of nanomaterials. We focused on their interaction with metal-oxide nanoparticles as these are largely used in medical and cosmetics applications. The discussion and summary of these studies can guide the development of new nanomaterials, which are, at the same time, safe and useful for new purposes, especially for health applications.

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Metal-oxide nanomaterials; protein corona; human macrophages; macrophage signalling; inflammation

## Introduction

Nanomaterials have been broadly used in different fields owing to their large surface to volume ratio. Their key properties are often compared to their bulk counterparts [1]. Metal-oxide nanomaterials, in particular, have found numerous applications mainly in the medical, cosmetic and food areas. Titanium dioxide nanoparticles act as photocatalysts and their optical and chemical properties make them attractive for applications in cosmetics and as coating materials for numerous purposes [2–4]. Iron oxide, zinc oxide and cobalt oxide nanomaterials have been extensively studied due to their magnetic properties [5–7]. The superparamagnetic behaviour of iron oxide nanoparticles allows their use in magnetic resonance imaging, drug delivery, biosensors and many other biomedical applications [8,9]. Zirconium oxides have demonstrated antibacterial activity [10]. Besides, cerium oxide ($CeO_2$), a potential catalytic antioxidant is another example of metal-oxide nanoparticles extensively studied from the past few years [11,12].

The remarkable variety of nanoparticle applications brings up the concern on their safety for human health. Biological effects can be triggered by nanoparticle exposure thanks to their unique physical and chemical properties. Cells in contact with foreign particles may induce an immunological response. The immune system consists of a group of cells and molecules responsible for defending the body against these foreign substances. Figure 1 presents the different cells of the immune system in various organs of the human body. The immune system is mainly divided into two subsystems: the innate immune system and the adaptive immune system. The innate immune system comprises the first front of immunological defence of the body after the anatomical and physical barriers, working in the recognition of the invader. On the other hand, the adaptive immune system is a more specific line of defence attacking the invader in different ways and generating immunological memories. Cells composing the innate immune system are phagocytic leukocytes, dendritic cells and natural killer cells.

Macrophages are leukocytes, which are originated by primitive cells from yolk sac, can be self-renew or derivate from bone marrow-derived monocytes [14–17]. They accumulate in different organs of the body, as shown in Figure 1, acting as scavengers and antigen-presenting cells [13]. During inflammation or tissue injury, macrophages are commonly exhibiting pro- or anti-inflammatory characteristics depending on the stimuli and their origins. Phenotype 1 (M1) is an inflammatory macrophage while phenotype 2 (M2) has a regulatory function acting as an anti-inflammatory macrophage and is involved in tissue repair. Mantovani et al. [18] reported a model presenting polarized macrophage and their specific properties under different stimuli (Figure 2). Monocytic cell lines submitted to differentiation treatments are usually used to investigate macrophage functions *in vitro*. Exposure of the cell to different stimuli implies the development of specific phenotypes. Human monocytic THP-1 cells and human peripheral blood monocytes differentiated by phorbol 12-myristate 13-acetate (PMA) and colony-stimulating

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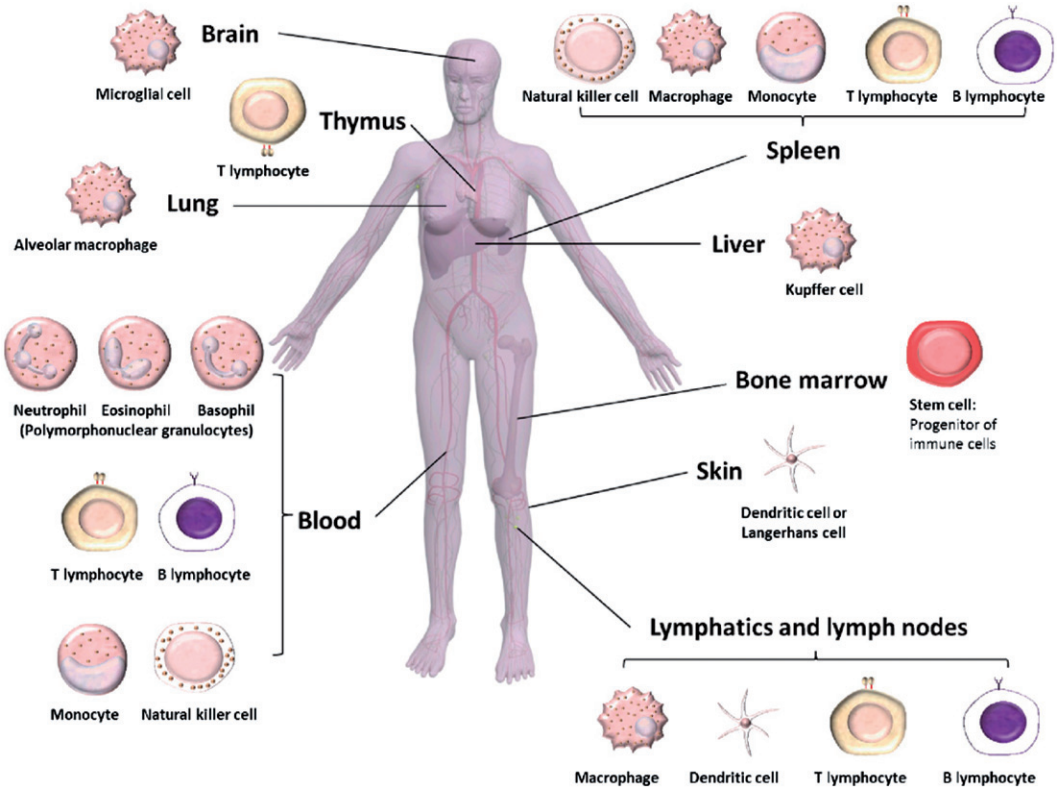


**Figure 1.** Distribution of immune cells in various organs of the human body. Reproduced with permission from [13]. Copyright 2013 Royal Society of Chemistry.

