Biomaterials Science

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Biomater. Sci.*, 2023, **11**, 4151

Immunotoxicity of metal and metal oxide nanoparticles: from toxic mechanisms to metabolism and outcomes

Jiaming Bi,†[a] Chuzi Mo,†[a] Siwei Li,[a] Mingshu Huang,[b] Yunhe Lin,[a] Peiyan Yuan,[a] Zhongjun Liu,*[a] Bo Jia *[b] and Shuaimei Xu *[a]

The influence of metal and metal oxide nanomaterials on various fields since their discovery has been remarkable. They have unique properties, and therefore, have been employed in specific applications, including biomedicine. However, their potential health risks cannot be ignored. Several studies have shown that exposure to metal and metal oxide nanoparticles can lead to immunotoxicity. Different types of metals and metal oxide nanoparticles may have a negative impact on the immune system through various mechanisms, such as inflammation, oxidative stress, autophagy, and apoptosis. As an essential factor in determining the function and fate of immune cells, immunometabolism may also be an essential target for these nanoparticles to exert immunotoxic effects *in vivo*. In addition, the biodegradation and metabolic outcomes of metal and metal oxide nanoparticles are also important considerations in assessing their immunotoxic effects. Herein, we focus on the cellular mechanism of the immunotoxic effects and toxic effects of different types of metal and metal oxide nanoparticles, as well as the metabolism and outcomes of these nanoparticles *in vivo*. Also, we discuss the relationship between the possible regulatory effect of nanoparticles on immunometabolism and their immunotoxic effects. Finally, we present perspectives on the future research and development direction of metal and metal oxide nanomaterials to promote scientific research on the health risks of nanomaterials and reduce their adverse effects on human health.

Received 17th February 2023,
Accepted 17th April 2023

DOI: 10.1039/d3bm00271c

rsc.li/biomaterials-science

1. Introduction

In recent decades, nanomaterials have been widely applied in industrial manufacturing and the food and medicine sectors, promoting the rapid development of related technologies. However, although nanomaterials have become increasingly popular, their toxic effects on humans are not clearly understood. In the field of biomedicine, metal and metal oxide NPs (including Ag, Au, ZnO, CuO, and CeO_2 NPs) are widely used as drug delivery systems and diagnostic, therapeutic and imaging systems due to their unique physical and chemical properties. However, the immunotoxicity caused when these NPs interact with immune cells has raised concerns about their safety. Numerous studies have shown that metal and metal oxide NPs pose a risk to the human body, inducing a series of reactions through its defence system.[1] The immune response refers to the defensive response of the body to foreign components or mutated autologous components, which can be divided into non-specific and specific immune responses. When metal and metal oxide NPs enter the human body, they are recognised as 'foreign' substances by the immune system and trigger a series of immune responses. The relevant tissue- and organ-level regulations enable the body to respond quickly and adapt to the NP stimulus within a short period.[2] Although this can lead to positive immune responses (*e.g.*, NPs can participate in and coordinate the immune response as a vaccine adjuvant), in some cases, negative immune responses occur[3] (*i.e.*, immunotoxicity).

The field of immunometabolism has developed rapidly in recent years, revealing the contribution of biochemistry to the development, fate, and behaviour of immune cells. The manipulation of specific components of the immunometabolism cycle (such as TORC1, TORC2, PTEN, AMPK, and PI3K) by genetic or pharmacological methods can affect the energy metabolism in immune cells, thereby regulating cell behaviour and function.[4] Therefore, metal and metal oxide NPs may also

[a]*Department of Endodontics, Stomatological Hospital, School of Stomatology, Southern Medical University, Guangzhou, Guangdong, China.*
E-mail: xushuaimei@smu.edu.cn, liuzhongjundr@smu.edu.cn
[b]*Department of Oral and Maxillofacial Surgery, Stomatological Hospital, School of Stomatology, Southern Medical University, Guangzhou, Guangdong, China.*
E-mail: dentist-jia@163.com
† Both authors contributed equally.

affect the differentiation and functions of immune cells by affecting immunometabolism, which may also contribute to the immunotoxicity caused by these NPs.

The toxic effects of metal and metal oxide NPs mainly depend on their physicochemical properties, such as composition, size, geometry, surface charge, and coating material.[5] After administration, the physical and chemical properties of NPs are altered. NPs can aggregate, agglomerate, dissolve or degrade, and eventually excreted through the metabolism-related tissues and organs.[1] During this process, NPs and their degradation products may affect the roles and functions of immune cells and metabolism-related tissues and organs. Therefore, a complete understanding of their effects on the immune system, especially their immunotoxicity and fate *in vivo*, are essential for developing better NPs with good biosafety.

Previous reviews mainly focused on the immunotoxicity of specific metal or metal oxide NPs or the toxic effects observed in particular tissues. Thus, a systematic summary of the possible mechanisms of the immunotoxicity caused by these NPs is lacking. Different types of metal and metal oxide NPs have different structures and properties and may exhibit different cellular mechanisms of immunotoxicity. To provide a comprehensive and systematic review, herein, we introduce the structure, properties, and applications of different types of metal and metal oxide NPs. Subsequently, we summarise and delineate the immunotoxic effects of metal and metal oxide NPs based on their toxicity mechanisms. In addition, we describe the metabolism and outcomes of these NPs *in vivo*. Finally, we discuss the possible effects of different physicochemical properties and experimental conditions on the degradation, metabolism, and excretion of metal and metal oxide NPs. The relationship between the regulatory effect of nanoparticles on immunometabolism and their toxic effects is also discussed to understand the immunological properties of these NPs and offer additional prospects for designing safer NPs.

2. Classification, characteristics and applications of metal and metal oxide NPs

NPs are tiny particles with a size in the range of 1 to 100 nm in a particular dimension within three-dimensional space. 'Nanometals', which are metallic materials manufactured *via* nanotechnology, have a nanoscale structure and contain nanoparticle impurities.[6] Using nanotechnology, it is possible to control the composition and microstructure of metallic materials with extreme precision and intricacy during their production. Consequently, the mechanical and functional properties of metals have been improved by leaps and bounds. NPs can be mono-metallic or consist of metal alloys or metal oxides (such as Au NPs, Cu–Ag NPs, and CuO NPs, respectively). Metallic materials can be classified into different groups, which may have different physical and chemical properties and characteristics. Therefore, the corresponding metal and metal oxide NPs may also have various types, features, and applications.

Nowadays, metallic materials are usually divided into two categories (ferrous and non-ferrous metals) according to their colour and properties.[7] Ferrous metals include iron, manganese, chromium, and their alloys. They are called ferrous metals because the surface of steel is often covered by a layer of black Fe_3O_4 film, while manganese and chromium are often used to make steel alloys with iron. Therefore, manganese and chromium, together with iron, are collectively referred to as ferrous metals. These three metals constitute the primary raw materials for steel smelting, accounting for about 95% of the total metal production globally and play an essential role in the industrial and medical sectors.[7] More than 60 types of metals, besides ferrous metals, are together called non-ferrous metals. Most of them are silver or white, except gold (yellow) and copper (red).[8] Nonferrous metals can be divided into four categories, as follows: (I) heavy metals (nonferrous metals with $\rho > 4.5$ g cm^{-3}): this category includes most transition elements in the periodic table, such as copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), tungsten (W), molybdenum (Mo), cadmium (Cd), and mercury (Hg), as well as antimony (Sb), bismuth (Bi), lead (Pb), and tin (Sn). They are difficult to biodegrade and are instead enriched *via* bio-amplification in the food chain, eventually entering the human body.[9] Heavy metals interact strongly with human proteins and enzymes, rendering them inactive. They can also accumulate in some organs, causing chronic poisoning and damage to tissues and organs.[10] (II) Light metals (metals with $\rho < 4.5$ g cm^{-3}): this category includes metals such as lithium (Li), sodium (Na), potassium (K), rubidium (Rb), caesium (Cs), aluminium (Al), magnesium (Mg), and calcium (Ca). Light metals have many excellent physical and chemical properties and are widely used in industrial fields such as manufacturing and metallurgy.[11] (III) Noble metals: noble metals are metals with stable physical and chemical properties. They typically show low reserves in the Earth's crust, have an elegant appearance, and are expensive. They include rhodium (Rh), ruthenium (Ru), palladium (Pd), silver (Ag), osmium (Os), iridium (Ir), platinum (Pt), and gold (Au).[12] Noble metals have unique properties and are physicochemically stable, with high oxidation and corrosion resistance, excellent processing characteristics, and little effects on human tissues.[13] Therefore, they are widely used in aerospace engineering, industry, and especially the medical field. (IV) Scarce metals: these metals are less abundant in the Earth's crust and are scattered or difficult to extract from raw materials.[14,15] They can be further subdivided into six categories according to their physicochemical properties and production methods, as follows: (i) rare light metals, such as beryllium (Be), lithium (Li), rubidium (Rb), and caesium (Cs), which have a low specific gravity and strong chemical activity. (ii) Rare precious metals, such as platinum (Pt), iridium (Ir), and osmium (Os). (iii) Rare scattered metals, such as gallium (Ga), germanium (Ge), indium (In), and thallium (Tl), which are typically present in minerals of other elements. (iv) Rare earth metals, including scandium (Sc), yttrium (Y), lanthanum

(La), cerium (Ce), and neodymium (Nd), which have very similar chemical properties and co-exist in minerals. (v) Refractory rare metals, including titanium (Ti), zirconium (Zr), tantalum (Ta), vanadium (V), and niobium (Nb), which have a high melting point, together with their compounds derived from carbon, nitrogen, silicon, and boron. (vi) Radioactive rare metals, such as polonium (Po), radium (Ra), actinium (Ac), uranium (U), and plutonium (Pu). This system of classification is not very strict, and some scarce metals are often included in multiple categories.[15,16] For example, rhenium can be included in rare scattered metals or refractory rare metals (Fig. 1).