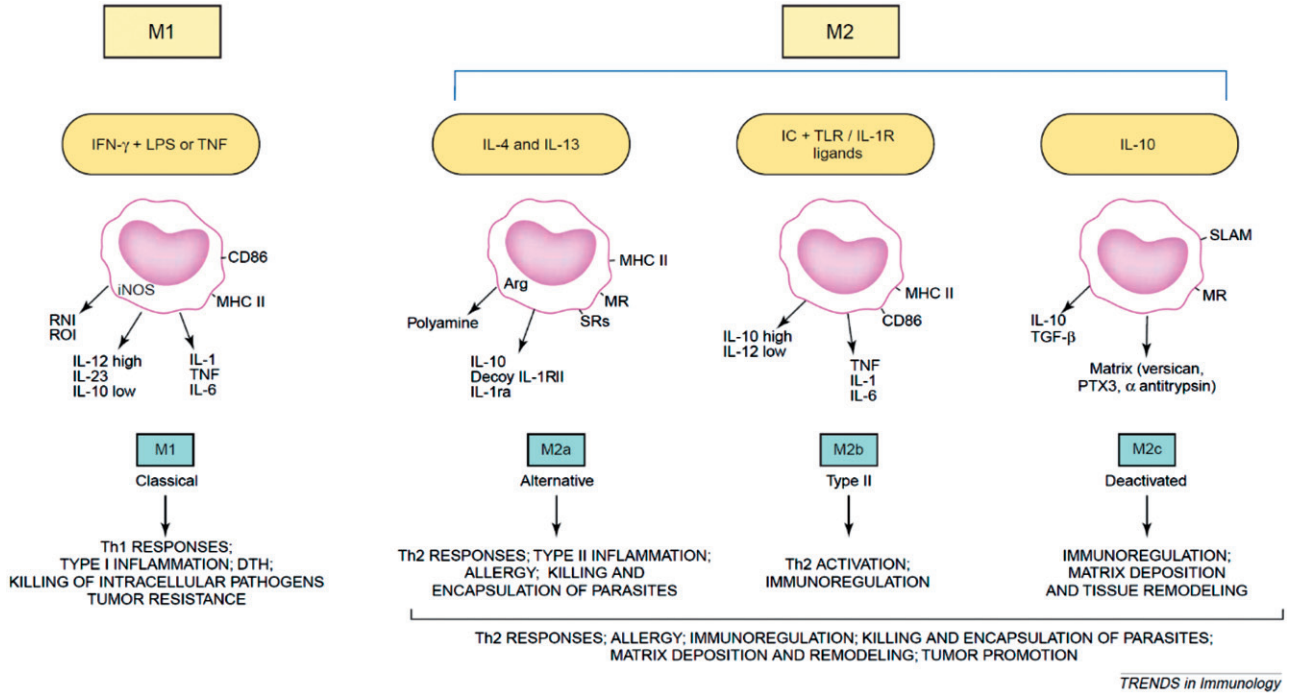


**Figure 2.** Responses of various macrophage populations under different stimuli. DTH: delayed-type hypersensitivity; IC: immune complexes; IFN-g: interferon-g; iNOS: inducible nitric oxide synthase; LPS: lipopolysaccharide; MR: mannose receptor; PTX3: the long pentraxin PTX3; RNI: reactive nitrogen intermediates; ROI: reactive oxygen intermediates; SLAM: signalling lymphocytic activation molecule; SRs: scavenger receptors; TLR: Toll-like receptor. Reproduced with permission from [18]. Copyright 2004 Elsevier.

factors are broadly used in studies as macrophage models. In humans, the granulocyte macrophage colony-stimulating factor (GM-CSF) leads to M1 and the macrophage colony-stimulating factor (M-CSF) to M2 being able to polarize towards M1 after specific stimulation [19].

Macrophages are characterized by the expression of specific proteins on their membranes, such as the glycoprotein CD14, which is a well-established differentiation marker for monocytes/macrophages [20]. CD80 and CD200R are specific markers for M1 and M2, respectively [18,21]. Human

macrophages express different markers compared to murine macrophages. The chemokine CCL2 is considered as a marker for polarization in murine macrophages but not in human macrophages [19]. Functionally, these proteins act as receptors recognizing foreign particles and substances activating immune responses. The activation of receptors induces macrophage phagocytosis as well as the production of cytokines and chemokines [22]. The investigation of the receptor expression and the presence of cytokines are useful parameters to evaluate the macrophages functions of defence. Accordingly, the interaction of nanomaterials with the human body can be evaluated by analysing how these nanomaterials interact with such cells.

In this review, we summarize the different reactions of human macrophages to metal-oxide nanoparticles and discuss the effect of modulated nanomaterial properties on this interaction.

## Nanomaterial recognition by macrophages

The first step of the immune response is related to the recognition of the foreign compounds by macrophages. When nanomaterials enter a biological environment, they interact with molecules presented in this system, coating their surface and forming the protein corona. A wide range of studies suggests that the protein corona of nanoparticles formed in the biological environment plays an important role in the macrophage behaviour [23–25]. Our group recently investigated the characteristics of the protein corona of $TiO_2$ nanoparticles and the interaction of these particles with primary cells of human macrophages. We observed preferentially secondary modified protein in the corona of nanoparticles and IL-6 gene secretion by the macrophages even in low nanoparticle concentrations [26]. Vogt et al. [27] have shown that the protein corona modulates cellular uptake of silica-coated superparamagnetic iron oxide nanoparticles (SPIONs) in primary human macrophages. The uptake of these nanoparticles was enhanced by the presence of a protein corona. On the other hand, no difference was noticed in the uptake of dextran-coated SPIONs with or without corona.

Macrophages recognize foreign molecules expressing immune receptors. Among these are toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors (NLRs), Fc receptors (FcRs), scavenger receptors (e.g. MARCO, SR-A1), integrins and carbohydrate receptors. The evaluation of these expressions is useful for the modulation of macrophage responses when exposed to nanoparticles.

TLR signalling is related to the initiation of the innate immune system reactions. The activation of TLR starts a complex intracellular signalling pathway. TLR1 to TLR10 have been well characterized in humans [22]. Each TLR recognizes specific substances inducing different responses. Some of these receptors were predicted to recognize viruses. TLR7 expression is related to single stranded RNA (ssRNA) recognition and TLR3 responds to double-stranded RNA (dsRNA), produced by viruses during their replication. TLR9 is a receptor for bacterial- and viral-derived CpG DNA being its agonists. This aspect is of interest in vaccine development [28,29].

Lucarelli et al. [30] have observed that the level of TLR7 is increasing in a population of human macrophages (PMA-differentiated U-937 cells) when in contact with $TiO_2$ and $ZrO_2$ at non-toxic concentrations. Moreover, it has been shown that $ZrO_2$ nanoparticles induced TLR3 expression and $TiO_2$ nanoparticles impaired by TLR9 expression. This finding demonstrates that these nanoparticles, especially $ZrO_2$ nanoparticles, can enhance the reactivity of macrophages against viral infections and that $TiO_2$ nanoparticles can decrease their ability to respond to vaccination. The increase in TLR10 expression also seen in their study possibly corroborates the hyperactivity of the body to viral infections in contact with both of metal-oxide nanoparticles. Even though the receptor TLR10 still has unknown functions, a study has demonstrated its role as a sensing receptor for virus in human macrophages [31].

While TLRs are receptors located in the cell membrane, NLRs (nod-like receptors) are cytoplasmic receptors that oligomerize to form multiprotein complexes called inflammasome, activating inflammatory signallings [32]. Yazdi et al. [33] have investigated the inflammatory ability of $TiO_2$ nanoparticles by the activation of the inflammasome NLRP3 (NLR pyrin domain containing 3) in human macrophages (PMA-differentiated THP-1 cells). Compared to different nanoparticles, $TiO_2$ nanoparticles trigger NLRP3 activation in contrast to ZnO counterparts. Later on, Baron et al. [34] have shown that the mechanisms mediating NLRP3 inflammasome activation by $TiO_2$ nanoparticles involve exogenous adenosine. The authors showed that extracellular adenosine at high concentrations enhance ATP release and promote NLRP3 inflammasome activation.

Besides the ability to recognize foreign compounds through receptors, another feature of macrophages is the ability to internalize compounds and communicate with other cells through endocytosis. The internalization of these compounds not only permits their clearance from the body but also their presentation to specialized cells of the immune system allowing the so-called “immunological memory”. Endocytosis includes non-specific endocytosis such as phagocytosis and pinocytosis, and receptor-mediated endocytosis (Figure 3) [35].

An efficient recognition of some particles by macrophages associated with the high endocytic activity of these cells can be useful for cell imaging in the diagnosis of specific diseases characterized by macrophage accumulation. Among these nanoparticles are the superparamagnetic iron oxide nanoparticles (SPIONs), used in the detection of atherosclerotic plaques and lymph-node metastases [36,37]. By using uptake inhibitors it is possible to determine which mechanism and cellular receptor are involved in the cellular uptake of nanomaterials. SPIONs and ultrasmall SPIONs (USPIONs) with a carboxydextran coating with 60 and 20 nm in diameter, respectively, were uptaken by primary human monocyte-derived macrophages (M-CSF-differentiated) *via* endocytosis mediated by the protein clathrin, as demonstrated by Lunov et al. [38].

This mechanism is also involved in the uptake of ZnO nanoparticles by THP-1-derived macrophages (PMA-differentiated) as shown by James et al. [39]. Lunov et al. [38] have