In addition to being widely used in industry, the aforementioned metals also play an essential role in biomedicine. For example, metal and metal oxide NPs are used as carriers for drug delivery, and they can exert anti-tumour and antibacterial effects.[17,18] Noble metal nanomaterials (NMNs) also show application prospects and great potential in energy, catalysis, biosensors, and medicine. Most NMNs possess excellent biocompatibility and exert low toxicity. Au and Ag NPs are the most widely studied due to their ease of preparation and high safety. Furthermore, gold NPs have attracted widespread attention due to their relative non-toxicity.[19,20] Gold NPs have been used clinically to treat rheumatoid arthritis since the 1920s, when they were called 'colloidal gold'.[21] Gold NPs are also widely used in electron microscopy probes and biomolecule delivery carriers, and as biosensors and imaging labels.[20,22,23] Similar to gold NPs, silver NPs have excellent antibacterial properties and are widely used in wound dressings, catheters, cosmetics, and clothing. In addition, silver NPs are also a potential anti-tumour angiogenesis drug, and they have prospects for application in the treatment of stroke, pulmonary oedema, myocardial infarction, rheumatoid arthritis, and other diseases.[24] NMNs have some remarkable unique properties compared to other metal NPs. For instance, NMNs show a plasma resonance spectral band, which is not available in other metal materials.[25] Nowadays, NMNs are widely used in biological imaging,[26] drug release, and biosensor technology due to their freely adjustable absorption and scattering spectra.[27,28] In addition, recent studies have shown that NMNs can reprogram the tumour microenvironment, thereby inhibiting tumour growth.[29]

In addition to NMNs, other metal and metal oxide NPs are also increasingly being used in biomedicine. In recent years, magnetic nanomaterials based on ferriferous oxide have attracted wide attention in the field of medicine. When reduced to a size on the nanometre scale, superparamagnetic iron oxide NPs can only be influenced by an external magnetic field, enabling them to form stable colloids in physical-physiological media. Their superparamagnetism and other intrinsic properties, such as low toxicity, colloidal stability, biodegradability, and traceability, make them ideal for biomedical applications *in vivo* and *in vitro*.[30,31] The characteristics, applications, and toxicity of heavy metal and metal oxide NPs (such as copper, copper oxide, and zinc oxide NPs) have been the focus of extensive research. Many studies have reported the applications of NPs in medicine. For example, copper is a relatively cheap metal and commonly used. Its NPs have low preparation costs and show excellent performance; thus, they

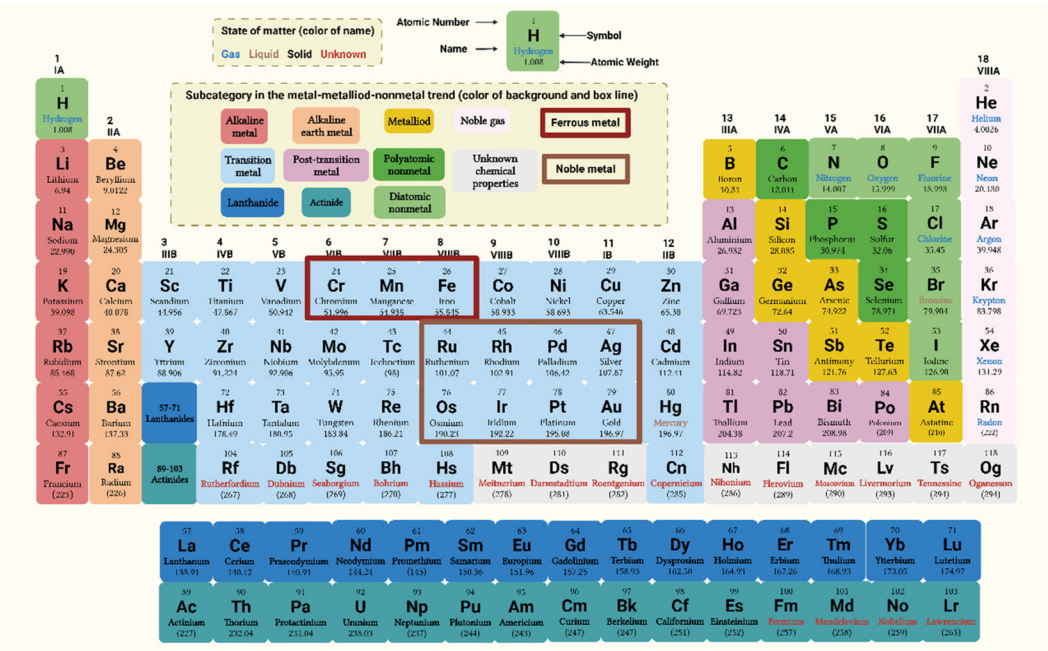

Fig. 1 Location of different types of metal elements in the periodic table. The positions shown in the red and brown boxes are ferrous metals and precious metals, respectively. Except for iron, manganese and chromium, all other metal elements belong to non-ferrous metals. Created with BioRender.com.

are used in various industries, especially the pharmaceutical industry. In addition, copper is a trace element, harmless to many living cells, and can participate in many metabolic reactions. In contrast, Cu NPs show significant antibacterial and bactericidal activity when cell membranes, nucleic acids, and proteins are damaged.[32] ZnO is relatively inexpensive, has good biocompatibility and low toxicity, and has shown good application prospects in many aspects of biomedical engineering. Several studies have confirmed that zinc oxide and its NPs are antibacterial. Compared with ordinary ZnO, ZnO NPs have a smaller particle size and a significant micro-quantum effect, exerting substantially improved antibacterial properties and application prospects.[32] In recent years, there has been an increase in research on rare metal and metal oxide NPs and their applications. For instance, CeO_3 and TiO_2 NPs have antioxidant effects. They are characterised by a small size, controllable and flexible modification, relatively low toxicity, and easy preparation. Thus, they have promising application prospects in medicines, cosmetics, and food additives.[33] Table 2 presents a summary of the properties and biomedical applications of some common metal and metal oxide NPs.

3. Immunotoxicity mechanisms of metal and metal oxide NPs

Metal and metal oxide NPs can enter the body through various pathways (*e.g.*, inhalation, gastrointestinal absorption, and biomedical application) and get absorbed by the spleen and bone marrow or distributed to other tissues, organs, and cells after entering the blood system.[12] Subsequently, these NPs continue interacting with the cells in the body, which may result in both positive and negative effects. Bulk metal and metal oxide NPs are recognised as foreign antigens, triggering an immune response. The immunotoxicity of Au, Ag, TiO_2, and Fe_2O_3 NPs has been reported previously.[34–38] The literature indicates that metal and metal oxide NPs can react with different immune cells, including macrophages, dendritic cells (DCs), natural killer (NK) cells, and B and T lymphocytes. During this interaction, the NPs are engulfed and processed by immune cells, thereby affecting their metabolism, function, and fate. Oxidative stress and inflammation have been studied extensively as immunotoxic mechanisms induced by metal and metal oxide NPs. Oxidative stress refers to the imbalance between oxidation and antioxidation in the body, where under the conditions of oxidative stress, there is an excess of reactive oxygen species (ROS), and biomolecules tend to be oxidized. Inflammation is the defensive response of the body against external agents. However, if not regulated, it can also have harmful effects. The processes of oxidative stress and inflammation are fundamentally related.[39] Therefore, the response of immune cells to NPs is bimodal. To understand the immunotoxicity mechanisms of metal and metal oxide NPs, it is essential to examine the anti-inflammatory and antioxidant responses that mediate the response of immune cells to NPs and their eventual impact on the human body. The toxic effects of some metal and metal oxide NPs on different types of immune cells have been reported. For example, a significant reduction in the number of NK cells was observed in mouse models after prolonged exposure to TiO_2 NPs.[40] Moreover, TiO_2 NPs were found to up-regulate the expression of MHC-II, CD80, and CD86 in DCs.[41] Overall, these results indicate that metal and metal oxide NPs can affect the function of immune cells through different mechanisms, producing immunotoxic effects. In the following sections, we summarise the modes of metal and metal oxide NP immunotoxicity reported thus far, in which the changes in NP immunometabolism may also affect immunotoxicity (Tables 3 and 4).