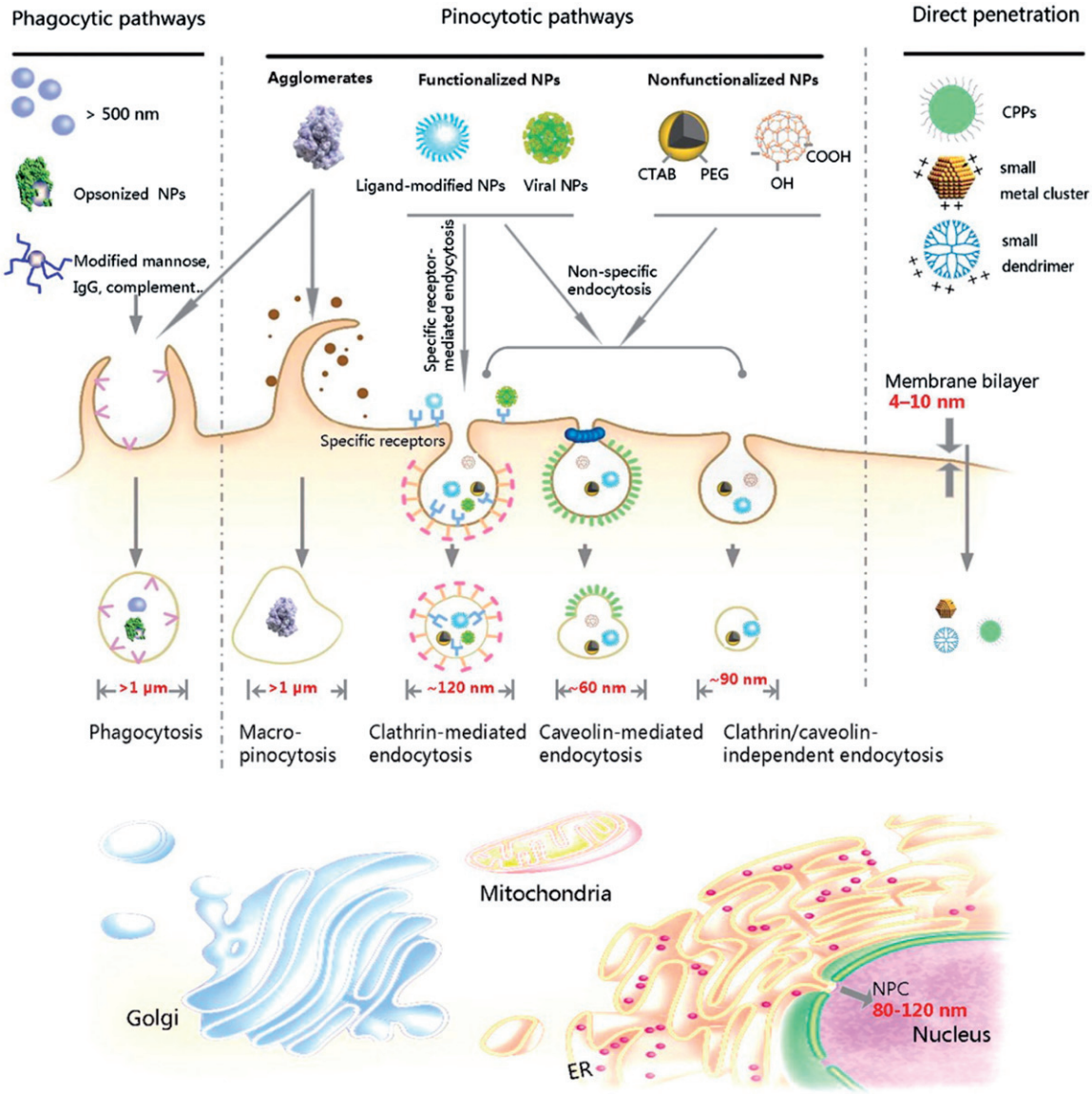


**Figure 3.** Mechanisms of endocytosis of nanoparticles (NP) and cellular components involved. Reproduced with permission from [35]. Copyright 2013 American Chemical Society.

also detected the involvement of the scavenger receptor A (SR-A) in the uptake of SPION and USPION nanoparticles by primary human monocyte-derived macrophages (M-CSF-differentiated). SR-A also mediated the uptake of dextran-coated SPIONs (120–180 nm) by THP-1-derived macrophages (PMA-differentiated) [40]. A peptidic SR-A type I ligand conjugated in USPIONs (15–30 nm) increased the uptake of these particles by macrophages (PMA-differentiated THP-1 and U937 cells) in the study of Segers et al. [41] reinforcing the involvement of this receptor in the internalization of some SPIONs used as magnetic resonance contrasts.

SR-A are phagocytic recognition receptors, as well as opsonic FcR, and bind a broad range of ligands such as modified low-density lipoprotein (LDL), polynucleic acids and bacterial components [42]. The pathogenesis of some diseases with high macrophage accumulation can be related to the high expression of this receptor [43,44]. ZnO nanoparticles upregulated the expression of SR-A and CD36 (SR-B) in THP-1-derived macrophages (PMA-differentiated cells) increasing the uptake of modified LDL by these cells. On the other hand, $TiO_2$ nanoparticles presented the opposite effect on CD36 expression and have no effect on SR-A expression. The effect promoted by ZnO nanoparticles suggests that these particles induce the progression of atherosclerosis in humans [45].

The interaction of macrophages with SPIONs can vary depending on the functionalization of these nanoparticles. The presence of amino groups on the surface of SPIONs allows their uptake by primary human monocyte-derived macrophages (PMA-differentiated) through the integrin receptor Mac-1 [46]. This receptor consists of integrins (αM and β2) involved in several immune responses besides phagocytosis. Zhou et al. [47] reported that Mac-1 receptors have also been involved in the extracellular dsRNA recognition. Folic acid functionalization of iron oxide nanoparticles with an organic and inorganic layers leads to the uptake by primary human macrophages (M-CSF differentiated) through endocytosis and folate receptor-α independently [48].

The charge of the nanoparticle will also define the nature of the protein adsorbed to their surface in a biological medium leading their interactions with cells. Deng et al. [49] suggested that negatively charged nanoparticles interact with Mac-1 receptor on THP-1 cells due to binding and unfolding induction of fibrogen by these nanoparticles. Conformational changes of proteins expose buried sequences influencing the interaction of nanoparticles with cells [50].

The recognition of nanomaterials by macrophage receptors activates intracellular pathways leading to the production of cytokines and chemokines, and are promoting the sequence of immune reactions.

## Macrophage activation by metal-oxide nanomaterials

The macrophages activation has several mechanisms. Elucidation of signalling pathways of macrophages upon a specific material is essential for understanding the inflammatory process. Therewith, regulatory mechanisms can be studied to tune macrophages activation.

Nanoparticles can elicit immune-modulatory effects on the body. Induction of inflammatory and anti-inflammatory cytokines reveals the immune-stimulation and immune-suppression ability of nanoparticles, respectively. Nanoparticles with high potential for imaging applications, for example, SPIONs, must avoid immune recognition. Müller et al. [51] have demonstrated that dextran-coated USPIONs (30 nm) did not trigger inflammatory responses on primary human macrophages. The authors have investigated the secretion of IL-12, IL-6, TNF-α and IFN-γ by the cells. Vogt et al. [27] also have not observed release of TNF-α on primary human macrophages under exposure of dextran-coated SPIONs (multiple iron core clusters of ∼50 nm) and silica-coated SPIONs (single core shell nanoparticles of ∼50 nm). On the other hand, a desired immune-activation can be useful in the development of therapeutic nanoparticles. Chattopadhyay et al. [52] demonstrated that primary human macrophages were activated by CoO nanoparticles loaded with tumour-associated antigens, under non-toxic concentrations, releasing TNF-α, IFN-γ and IL-12. The human oral carcinoma cell line KB was used to investigate antitumor effects under nanoparticles activation. Since macrophages are present in or around tumours, this augment of the cytokine production induces the adaptive immunity resulting in an efficient immune activity around the region to be treated [52,53]. Upon studying cell elasticity of human macrophages, Gonnissen et al. [54] demonstrated a decrease of the elasticity in the presence of starch coated iron oxide nanoparticles, which also resulted in a decrease of transmigratory activity of such cells. The latter effects might be useful, for example, to develop anti-inflammatory active nanoparticles, and demonstrate that the effects of nanoparticles can be more delicate than just affecting the survival of cells.

The ability of some metal-oxide nanoparticles to induce cytokine production was presented by several authors. *In situ* studies performed by Andujar et al. [55] showed that the chemical ingredient of metal-oxide nanoparticles detected in macrophages presented in the lung tissue of welders, workers commonly exposed to metal fumes, were mainly Fe, Cr and Mn. By analysing THP-1 macrophages (PMA-differentiated cells) *in vitro* the authors demonstrated that $Fe_2O_3$ and alloyed metal oxide $MnFe_2O_4$ nanoparticles induce mainly the secretion of pro-inflammatory cytokines TNF-α, IL-1β, CCL-2, CCL-3, CCL-4 and CXCL-9. $Fe_3O_4$ was the lowest reactive nanoparticle.

Yazdi et al. [33] observed that anatase $TiO_2$ nanoparticles (20 nm and rutile 80 nm) trigger the secretion of IL-1β in the same type of macrophages, whereas ZnO nanoparticles (15 nm) present no effects on these cells. The inflammatory pathway of IL-1β starts with the activation of the adaptor protein Myd88 to the receptor leading to the translocation of the transcription factor NF-κB into the nucleus. IL-1β pathway also comprises the proteinase caspase-1 activation. Thereafter, Baron et al. [34] have also shown the involvement of adenosine on the IL-1β secretion. $TiO_2$ nanoparticles induced the release of ATP leading to IL-1β secretion. The authors demonstrated that the inhibition of the P2X7 receptor (purinergic receptor for ATP) of THP-1 macrophages reduced IL-1β secretion. Therefore, investigation on the activity of these compounds is important to determine the secretion of mature forms of cytokines [56,57]. It is well known that IL-1β contributes to inflammatory diseases [56,58]. Using the same type of macrophages, the results by Yazdi et al. [33] for $TiO_2$ nanoparticles and ZnO nanoparticles were corroborated by Morishige et al. [59] and Xia et al. [60], respectively. On the other hand, $TiO_2$ with a belt shape (7000 nm length ×200 nm width ×10 nm thickness), rather than particles (81/19% anatase/rutile, 24 nm and 100% anatase, 28 nm) was the only nanomaterial that presented the ability to induce IL-1β expression in the study of Xia et al. [60]. Considering that the studies of Yazdi et al. [33] and Morishige et al. [59] range $TiO_2$ nanoparticle concentrations of 20–500 μg/mL for cell stimulation, the inconsistency of their results with those obtained by Xia et al. [60] (nanoparticle concentrations up to 100 μg/mL) is possibly due to the characteristics of the cell population investigated. While the latter authors used 5 nM PMA associated with vitamin D3 on THP-1 cells differentiated by 10 ng/mL LPS, the former authors used a 100 times higher PMA concentration. Concerning PMA treatment on THP-1, the conditions for the culture have to be carefully monitored to obtain a homogeneous culture expressing reliable responses [61]. Thus, the precise definition of a cell population in studies involving bioactivity is essential.