3.1 Inflammatory responses

Metal and metal oxide NPs can trigger the release of many inflammatory factors. It has been reported that ZnO NPs of

Table 1 Different metabolic features of immune cells

		Main ways of energy metabolism				
Type	Cell subtypes	Glycolysis	OXPHOS	FAO	Glutaminolysis	Ref.
T cells	Naive T cells	–	+	++	–	210 and 211
	TH1 cells	++	–	–	+	210
	TH2 cells	++	–	–	+	210
	TH17 cells	++	–	–	+	210
	Treg cells	–	–	++	–	212
	Memory T cells	–	–	++	–	212
	Cytotoxic T cells	++	–	–	+	210
B cells	Pro-B cells	++	–	–	+	213 and 214
	Immature B cells	++	–	–	+	213 and 215
	Mature B cells	++	–	–	+	213 and 215
Innate immune cells	Dendritic cells (resting)	–	++	–	–	216 and 217
	Dendritic cells (active)	++	–	–	–	217 and 218
	Macrophages (M1)	++	–	–	–	219
	Macrophages (M2)	–	++	++	–	216 and 220
	Neutrophils	++	–	–	–	221 and 222
	NK cells	++	–	–	+	223–225

++: major metabolic pathway; +: minor metabolic pathway; –: neither major nor minor.

Table 2 The properties of different types of metal and metal oxide NPs and their main applications in academic studies

Classification of metal materials	Type of NPs	Properties	Applications	Ref.
Ferrous metal	Fe_2O_3 NPs	Magnetic, traceability, imaging	Cell labeling, tumor therapy, MRI	226–228
	Fe_3O_4 NPs	Magnetic, traceability, near-infrared plasma absorption, thermal ablation characteristics	Biosensors, hyperthermia, PA imaging of tumors, PTT, gene transfer, protein separation	22, 229 and 230
	SPIONPs	Superparamagnetic, traceability, low toxicity, colloidal stability, biodegradability	Tumor cell markers, targeted tumor cells, MRI, drug delivery	231–233
	Mn_3O_4/ Mn_2O_3/ MnO_2 NPs	Antioxidant activity	ROS scavengers	234–236
	Cr_2O_3 NPs	Antibacterial activity	Antibacterial agents	237
	CoCr NPs	Low wear and a low incidence of osteolysis	MoM arthroplasties	238 and 239
Heavy metal	Cu NPs	DNA degradation potential, anticancer and antibacterial activity	Tumor therapy, antimicrobial agents	240 and 241
	Bi NPs	High stability, strong diamagnetism, high near-infrared absorption and photothermal conversion efficiency	Drug carriers, cancer combination therapy, photothermal and radiation therapy, bioimaging, tissue engineering	242 and 243
	ZnO NPs	Low toxicity, antibacterial activity	Antibacterial agents	32 and 244
	CuO NPs	Antibacterial, antiviral, antioxidant, anticancer, high temperature superconductivity	Antibacterial agents, antiviral drugs, dentin binding agents, tumor therapy, imaging agents, drug delivery agents	245–247
	Ni_2O_3/ Co_3O_4/CoO NPs	Antibacterial activity	Antibacterial agents	237
Light metal	Al_2O_3 NPs	Good biocompatibility, high strength, antibiosis	Antibacterial agents, self-healing	248 and 249
	MgO NPs	Antibacterial activity	Antibacterial agents	248 and 250
Noble metal	Au NPs	Large absorption of near-infrared light, antibacterial, antiviral, anti-angiogenesis, SERS, osteoinductive	Photothermal therapy, anti-angiogenic agents, immunoassays, cancer therapy, inhibition of HIV-1, biomedical imaging, bacterial screening, osteoinductive agent for implant dentistry	251–253
	Ag NPs	Antibacterial, anti-angiogenesis, anti-fungal, antiprotozoal, promoting reparative regeneration, sturdy and durable	Antibacterial agents, bone regeneration, nerve regeneration, tumor diagnosis and treatment, biosensors, dental resin filler composites	254–257
	Pt NPs	Antioxidant, antibacterial, strong affinity with dopamine, electrocatalysis	ROS scavengers, bacteriostatic agents, dopamine sensors, targeting tumor cells	258–261
	Pd NPs	Catalytic performance, photothermal ablation	PTT agents, cancer treatment	262 and 263
	Ru NPs	Antibacterial and antioxidant, osteogenesis	Bacteriostasis, ROS scavengers, regulating the behavior of stem cells	264–266
	Rh NPs	Photothermal	Cancer phototherapy	267
	Ir NPs	Photosensitive, hydrophobicity, charge transfer	Enhanced photodynamic performance, drug delivery, bioimaging	268–270
	AgO NPs	Antiviral	Fighting the drug-resistant types of viruses	271
Scarce metal	CeO_2 NPs	Anti-inflammatory, antioxidant, antibacterial, osteogenic, pro-angiogenic	Antibacterial agents, ROS scavengers, anti-inflammatory therapy, bone regeneration, vascular regeneration	244 and 272–274
	TiO_2 NPs	Antioxidant, stability, antiangiogenic, photocatalytic activity	ROS/RNS scavenging, PDT, SDT, photo-controlled drug release and targeted therapy	33 and 275–277
	Ta NPs	High corrosion resistance, anti-inflammatory, anti-apoptotic, osteogenic	Bone regeneration, *in vivo* treatment of transplanted vascular lesions	65, 278 and 279

different sizes and charges can cause immunotoxicity by inducing inflammation[42] and cytokines and chemokines determine the severity of the inflammatory response. Therefore, understanding the timing and mechanisms of the inflammatory responses mediated by metal and metal oxide NPs is vital for developing safe NPs.

3.1.1 Cytokine storm. Multiple studies have confirmed that metal and metal oxide NPs up-regulate multiple cytokines/chemokines (Fig. 2). For example, exposure to alumina NPs (Al NPs) can alter the cytokine levels in tissues and organs such as the spleen and serum. This causes immune-related organ and cell dysfunction and leads to the abnormal expression of various cytokines.[43] Rodent experiments have shown that the main target organs of gold NPs are the liver and spleen, which regulate immune organs in a dose-dependent manner.[44] Gold NPs at a concentration of 0.25 ppm can stimulate the immune response and enhance the expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in the body. However, at a concentration of 25 ppm, gold NPs are considered to have pro-inflammatory or immunotoxic effects because they induce

Table 3 Immunotoxicity mechanisms induced by different types of metal and metal oxide NPs *in vitro*

Classification of metal materials	NP types	NP properties	Models	Mechanisms	Results	Ref.
Ferrous metal	Fe_2O_3	30–35 nm	Lymphocytes of healthy Wistar rats	Induced concentration-dependent oxidative stress and increased ROS, lipid peroxidation levels, antioxidant enzymes and GSH consumption in lymphocytes (male). Imbalance of lipid peroxidation and antioxidation in all vital organs (female).	Morphological changes of lymphocytes and induction of ROS-mediated cytotoxicity.	280
	CoCr	50–150 nm	Monocytes (U937 cells)	Increased secretion of TNF-α (resting cells); increased IFN-γ production (activated cells).	Contribute to *in vivo* osteolysis process; protection of cells against tissue injury.	281
		30–35 nm	RAW 246.7	Reduce cell viability, induce DNA damage, chromosome aberration, metal hypersensitivity increased.	Soft-tissue reactions (local) and arthroprosthetic cobaltism (systemic).	238 and 282
Heavy metal	NiO	5–100 nm	Human peripheral blood lymphocytes	ROS production and lipid peroxidation.	Induce oxidative stress and inflammatory response.	283
	ZnO	20 or 100 nm; positive or negative charge	RAW 246.7	The positively charged NPs exerted higher cytotoxicity.	Lead to immunotoxicity *in vitro*.	42
	Co_3O_4	≤50 nm	Human lymphocytes	Oxidative stress.	Decreased cell viability and increased cell membrane damage (dose-dependent).	142
Noble metal	Au	12, 35, 60 nm; PEG and OVA-coated	RAW 246.7	The OVA-coated GNPs induce higher secretion of TNF-α, IL-6, and IL-1β.	Smaller and the OVA-coated GNPs induced stronger inflammatory responses.	284
	Ag	<30 nm	THP-1 cells	Downregulation of CD11b and response to LPS stimulation, blocking the degradation of p62, inducing lysosomal damage.	Prevent THP-1 cells from differentiating into macrophages.	116
		100 nm; AOT/PVP/PLL/BSA-coated	hPBMC	Oxidative stress, mitochondrial membrane damage, DNA damage.	Apoptosis and cell death (dose and time-dependent); genetic toxicity potential.	285
Scarce metal	TiO_2	20–80 nm	Murine dendritic cells	Upregulate MHC-II, CD80 and CD86, activate inflammasome, enhance ROS production.	Strong influence on the activation state of DCs.	41
		10–30 nm	HUVECs	Induce intracellular ROS production, cell membrane oxidative damage, IKKα/β and Akt phosphorylation and p38 dephosphorylation.	Oxidative stress and apoptosis.	88
		17, 117 nm	THP-1 cells	Glutathione depletion, increased IL-8 and IL-1β, DNA damage and cytotoxicity.	Large agglomerates of 17 nm TiO_2 induced stronger responses than small agglomerates, while no effect of agglomeration was observed with 117 nm TiO_2.	286

a sharp decline in lymphocyte proliferation activity. It is worth noting that the regulatory effects of all doses of IL-2 indicate their effects on the immune regulation mechanism in the spleen.[44] Zhu *et al.*[45] observed elevated levels of IL12, TNF-α, and interferon (IFN)-γ in *in vitro* cultures of macrophages and immature DCs treated with magnetic iron oxide NPs (MIONs). However, they did not detect any elevation in IL-4. They speculated that the changes in cytokine levels were induced by exosomes activating the inflammatory response and Th1-type immune response.[45] Similarly, *in vivo* experiments have also confirmed that the chronic accumulation of iron oxide NPs (Fe NPs, Fe_2O_3) in the lungs can induce a Th1-polarized inflammatory response accompanied by an increase in the secretion of chemokines. In addition, they can also elevate the levels of antigen-presentation proteins such as CD80, CD86, and MHC II in bronchoalveolar lavage (BAL) fluid and enhance the function of antigen-presenting cells (APCs).[46] However, some studies suggest that IONPs can also inhibit the expression of cytokines. For example, the IFN-γ levels were reduced in ovalbumin (OVA)-activated T cells exposed to carboxyl-dextran-coated IONPs.[47] Zinc oxide NPs can induce significant immunotoxicity in immune cells and organs, and inflammation and oxidative stress may by the underlying cause of these effects. In male Wistar albino rats, ZnO NPs (26.6 nm, 350 mg kg^{-1} by oral gavage) were found to significantly increase the expression of immune regulatory genes (CD3, CD11b, and HO-1) and inflammatory genes (TLR4 and TLR6), DNA strand breaks, and the malondialdehyde levels in the thymus and spleen, as well

Table 4 Immunotoxicity mechanisms induced by different kinds of metal and metal oxide NPs *in vivo*

Classification of metal materials	NP types	NP properties	Models	Mechanisms	Results	Ref.
Ferrous metal	Fe_2O_3	Needle-like shape	5-Week-old male ICR mice	Increased secretion of chemokines; enhanced expression of CD80, CD86 and MHC II (BAL).	Enhance the function of pulmonary antigen presenting cells by inducing Th1 polarized immune response.	46
	SPIO	15–20 nm; 300 nm (PLGA-coated)	Female NIH mice	Extensive damage to lysosomes, accumulation of LC3-positive autophagosomes, mitochondrial damage, ER and Golgi stress, and PLGA-coated Fe_3O_4 NPs reduced the damage to these organelles.	Autophagosomes accumulated in the kidney and spleen (detection of endogenous LC3 protein distribution).	287
		Resovist®, 28 mg Fe per mL	Male BALB/c mice	Inhibition of inflammatory cytokines (IFN-γ, IL-4) and antigen-mediated antibody responses.	Impaired antigen-specific immune responses.	157
		45 ± 9.8 nm, 89 ± 0.4 nm, 67 ± 4.6 nm; DEX-coated and PEG-coated	Female Wistar rats	Affect anti-oxidant and tissue nitrite levels.	Mast cell infiltration in liver, lung and heart.	288
	CrNano	40–70 nm	Male Sprague-Dawley rats	Increase the serum level of IgG; enhance lymphoid tissue proliferation response of peritoneal macrophages, anti-SRBC PFC response and phagocytic activity.	Affect hormone and immune responses in heat-stressed rats.	289
Heavy metal	NiO	20 nm	Male Wistar rats	NF-κB activation and Th1/Th2 imbalance.	Enhance the nitrative stress and inflammatory response in lung tissue.	290
		5–100 nm	Rodents	ROS production and lipid peroxidation.	Induce oxidative stress and inflammatory response.	283
	Cu	45–115 nm	Male Sprague-Dawley rats	Induce oxidative stress and overexpression of pro-inflammatory/anti-inflammatory cytokines.	Repress the immune function of the spleen.	291
		32.7 ± 10.45 nm	Male Sprague-Dawley rats	Activate TGF-β1/Smad-dependent and -independent pathways (MAPK and Akt/FoxO3).	Hepatic damage and markedly increased oxidative stress in liver tissues.	292
		40 nm (5, 10, 15 mg kg^{-1})	Male BALB/c mice	Oxidative stress, chromosome aberration, DNA degradation.	Induce significant genotoxicity at the highest concentration.	293
	ZnO	20 or 100 nm; positive or negative charge	C57BL/6 mice	Inhibition of NK cell activity and serum levels of pro/anti-inflammatory cytokines and T helper-1 cytokines.	Lead to immunotoxicity *in vivo*; immunosuppression.	42
		<40 nm	Male Wistar albino rats	Oxidative/inflammatory pathway.	Induce obvious immunotoxicity in the thymus and spleen.	48
	CdO	9.82 nm	Female ICR mice	The percentage of CD3e + CD8a + cells in the thymus increased, and the production of spleen cells, inflammatory cytokines and chemokines increased.	Stimulation of immune/inflammatory response, oxidative stress in the intestine.	294
Noble metal	Ag	20 nm, 100 nm	Wistar rats	Affect multiple immune parameters.	Adverse effects on the immune system.	295
Scarce metal	TiO_2	21 nm	Sprague-Dawley rats	Change the expression levels of IFN-γ, IL-4, T-bet and GATA-3; Th1/Th2 cytokine imbalance.	Increase accumulation of pulmonary macrophages, lung injury.	296
		5–6 nm	Female ICR mice	Alteration of inflammatory and apoptotic cytokines expression.	Lymphocyte subsets and immune capacity decreased, spleen damage.	40
				Significantly increase the levels of various inflammatory factors and chemokines, while decreasing NKG2D, NKp46 and 2B4.	Significantly increase the spleen and thymus indices, spleen damage.	297
				Activation of NF-κB-mediated MAPKs pathway.	Exert toxic effects on lymphoid organs and T cells and innate immune cell homeostasis.	76
		10, 50, 100 nm	Female C57BL/6J mice	Alteration of T lymphocyte proliferation and phenotype.	Cause low-grade intestinal inflammation and aggravate immunological response to external stimulus.	298

Table 4 *(Contd.)*

Classification of metal materials	NP types	NP properties	Models	Mechanisms	Results	Ref.
		17, 117 nm	C57BL/6JRj mice	Glutathione depletion, increased IL-8 and IL-1β, DNA damage and cytotoxicity.	Large agglomerates of 117 nm TiO_2 induced higher pulmonary responses and blood DNA damage compared to small agglomerates.	286
		22.75 ± 7.04 nm	Female Kunming mice	Th1/Th2 imbalance.	Induced ileal physical barrier dysfunction (dose-dependent).	299
Light metal	AlO	Aspect ratios (6.2 ± 0.6, 2.1 ± 0.4)	6-Week-old male ICR mice	Alter the levels of redox response-related elements.	May influence immune functions in an exposed host.	64
	Al_2O_3	13, 50 nm	3-Month-old male ICR mice	The levels of SOD and GSH decreased, the malondialdehyde increased.	Immune organs damage and immune cells dysfunction, leading to abnormal immune-related cytokine expression.	43
		20 nm	Male Sprague-Dawley rats	DNA damage.	Induce genetic toxicity of bone marrow.	300

as the levels of the IL-10, IL-1β, TNF-α, and INF-γ pro-inflammatory factors. Notably, they were also found to enhance the importance macrophage activation marker CD11b.[48] In addition, studies showed that TiO_2 NPs can also induce pro-inflammatory mediators such as MIP-1α/β, IL-6, IL-8, and Gro-α, which activate macrophages, DCs, NK cells, and lymphocytes to promote inflammation.[49]