Macrophages also differ in their ability to respond to nanoparticles depending on their activation state. $TiO_2$ (70 nm) and $ZrO_2$ (5–30 nm) nanoparticles have increased the level of anti-inflammatory IL-1Ra, the receptor antagonist of IL-1, on PMA-differentiated U-937 macrophages and decreased its level on the same cells exposed to LPS (M1 type macrophage) in the study of Lucarelli et al. [30]. $ZrO_2$ nanoparticles have enhanced IL-1β production on LPS-activated macrophages. On the other hand, some metal-oxide materials demonstrate effects on macrophage polarization. SPIONs formulated with different coatings evince distinct reactions on these cells. Laskar et al. [62] have demonstrated the carboxydextran-coated SPIONs (45–60 nm) accumulation

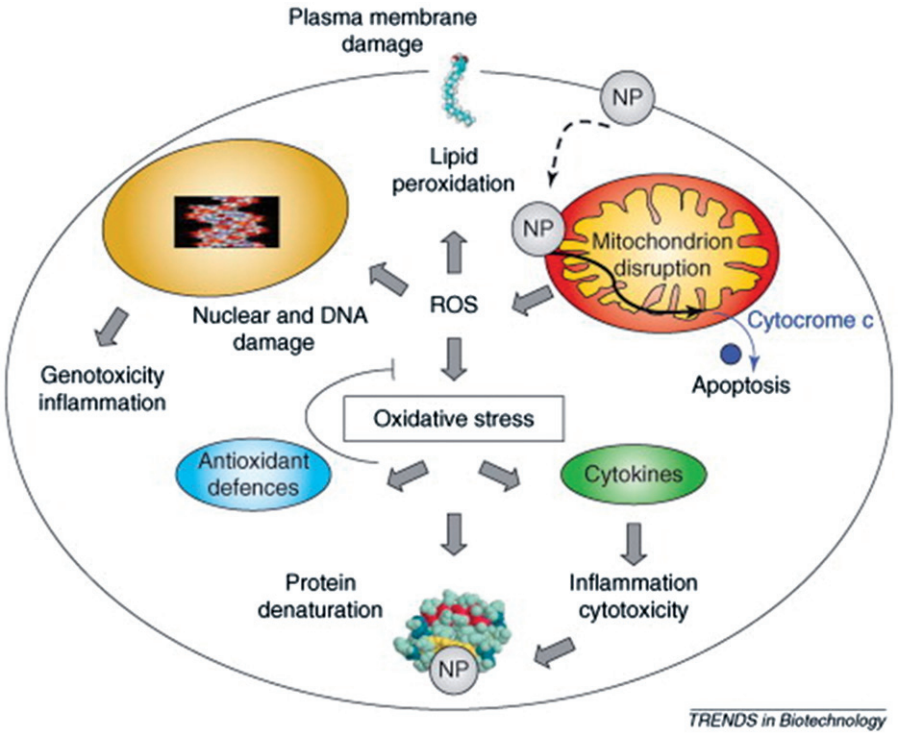


**Figure 4.** Responses of the cells under oxidative stress induced by nanoparticles (NP). Reproduced with permission from [64]. Copyright 2008 Elsevier.

in M2-differentiated THP-1 cells affecting their phenotype and leading to a shift towards an M1-like phenotype. Later, Rojas et al. [63] showed that the same cells have their activation profiles changed by DMSA-, APS- and AD-SPIONs without switching to M1 phenotype. The authors also observed differences in the gene expression profiles of these cells and murine primary IL-4-activated bone marrow-derived macrophages differentiated with M-CSF after interaction with SPIONs, suggesting that the choice of the model to be studied and its initial activation state are of great importance in the evaluation of the nanoparticles effects.

Nanomaterials can induce the production of some toxic mediators, such as reactive oxygen species (ROS), since they are able to reach mitochondria directly, and thereby induce inflammatory pathways on activated macrophages. Morishige et al. [59] and Yazdi et al. [33] demonstrated that ROS are responsible for inflammasome activation leading to IL-1β maturation in PMA-differentiated THP-1 macrophages, under $TiO_2$ nanoparticle stimulation.

Oxidative stress occurs when the concentration of oxidizing compounds increases causing inflammatory and toxic effects to the cells (Figure 4) [64].

Rahman et al. [65] showed that human alveolar macrophages produce more ROS than rat alveolar macrophages in contact with ultrafine $TiO_2$ nanoparticles making them a more reliable indicator for the toxicity of some nanomaterials.

The ability of metal ions to enhance the ROS response on macrophages has been observed by Mokgobu et al. [66] and Petit et al. [67]. These studies revealed that uncoated iron oxide nanoparticles are degraded into toxic metal ions inside the cells [68–70].

The toxicity is enhanced in the case of macrophages that have higher ability to reduce iron than other cells being the ferrous state catalyser of hydroxyl radical generation *via* the Fenton reaction [71,72]. Lunov et al. [73] showed that carboxydextran-coated SPIONs, small (∼60 nm) and ultrasmall (∼20 nm), also induce toxicity on monocyte-derived macrophages (M-CSF-differentiated) by ROS production after exposure times over 24 h. These nanoparticles trigger cell apoptosis due to the degeneration of the coating and exposure of the reactive core. Exposure times under 24 h did not induce any cytotoxicity on these cells as was also shown by other studies for human fibroblasts and human carcinoma cells [74–76].

Strehl et al. [77] recently demonstrated that amino-polyvinyl alcohol-coated SPIONs even increase the same type of cell survival under 20 h of exposure.

ROS scavengers are useful to reduce the damage of oxidative stress. The body has an antioxidant defence system composed of enzymes (e.g. superoxide dismutase) and substances like glutathione and some vitamins. However, facing the abundance of ROS, several substances such as Trolox, PEG, BHA and NAPDH oxidase have been used in studies presenting efficient ROS scavenging abilities [59,73,78–80]. Interestingly, some metal oxide nanoparticles present this property, as is the case of cerium oxide nanoparticles. Cerium oxide exhibit catalytic properties since they present a reversibility of the oxidation states $Ce^{+3}$ and $Ce^{+4}$ that leads to oxygen vacancies [81]. Lord et al. [82] investigated the antioxidant properties of cerium oxide nanoparticles in U-937 derived macrophages (PMA-differentiated) exposing the cells for up to 72 h to the nanoparticle. The authors demonstrated the uptake of the nanoparticles by the cells leading to a continuous intracellular ROS scavenger action.

For an overview, some interactions of metal-oxide nanoparticles with human macrophages are listed in Table 1.

**Table 1.** Interaction of different metal-oxide nanomaterials with human macrophages.

| Material | Size (nm) | Characteristics | Dose (μg/mL) | Exposure time (h) | Type of cells | Differentiation protocol | Treatment of macrophages | Cellular responses | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| $TiO_2$ | 20–160 | n.s. | 400 μg/$10^6$ cells | 24 | U-937 | 10 nM PMA, 72 h | – | ↑TLR7, ↑TLR10, ↓TLR9, ↑IL-1Ra | [30] |
| | | | | | | | 100 ng/mL LPS | ↓IL-1Ra | |
| | ~24 | 81% anatase 19% rutile | 10, 25, 50, 100 | 24 | THP-1 | 5 nM PMA +150 nM Vit $D_3$, overnight | 10 ng/mL | n.d. IL-1β | [60] |
| | ~28 | Anatase | 10, 25, 50, 100 | 24 | THP-1 | 5 nM PMA +150 nM Vit $D_3$, overnight | 10 ng/mL | n.d. IL-1β | [60] |
| | 20 | Anatase | 20 | 6 | THP-1 | 0.5 μM PMA, 3 h | – | ↑IL-1β (*via* NLRP3 activation) | [33] |
| | 10 | Anatase | 500 | 6 | THP-1 | 0.5 μM PMA, 24 h | – | ↑IL-1β (mediated by caspase-1, cathepsin B, ROS) | [36] |
| | 80 | Rutile | 20 | 6 | THP-1 | 0.5 μM PMA, 3 h | – | ↑IL-1β (*via* NLRP3 activation) | [33] |
| | 30–40 | Rutile | 500 | 6 | THP-1 | 0.5 μM PMA, 24 h | – | ↑IL-1β (mediated by caspase-1, cathepsin B, ROS) | [36] |
| | 7000 × 200 × 10 | Anatase nanobelts | 10, 25, 50, 100 | 24 | THP-1 | 5 nM PMA +150 nM Vit $D_3$, overnight | 10 ng/mL | ↑IL-1β (dose dependent) | [60] |
| | 10 × 40 | Rutile spicula | 500 | 6 | THP-1 | 0.5 μM PMA, 24 h | – | ↑IL-1β (mediated by caspase-1, cathepsin B, ROS) | [36] |
| $ZrO_2$ | 5–30 | – | 400 μg/$10^6$ cells | 24 | U-937 | 10 nM PMA, 72 h | – | ↑TLR7, ↑TLR10, ↑TLR3, ↑IL-1Ra | [30] |
| | | | | | | | 100 ng/mL LPS | ↓IL-1Ra, ↑IL-1β | |
| ZnO | 15 | – | 20 | 6 | THP-1 | 0.5 μM PMA, 3 h | – | n.d. IL-1β (*via* NLRP3 activation) | [33] |
| | 20 | – | 10 | 3 | THP-1 | 162 mM PMA, 16 h | – | ↑SR-A, ↑CD36 | [44] |
| | 20 | – | 10, 25, 50, 100 | 24 | THP-1 | 5 nM PMA +150 nM Vit $D_3$, overnight | 10 ng/mL | Toxic (≥50 μg/mL), n.d. IL-1β | [60] |
| SPION | 60 | Carboxydextran coated | 500 | 6 | Blood monocyte | 15 ng/mL M-CSF, n.s. | – | ↑SR-A | [38] |
| | | | 10 | 72 | Blood monocyte | 15 ng/mL M-CSF, n.s. | – | ↓viability (↑caspase-3), n.d.TNF-α, ↑ROS | [73] |
| USPION | 30 | Amino PVA-coated | 120 μg Fe/mL | 2 | Blood monocyte | 100 ng/mL PMA, 15 min | – | ↑Mac-1 | [46] |
| | | Dextran-coated | 10 | 2 | Blood monocyte | 100 ng/mL PMA, 15 min | – | ↑Mac-1 | [46] |
| | 31 | Amino-polyvinyl alcohol coated | 1, 10,100, 1000 | 20 | Blood monocyte | 8.3 ng/mL M-CSF, 6 days | – | ↑viability | [77] |
| | 20 | Carboxydextran- coated | 500 | 6 | Blood monocyte | 15 ng/mL M-CSF, n.s. | – | ↑SR-A | [38] |
| | | | 10 | 72 | Blood monocyte | 15 ng/mL M-CSF, n.s. | – | ↓viability (↑caspase 3), n.d.TNF-α, ↑ROS | [73] |

n.d.: not detected; n.s.: not shown.

## Physicochemical properties of metal-oxide nanomaterials on macrophage behaviour

As mentioned previously, the characterization of the working population of cells is highly important for the correct analysis of macrophage responses upon contact with nanoparticles. Besides, the properties of the nanoparticles can greatly affect their interaction with macrophages and sequential intracellular signalling.