3.1.2 Signalling pathways for the regulation of inflammation. As described earlier, inflammatory factors can induce a range of cellular responses. Studies have shown that gold and silver NPs have anti-cell proliferation effects on leukaemia cell lines, including T lymphocytes and monocytes. They can affect different signalling pathway responses, inhibiting or stimulating cytokine production[50] (Fig. 2). For instance, Ag NPs down-regulated the TNF-α levels in Jurkat cells, and this effect was mediated by the ERK but not the JNK pathway. However, Au NPs reduced the levels of IL-2 in Jurkat cells and IL-6 in U937 cells and induced TNF-α production through the JNK pathway in U937 cells.[50] The anti-proliferative effect of Ag NPs (<100 nm) was observed in IL-2-dependent T lymphoblastic cells. The mechanism involves the overexpression of CD25 without any significant alteration in the levels or phosphorylation of three essential signalling proteins activated by IL-2 receptors (ERK1/2, Stat5, and JNK). However, the exact mechanism of action still warrants further research.[51] JAK and STAT are critical components of the signalling pathways that regulate cell proliferation, differentiation, survival, and pathogen resistance.[52] These pathways consist of three parts, *i.e.*, signal-receiving tyrosine kinase-related receptors, the signal-transmitting tyrosine kinase JAK, and the effector transcription factor STAT. This is the primary signal transduction mechanism for various cytokines and growth factors.[53] Xu L. *et al.* reported that an Ag NP hydrogel could up-regulate inflammatory genes such as *IL* genes. Although these inflammatory factors are involved in the immune response, they may also stimulate the JAK/STAT signalling pathway.[54] A study showed that Ni NPs induced the production of pro-inflammatory cytokines. In this study, the increased levels of IL-6 and CXCL1 and the activation of STAT3 in male mice increased their susceptibility to acute neutrophil inflammation, demonstrating sex-related differences in the lung inflammatory response to Ni NPs in mice.[55] It is worth noting that PTPN6 is a negative regulator of the JAK/STAT pathway. Exposure to Al_2O_3 NPs led to the phosphorylation of STAT3 and inhibition of PTPN6, eventually leading to the increased expression of the apoptosis marker PDCD4.[56] Consistent with these findings, Zeng F. *et al.* reported that cerium oxide NPs (CENPPEG) may down-regulate ROS and numerous pro-inflammatory cytokines by inhibiting NF-κB and the JAK2/STAT3 signalling pathways, thereby countering the pro-inflammatory microenvironment and inhibiting the pro-inflammatory actions of macrophages and the Th1/Th17 response,[57] which highlights their potential anti-inflammatory effect.

3.1.3 Activation of inflammasomes. Inflammasomes can recruit and activate the pro-inflammatory protease caspase-1, which cleaves the precursors of IL-1β and IL-18, thereby promoting the production of corresponding cytokines. Hence, this protein is closely related to the inflammatory response.[58] Tao X. *et al.* reported that exposure to 50 nm CuO NPs caused lysosomal damage and led to the release of CTSB in J774A.1 macrophages. Further, it promoted an IL-1β-mediated inflammatory response through the MyD88-dependent TLR4 and NF-κB signalling pathways.[59] In addition, the released Cu^{2+} ions could further activate the NLRP3 inflammasome and cause oxidative stress[59] (Fig. 2). Another study showed that Ag NPs induced ATF-6 sensor degradation and endoplasmic reticulum (ER) stress and activated the NLRP-3 inflammasome.[60] Murphy *et al.* also confirmed that Ag NPs can induce the release of pro-inflammatory factors such as IL-1, IL-6, and IL-1β in THP-1 cells and primary blood monocytes, suggesting their potential pro-inflammatory effects.[61] In addition, gold NPs can activate innate immune signalling pathways in a size-dependent manner. Ag NPs with a size of less than 10 nm promoted NLRP3 inflammasome and caspase-1 activation in

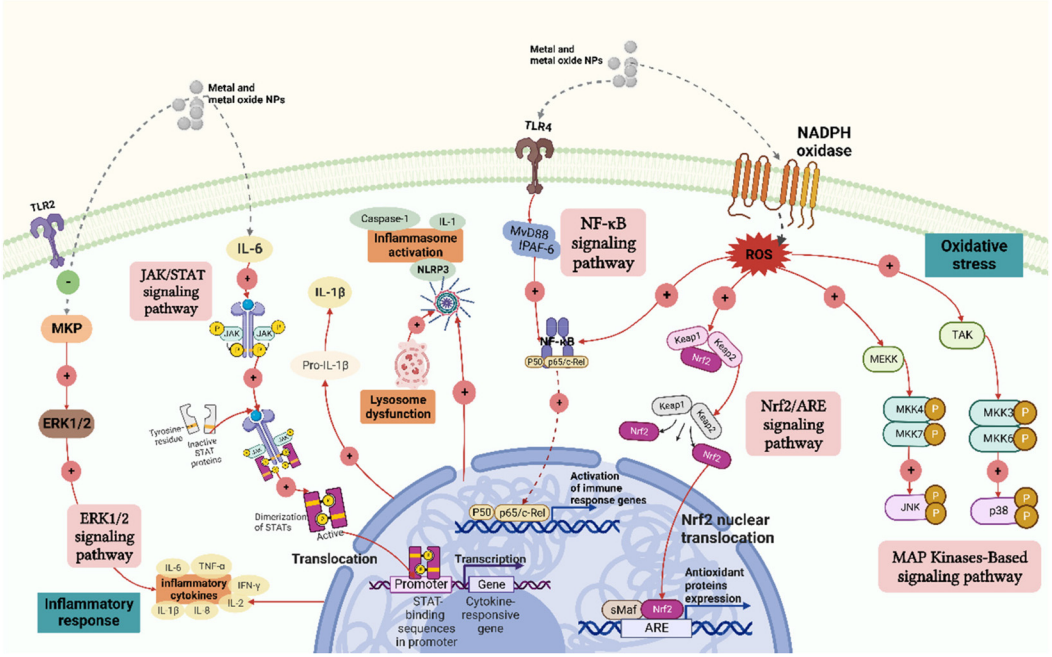

Fig. 2 Mechanisms of metal and metal oxide NP-induced inflammatory response and oxidative stress in immunotoxicity. Metal and metal oxide NPs can cause the activation of inflammasomes and the release of inflammatory factors such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α and induce inflammation and oxidative stress through a variety of different signal transduction molecular mechanisms, including the ERK1/2, JAK/STAT, NF-κB, Nrf2/ARE and MAPK signalling pathways. These effects are closely related to the immunotoxic mechanism of metal and metal oxide NPs. Created with BioRender.com.

mouse bone marrow-derived dendritic cells (BMDCs), resulting in the increased secretion of IL-1β. Meanwhile, Ag NPs greater than 10 nm in size activated the NF-κB signalling pathway.[62] *In vivo* experiments also confirmed the activation of the inflammasome by TiO_2 NPs. For example, Kim B. *et al.* investigated the effect of TiO_2 NPs on inflammasomes in a mouse model of allergic asthma. The results showed that these NPs activated caspase-1 in the lungs of OVA-sensitized/challenged mice, resulting in the increased secretion of IL-1β, IL-18, NLRP3, and caspase-1.[63] Hence, targeting inflammasomes may help in controlling the airway inflammation and hyperresponsiveness induced by TiO_2 NPs.[63] Similarly, Park E.-J. *et al.* compared the *in vivo* distribution and toxicity of two rod-shaped (long and short) alumina NPs (AlO NPs) in mice. They found that exposure to both types of AlO NPs increased the secretion of IL-1β, IL-8, and MCP-1 in the blood, and long NPs (5 mg kg^{-1}) increased the proportion of neutrophils and monocytes.[64] Tantalum (Ta) is emerging as a promising biomaterial for bone tissue engineering. Examination of the cytotoxicity of Ta NPs showed that they induce negligible ROS production in macrophages and pro-inflammatory cytokine alterations (TNF-α and IL-1-β), indicating that Ta NPs are inert, non-toxic, and non-inflammatory.[65]

Overall, the current evidence shows that metal and metal oxide NPs can alter anti-inflammatory and pro-inflammatory pathways *in vivo*, affect different signalling pathways in the immune system, and trigger inflammatory responses (Fig. 2). However, due to the differences in the types of immune cells and the physical and chemical properties of NPs as well as their concentration, dose, route of administration, and timing of use, comparisons across studies are complex, and thus more scientific and systematic analyses are required.

3.2 Oxidative stress

Oxidative stress can induce the production of a large number of oxidative intermediates, resulting in an oxidation–antioxidation imbalance *in vivo*, which eventually leads to an inflammatory response[66] (Fig. 2). Metal and metal oxide NPs can produce ROS through different mechanisms, and excessive ROS can cause cellular oxidative stress responses such as lipid peroxidation, DNA damage, and abnormal signal transduction. A previous study reported that ROS induced by metal and metal oxide NPs activate the Fenton or Haber–Weiss reaction, thereby aggravating oxidative stress damage,[67] even in immune cells (Fig. 5). Hence, regardless of their subcellular source, the excessive ROS induced by metal and metal oxide NPs have toxic effects on healthy cells. Oxidative stress is one of the primary mechanisms through which these NPs cause toxicity in immune cells.

3.2.1 Mitogen-activated protein kinase (MAPK) signalling pathway. MAPKs include three significant subsets, *i.e.*, p38, JNK, and ERK.[68] ROS activates MAPKs, and ROS-induced toxicity can be reduced by inhibiting p38 MAPK, thereby affecting cellular oxidative stress, gene transcription, and immune response processes[69,70] (Fig. 2). For example, Ag NPs can up-regulate and activate NADPH oxidase 2 (NOX2) and increase

ROS production through the p38 and ERK pathways.[71] In addition, one study showed that PEI-coated IONPs can activate TLR4-mediated signal transduction and ROS production in mouse and human macrophage cell lines through multiple pathways (p38, ERK1/2, and JNK MAPK).[72] They induced M1 polarisation, which manifested as an increased expression of IL-12, CD40, CD80, and CD86 and the activation of macrophages.[72] However, this study did not screen for endotoxin, a common contaminant that activates immune cells through TLR4-dependent signal transduction pathways. Notably, in another study by Venofer, Ferinject, and Ferrlecit, the differentiation of monocytes into M1 macrophages and BMDCs was inhibited upon treatment with IONPs.[73] This indicates that in the earlier study, the coating material likely drove IONP-induced M1 polarisation.

It is well-known that inflammation and oxidative stress can interact, with inflammation increasing the production of ROS and ROS aggravating inflammation. Senapati V. A. *et al.* exposed human THP-1 cells to 30 nm ZnO NPs to investigate the immunotoxic potential of the NPs.[74] The NPs induced oxidative and nitrosative stress in a dose-dependent manner, down-regulated the antioxidant glutathione (GSH), and increased the TNF-α and IL-1β levels by activating the NF-κB and MAPK signalling pathways to promote inflammation.[74] Current evidence suggests that TiO_2 NPs can induce oxidative stress through p38. A study exploring the *in vitro* immunotoxicity of TiO_2 NPs (20 nm, negatively charged) against RAW 264.7 mouse macrophages and the underlying molecular mechanisms showed that these NPs can induce immune cell apoptosis and toll-like receptor (TLR)-mediated signal transduction through the oxidative stress-sensitive SAPK/JNK and p38 MAPK pathways, resulting in a decrease in immune cells.[75] In addition, a reduction in lymphocyte subpopulations such as CD3+, CD4+, CD8+, and NK cells was observed in female ICR mice treated with TiO_2 NPs (continuous intragastric administration for 9 months), indicating the toxic effects of these NPs on mouse lymphoid organs, T cells, and innate immune cells. The findings indicated that these NPs may activate the NF-κB-mediated MAPK signalling pathways, causing immunotoxicity.[76]

3.2.2 Nrf2/ARE signalling pathway. The Nrf2/ARE signalling pathway is an intrinsic protective cellular signalling pathway. The downstream molecules expressed in this pathway have various cytoprotective effects, such as preventing oxidative stress, regulating inflammatory damage, antagonising apoptosis, and alleviating calcium overload.[77,78] Studies have shown that Au, Ag, and TiO_2 NPs can increase ROS and malondialdehyde levels, thereby activating Nrf2 and its downstream cascade[79,80] (Fig. 2). Fundamental regulatory mechanisms of the antioxidant response suggest that the ROS induced by ZnO NPs can promote an increase in Nrf2 in a dose- and time-dependent manner.[81] The absence of HO-1 inhibited the protective effects of Nrf2 in ZnO NP-treated endothelial cells, suggesting that ZnO NPs may induce endothelial injury *via* the Nrf2-HO-1 axis.[81,82] Liu J. *et al.* also evaluated the immunotoxicity of sub-10 nm monoclinic Gd_2O_3:Eu^{3+} NPs in BALB/c mice. They observed an increase in the expression of ROS, CD11b, and CD206 after treatment with these NPs, suggesting an increase in the ROS levels in peripheral blood neutrophils and the number of peripheral blood monocytes.[83] This study also reported that pristine NPs did not cause any apparent cytotoxicity *in vitro*. Nevertheless, the *in vivo* immunotoxicity remained significantly higher than that of Gd-DTPA, indicating that the negative surface charge and particle aggregation were the main contributors to their immunotoxicity.[83] However, multiple studies have shown that long-term and high-dose exposure to metals and metal oxide NPs reduces the levels of Nrf2 and HO-1 in the body.[84–87] Therefore, the Nrf2 pathway may not fully ameliorate the oxidative stress induced by metal and metal oxide NPs.

The above-mentioned studies revealed that oxidative stress is indispensable in the immunotoxic effects induced by metal and metal oxide NPs, which cause cell dysfunction through oxidative damage and signalling pathways such as the p38, PI3K/Akt, and NF-κB pathways[88] (Fig. 2). However, some rare metal and metal oxide NPs have been reported to scavenge intracellular ROS.[89] For example, cerium oxide NPs reduced oxidative stress in PC12 cells by 50%, providing a substantial anti-ROS effect.[90] In addition, a study by Zheng C. *et al.* examining the *in vivo* immunotoxicity of Gd_2O_3:Eu^{3+} NPs showed that the NPs produced almost no immunotoxicity in BALB/c mice.[91] They found that ROS can act as a secondary messenger in signal transduction and inhibit the expression of phosphoinositide 3-kinase (PI3K) in the liver. This immunosuppression caused by PI3K inhibition helped the mice to adapt to stress, and thus tolerate Gd_2O_3:Eu^{3+} NP-induced immunotoxicity both *in vitro* and *in vivo*.[91]

3.3 Autophagy and apoptosis

Autophagy is the process in which cells engulf their own cytoplasmic proteins or organelles, inserting them into vesicles, which later fuse with lysosomes to form autophagic lysosomes. The components encapsulated within autophagic lysosomes are degraded to meet the metabolic needs of the cell and renew organelles.[92] Apoptosis refers to the independent and orderly death of cells and is controlled by genes. Apoptosis serves to maintain the stability of the internal environment of the human body. In contrast to necrosis, apoptosis is not a passive process but an active one and is closely related to cell proliferation and senescence.[93] Evidence showed that the inhibition or activation of autophagy and apoptosis by metal and metal oxide NPs also plays an essential role in their toxic effects. MAPK, death protein kinase, PI3K, AKT, mTOR, and AMP kinase are known to be the main components inducing or inhibiting autophagy in response to metal/quasi-metal NPs.[94] Autophagy is associated with many cellular functions, including immunity, inflammation, and apoptosis. For example, Johnson B. *et al.* revealed that ZnO NPs are immunotoxic to primary and immortalised immune cells. *In vivo* spleen cell death was observed in mice after intranasal exposure to ZnO NPs.[95] Therefore, autophagy and apoptosis can be activated or inhibited when NPs enter immune cells,

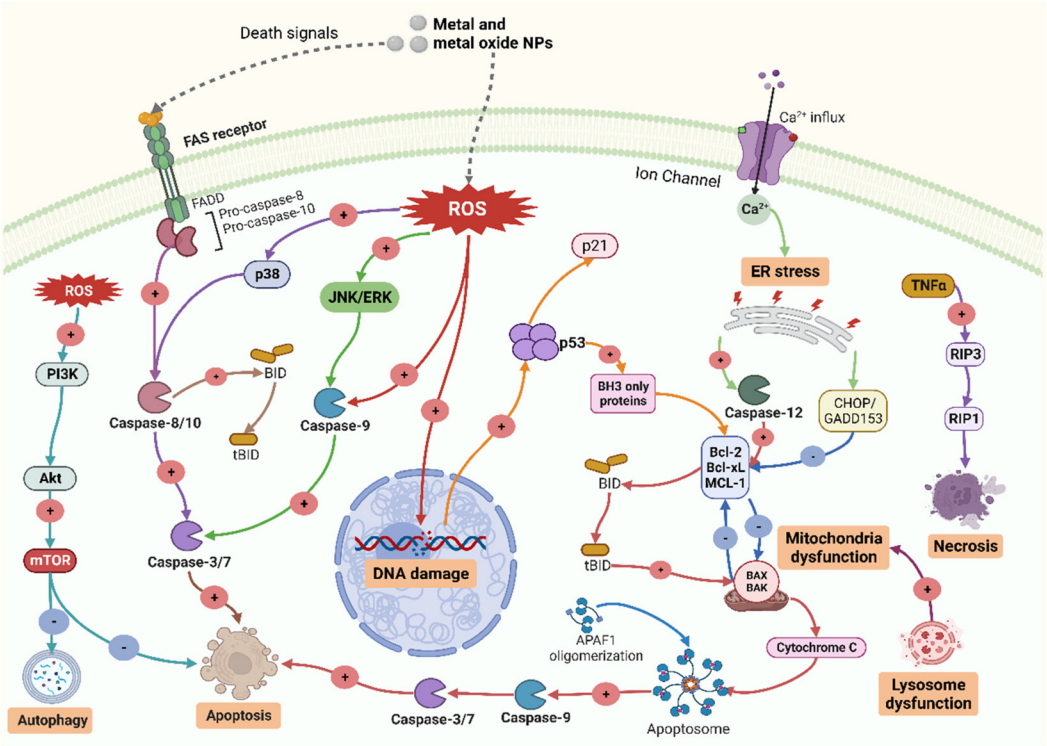

Fig. 3 Mechanisms of metal and metal oxide NP-induced apoptosis and autophagy in immunotoxicity. Apoptosis is caspase-dependent cell death involving three main pathways including death receptor pathway, mitochondrial pathway and endoplasmic reticulum stress pathway. The PI3K-Akt-mTOR pathway may have a negative regulatory effect on autophagy and apoptosis, while RIP3, which responds to the TNF cytokine family, binds to the kinase RIP1 and plays an important role in the necroptosis pathway. Created with BioRender.com.

producing adverse effects in cells or organisms through a range of signalling pathways (Fig. 3).