Studies involving nanomaterials had aroused the interest of the scientific community mainly due to their nanoscale sizes (i.e. high surface area to volume ratio), which lets them display quite different properties from their bulk counterparts. The ability of ultrasmall metal-oxide nanoparticles (<30 nm) to induce more pronounced effect on human macrophages than small (~60 nm) nanoparticles was demonstrated in some studies [40,73]. An impairment of primary human monocyte-derived macrophage (M-CSF-differentiated) function and an enhanced long-term toxicity (>24 h) after exposure of carboxydextran-coated ultrasmall SPIONs (20 nm) was observed by Lunov et al. [73] measuring iron uptake and ROS generation compared to carboxydextran-coated small SPIONs (60 nm). Cell viability measurements demonstrated that macrophages only exhibit translocation of membrane components after 3-days exposure of small SPIONs, characteristic of the early signs of cell death by apoptosis, while the same time exposure with ultrasmall SPIONs resulted in a loss of integrity of the cell membrane, an evidence of the late stage of apoptosis. These results were confirmed by the detection of a higher activity of caspase-3 on macrophages exposed to ultrasmall than small SPIONs. This enzyme is activated by mitochondrial components released during apoptosis [72]. The authors also developed the mathematical modelling of the uptake of these nanoparticles by the macrophages enabling the prediction of parameters such as wrapping time and uptake rate of the nanoparticles. The uptake mechanism was the same for both nanoparticles being clathrin-mediated endocytosis, however, ultrasmall SPIONs were uptaken faster than small SPIONs [38].

Of note, studies with alternatively labelling human monocytes for imaging with SPION and USPIONs also showed higher internalization of SPIONs than USPIONs by these cells [83].

Some metal-oxide nanomaterials present unique characteristics that have to be taken into account together with the size on the evaluation of their effects. Among these are $TiO_2$ nanoparticles. $TiO_2$ may exhibit three different crystal structures: anatase, rutile and brookite, with anatase and rutile vastly studied. Morishige et al. [59] have reported that smaller anatase and larger rutile induced IL-β secretion by PMA-differentiated THP-1 macrophages. The authors also demonstrated higher stimulation ability of rutile than anatase $TiO_2$ nanoparticles on the macrophages.

Proteins bound to the nanomaterial surface mediate immune responses, as discussed previously. The physicochemical properties of materials influence the amount and the profile of proteins adsorbed to their surfaces leading to different cell outcomes [24,83]. Surface coating, a preset to alter undesirable properties of nanoparticles, can highly affect

the nanoparticle-protein corona since the surface is the first contact site of the nanoparticles with the cellular environment. Consequently, the interaction of nanoparticles with cells occurs *via* this protein corona [26].

Uncoated $TiO_2$ nanoparticles adsorbed more proteins than alumina and silicone-coated particles in contact with primary human monocyte-derived macrophages (GM-CSF-differentiated) as demonstrated by Sund et al. [84]. Later on, the authors studied the cytoplasmic protein changes of the macrophages in contact with uncoated- and silica coated-$TiO_2$ nanoparticles. These proteomic changes of macrophages can be considered as an indicator of certain biological activities, for example, phagocytosis. This study has shown that $TiO_2$ nanoparticles induce pronounced changes in the proteome of the same human macrophages when they are coated [85]. The difference on the protein binding due to different surface charges of silica-coated (30–120 nm) and dextran-coated (20 and 50 nm) SPIONs was suggested by Kunzmann [86] to be the reason for the higher uptake detected for silica-coated SPIONs by primary human monocyte-derived macrophages (M-CSF differentiated). Using inductively coupled plasma-mass spectrometry (ICP-MS) with an inhibitor of actin polymerization, the authors monitored the uptake of these particles and verified that silica-coated SPIONs were taken up to a higher degree using an actin-cytoskeleton dependent mechanism. On the other hand, Deng et al. [87] showed that some metal oxide nanoparticles, such as $TiO_2$ and ZnO, have the ability to bind different proteins in contact with human plasma even though they present similar surface charges. Therefore, the degree of complexity for protein–nanoparticle interaction is high and many factors have to be considered together with the nanoparticle characteristics, like the agglomeration state in biological systems, and further *in vivo* studies have to be developed since the protein binding status is highly variable within protean biological conditions.

Compared to the size, the surface properties of SPIONs have higher impact on the phagocytosis in human monocytes and rat macrophages cell lines [83].

The shape of nanomaterials is another factor to consider since the material surface area interacting with the environmental changes. The study of Xia et al. [60] showed that the cytokine release by PMA-differentiated THP-1 macrophages was influenced by their shape once exposed to $TiO_2$ nanomaterials (nanoparticles and nanobelts). $TiO_2$ nanospheres attract qualitatively more protein than $TiO_2$ nanorods (ellipsoid shaped nanomaterials) and $TiO_2$ nanotubes, which will trigger different immune responses [87].

Therefore, the characterization of the nanomaterials is of great importance and a prerequisite for their safe and actual medical application toward human beings.

## Conclusion

Evaluation of the interaction of macrophages with different metal-oxide nanomaterials, as discussed in this report, is important for the handling the risk in the development of new nanomaterials. Interactions between cells and nanoparticles are regulated by a plethora of factors including the design, shape and surface chemistry of the nanoparticle on the one hand and the characteristics of the cells and the experimental conditions on the other hand. That is why general conclusions can only be made including all of the above-mentioned factors.

## Disclosure statement

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