3.3.1 mTOR signalling pathway. In mammals, the mammalian target of the rapamycin (mTOR) pathway, including mTORC1 and mTORC2, is the primary signalling pathway for autophagy.[96] Song *et al.* reported that PI3K/AKT (upstream of mTOR-mediated autophagy) and MAPK are closely associated with ZnO NP-induced autophagy.[97] In addition, ZnO NPs released Zn^{2+} ions under the acidic conditions in human THP-1 cell lysosomes, resulting in the loss of lysosome integrity and stability. However, TiO_2 NPs did not produce these effects.[98] Chen *et al.*[99] studied the effect of TiO_2 nanotubes loaded with silver NPs (Ag@TiO_2-NTs) on macrophage polarisation. They found that Ag@TiO_2-NTs could promote the differentiation of M2 RAW 264.7 macrophages and exert anti-inflammatory effects by inhibiting the PI3K/Akt pathway and activating autophagy. In another study, Au NPs of different sizes entered cells and accumulated within acidic lysosomes, which led to lysosomal alkalization.[100,101] The autophagy substrate p26 was degraded, indicating that the accumulation of autophagosomes was due to the blockade of autophagy flux rather than the induction of autophagy[100,101] (Fig. 5).

3.3.2 Other autophagy-related pathways. Previous studies reported that different types of IONPs can induce autophagy in immune cells such as macrophages, DCs, and lymphocytes, both *in vitro* and *in vivo*. For example, the dextran-coated SPIONs Feraheme (Ferumoxytol) and Reservist (Ferucarbotran) induced autophagy in RAW 264.7 cells by activating TLR4-p38-Nrf2-p62 signalling, and also induced inflammation (manifesting as a significant increase in the pro-inflammatory cytokines IL-1β, IL-2, IL-12p40/70, TNF-α, and IL-10, as well as MCP-1 and SDF-1α).[102] In addition, lactosylated *N*-alkyl polyethyleneimine-coated SPIONs induced the conversion of LC3-I to LC3-II in RAW 264.7 macrophages, thereby promoting protective autophagy.[103] A similar phenomenon was also observed in DCs in BALB/c mice.[104] Meanwhile, the autophagy induced by these NPs could promote DC maturation, thereby enhancing therapeutic immune activation. Further, 3-methyladenine reduced autophagy flux and induced apoptosis.[104] ZnO NPs up-regulated ROS and LC3A (essential component of autophagic vacuoles) in immune cells, resulting in autophagic death. This effect was mediated by the release of free Zn^{2+} from ZnO NPs.[95] Similarly, ZnO NP-induced autophagy could promote the transfer of NPs to lysosomes and promote NP degradation and continuous Zn^{2+} release under acidic conditions.[105] These Zn^{2+} ions destroyed lysosomes, leading to impaired autophagy flux and mitochondrial damage, thus resulting in excessive ROS production and cell death.[105] Lin Y.-R. *et al.*[106] reported that exposure to 5–10 nm dextran-coated SPIONs led to an increase in LC3-II and autophagosome formation in human peripheral blood monocytes. They also noted that regulating the autophagy induced by these NPs could modulate the sub-

sequent inflammatory responses. If autophagy is inhibited, cell survival may be reduced and inflammatory responses may be enhanced.[106]

3.3.3 Caspase signalling pathway-mediated apoptosis. There are three main pathways of apoptosis *in vivo*, *i.e.*, the death receptor pathway, mitochondrial pathway, and ER stress pathway. All these pathways are mediated by serine protease caspases. In addition, these three pathways are also directly linked and interact with each other[107] (Fig. 3). Caspases are a group of structurally related cysteine proteases found in the cytoplasm. One of their significant commonalities is the specific cleavage of peptide bonds after aspartic acid residues.[108] Hence, cell lysis occurs due to the effect of caspases. Caspase-9 is the initiator of apoptosis and it can activate caspase-3, thereby initiating a caspase–enzyme cascade and inducing apoptosis. Several studies revealed that exposure to ZnO NPs can lead to the activation of caspase-9, caspase-7, and caspase-3, thereby inducing apoptosis.[109,110] TiO_2 NPs can also induce apoptosis by causing nuclear pyknosis, activating caspase-3, increasing Bax (pro-apoptosis), and inhibiting Bcl-2 (anti-apoptosis).[111,112] Notably, larger NPs usually induce more robust apoptosis. In addition, TiO_2 NPs could alter the morphology and function of neutrophils in a time- and concentration-dependent manner (20, 500, and 100 mg mL^{-1}), indicating their potential to activate these cells.[49] They induced the rapid phosphorylation of p38 MAPK and Erk-1/2, thereby participating in apoptosis.[49] *In vitro* experiments showed that exposure to CuO NPs for 24 h led to excessive ROS production in BRL-3A cells, resulting in decreased mitochondrial membrane potential and cell death *via* enhanced apoptosis.[113] Furthermore, oxidative stress could also trigger the ER stress pathway *in vitro* and *in vivo*, resulting in the activation of the CHOP, JNK, and caspase-12 apoptotic pathways[113] (Fig. 5).

Overall, the above-mentioned studies showed that autophagy may be a protective mechanism against the cytotoxicity of metal and metal oxide NPs in immune cells.[106,114] However, autophagy may also trigger apoptosis or cell death, and autophagy may also be activated due to the organelle dysfunction caused by these NPs in immune cells, leading to immune system dysfunction and immunotoxicity.

3.4 Organelle damage and dysfunction

Another effect of metal and metal oxide NPs on immune cells is organelle (*e.g.*, mitochondria, ER, and lysosomes) damage or dysfunction. After exposure to NPs, the morphology of organelles is altered due to direct NP accumulation or indirect subcellular interactions. In addition, NP-induced adaptive changes in subcellular morphology modify cell behaviour and organelle-related functions[2] (Fig. 5).

3.4.1 Lysosome damage. Lysosomes are endpoints of the endocytosis pathway and act as digestive organelles for both intracellular and exogenous substances. They are essential for maintaining cellular homeostasis. The accumulation of NPs in lysosomes significantly affects cell digestion and leads to lysosomal dysfunction[115] (Fig. 5). For example, lysosomal alkalisation and decreased lysosomal membrane stability were detected in THP-1 cells treated with Ag NPs and were found to affect the differentiation of THP-1 cells.[116] In addition, it was reported that transforming the geometry of titanite TiO_2 nanomaterials into a fibrous structure larger than 15 μm produced highly toxic particles and triggered inflammatory responses in alveolar macrophages in C57BL/6 mice.[117] Notably, these macrophages could not chelate TiO_2 nanofibers into lysosomes, resulting in the instability and destruction of lysosomes and the release of cathepsin B, which activated the NALP3 inflammasome and led to the release of inflammatory factors.[117]

3.4.2 Mitochondrial damage and metabolic changes. Mitochondria are prominent metabolism-related cellular organelles. In addition to providing energy to cells, they also play a role in processes such as cell differentiation, information transmission, and apoptosis. Further, they can regulate cell growth and the cell cycle and determine cell function and fate. Shah A. *et al.* studied the immunotoxic mechanisms of Feraheme® and found that it induced mitochondrial stress in cultured primary human T cells. It changed the structure, membrane potential, and dynamics of the mitochondria, decreasing the cytokine levels and proliferation in T cells.[118] Thus, Feraheme® can inhibit the immune function of T cells. Compared with other iron-containing pharmaceutical preparations, Feraheme® has unique immunotoxicity mechanisms with regard to its detrimental effects on mitochondrial and T cell function.[118] In addition, it was reported that PEG-Fe_3O_4 NPs could impair mitochondrial dynamics by activating the PGC-1α pathway and inducing a loss of mitochondrial stability in DCs.[119] PEG-Fe_3O_4 NPs also reduced autophagy to inhibit mitochondrial degradation and promote mitochondrial rupture, altering the immature state of DC function[119] (Fig. 5). Few studies explored the effects of metal and metal oxide NPs on immunometabolism. There is evidence that Au and Ag NPs can alter the function of immune cells by modulating metabolic pathways.[120] It is known that when APCs are stimulated by lipopolysaccharides (LPSs), they tend to differentiate into the pro-inflammatory M1 phenotype, wherein glycolysis is the primary mode of metabolism. However, under IL-4 stimulation, they transform into the anti-inflammatory M2 phenotype, which is largely dependent on mitochondrial metabolism. The exposure of primary macrophages and DCs to different concentrations of Au NPs moderately affected the metabolism of BMDCs in mice. Meanwhile, the mitochondrial and non-mitochondrial respiratory capacity in BMDMs significantly increased. Furthermore, Au NPs increased the glycolysis-dependent energy requirements in BMDCs and BMDMs, depending on the dose and stimulation state.[121] This evidence indicates that NMNs can affect metabolic pathways in immune cells and cause them to differentiate into different cellular phenotypes, thus affecting cell function and fate, which may be related to immunotoxicity.

3.4.3 Endoplasmic reticulum stress. The ER is the largest organelle in the cell, which is mainly responsible for protein synthesis and lipid metabolism. It can also regulate the response of cells to stress and various signalling pathways.

Metal and metal oxide NPs may cause protein misfolding, and then these misfolded proteins accumulate in the ER, resulting in ER stress[122] (Fig. 5), which is associated with NP toxicity. For example, PEGylated nanogels containing gold NPs accumulated in the cytoplasm and up-regulated ER stress-related proteins.[123] In one study, THP-1 cells were treated with non-toxic doses (25 μg mL^{-1}) of Ag NPs (15 nm). After 24 h, the degradation of ER stress sensors and the activation of ATF6, an indicator of ER stress, were observed. The NLRP-3 inflammasome was also activated.[60] Numerous studies indicated that metal and metal oxide NPs affect the metabolism, function, and fate of immune cells through mitochondrial damage and ER stress. For instance, magnetic iron oxide NPs (M-Fe NPs) impaired mitochondrial function in RAW 264.7 cells and induced ER stress, thereby causing pre-apoptotic autophagy.[124] The overexpression of superoxide dismutase 2 (SOD2), but not cytoplasmic SOD, was detected in primordial macrophages exposed to M-FeNPs (50 μg mL^{-1}); notably, the increase was associated with an increase in ROS. After 24 h of exposure, chromatin condensation and mitochondrial swelling increased, without any increase in mitochondrial calcium levels and apoptosis.[124] In addition, after 28 days of the systemic inhalation of TiO_2 NPs (19.3 ± 5.4 nm) in A/J Jms Slc mice (male and female), ER stress and mitochondrial abnormalities were observed in the lung, and LC3, p62, and Beclin1 protein levels were altered, indicating that the NPs may cause abnormal dose-dependent autophagy.[99]

3.4.4 Golgi fragmentation and exosome formation. The Golgi apparatus is the final processing and packaging organelle for proteins. The ER and Golgi apparatus are structurally and functionally continuous. Therefore, ER stress induced by metal and metal oxide NPs can also affect the Golgi *via* regular protein transport (Fig. 5). For example, Ag@ZnO NPs were reported to cause oxidative stress, leading to Golgi fragmentation.[125] Ma X. *et al.* first discovered that Au NPs impaired normal Golgi function without affecting cell viability by inducing size-dependent cytoplasmic calcium elevations and Golgi fragmentation.[126] Previous studies revealed that NPs can promote the production of exosomes (Fig. 5), which are small, single-membrane secretory organelles about 30 to 200 nm in diameter. Importantly, exosomes are rich in selected proteins, lipids, nucleic acids, and glycoconjugates. The release of their contents can activate various signal transduction pathways, including immune responses, thereby affecting various aspects of health.[127] For example, after respiratory exposure to MIONs (43 nm), a large number of exosomes was observed in the alveolar region in BALB/c mice. These exosomes activated splenic T lymphocytes and induced DC maturation.[128]

The above-mentioned results indicate that after exposure to metal and metal oxide NPs, immune cells can experience a range of organelle impairments through various mechanisms and signalling pathways. These may involve changes in immunometabolism, cause metabolic reprogramming, and even lead to cell death due to immunotoxicity. These processes are central to the biomedical functions and toxic reactions of NPs *in vivo*. However, the specific molecular mechanism is still unclear and needs further elucidation.

3.5 Changes in genetic material

Another mechanism of the immunotoxicity induced by metal and metal oxide NPs is the destruction of genetic information. This can occur *via* alterations in the sequence or structure of DNA and epigenetic modifications[129–131] (Fig. 5). Studies have shown that the effects of NPs on genes are related to their characteristics and experimental conditions, such as composition, size, shape, surface characteristics, timing, cell type, and treatment options.[132] Moreover, metal and metal oxide NPs can also induce epigenetic toxicity in immune cells.[131,133,134]

3.5.1 DNA damage. The genotoxicity of metal and metal oxide NPs has been widely reported, including in immune cells. For example, the genotoxicity of Ag NPs (10–100 nm) in leukocytes, Jurkat cells, and CloneE6-1 and THP1 cells was size-dependent, with smaller NPs inducing more genotoxic responses and DNA damage and micronucleus formation detected.[135] In one study, a micronucleus test was used to evaluate the genotoxicity of Ag NPs and Ag^+ in human splenocytes and TK6 cells.[136] The results showed that both entities caused genotoxicity through oxidative stress. However, it was mainly the intact NPs that contributed to the genotoxicity of Ag NPs.[136] Notably, although Au NPs of different sizes (5, 20, and 50 nm) caused DNA strand breaks, no significant difference in the frequency of chromosomal aberrations was observed between cells with and without exposure to NPs,[137] suggesting the repair of DNA damage. The surface coating of iron oxide NPs is likely to play a decisive role in their genotoxicity. For example, some researchers studied the potentially toxic effects of pristine Fe_3O_4 NPs and oleate-coated Fe_3O_4 NPs and found that the latter have dose-dependent cytotoxicity and cause DNA damage in TK-6 cells, with genotoxic potential.[138] In addition, Ghosh S. *et al.* synthesised two types of PLGA-PEG-COOH-encapsulated SPIONs using TPGS and DMAB as surfactants.[139] SPION (10 nm), SPION-DMAB (25 nm), and SPION-TPGS (180 nm) could all induce genotoxicity and ROS production in cells. However, the coating reduced the induced genotoxicity, and SPION-DMAB had the least toxicity among the three NPs.[139] Interestingly, both PAA-coated and uncoated iron oxide NPs showed no obvious genotoxicity in human T cells.[140] Therefore, coatings can change the uptake and response of cells to NPs and induce pathomorphological changes in cells. Surface modification may significantly affect the oxidative stress and DNA damage induced by iron oxide NPs.

The genotoxicity of heavy metal and metal oxide NPs has been widely reported both *in vitro* and *in vivo*. For example, ZnO NPs can cause significant genotoxicity and DNA damage in human monocytes and peripheral blood lymphocytes.[74] In one study, comet assays showed a significant increase in micronuclei and DNA damage in THP-1 cells exposed to ZnO NPs (20 μg mL^{-1}).[74] Similarly, studies on the genotoxicity of ZnO NPs of different sizes (4.175, 9.058, and 19.8 nm) in human peripheral blood lymphocytes showed that ZnO NPs can cause genotoxicity at low doses (≥12.5 ppm) and induce

lymphocyte death at higher concentrations (500 ppm and above). Notably, Jiang H. *et al.* explored the possible underlying mechanism for the effect of Co NPs on human T lymphocytes by measuring the levels of SOD, catalase, and glutathione peroxidase.[141] They found that Co NPs induced primary DNA damage in a concentration-dependent manner and led to a higher degree of DNA damage than Co ions.[141] DNA damage and chromosomal aberrations were also observed in human lymphocytes following exposure to Co_3O_4 NPs at concentrations of 100 μg mL^{-1}, and the effects were mediated by changes in antioxidant levels.[142] Similarly, the genotoxicity of TiO_2 NPs is mainly mediated by the generation of oxidative stress.[143] Kazimirova A. *et al.* tested the genotoxicity of TiO_2 NPs *in vitro* and *in vivo* using a comet assay and micronucleus test.[144] Increased DNA strand breaks were observed in the peripheral blood mononuclear cells (PBMCs) of female Wistar rats 1 day after exposure to TiO_2 NPs (approximately 21 nm in size) induced DNA breaks in human PMBCs in a time- and dose-dependent manner without causing DNA oxidation (75 μg cm^{-2} after 4 h of exposure, 75 μg cm^{-2} after 24 h of exposure; 15 and 75 μg cm^{-2}).[144] It was also reported that alumina NPs with a concentration of up to 0.5 mM produced genotoxic effects in human peripheral blood lymphocytes by inducing oxidative DNA damage and strand breaks, which led to a concentration-dependent increase in DNA single-strand breaks but had no impact on alkali-unstable sites.[145]

In summary, the interaction of metal and metal oxide NPs with the immune system can cause DNA damage and genotoxicity. The specific mechanisms and degree of severity may be closely related to the oxidative stress caused by NPs as well as their concentration and physicochemical properties. When the genetic changes induced by NPs exceed the repair capacity of cells, apoptosis or necrosis may occur,[146] causing toxic effects on the immune system (Fig. 4).

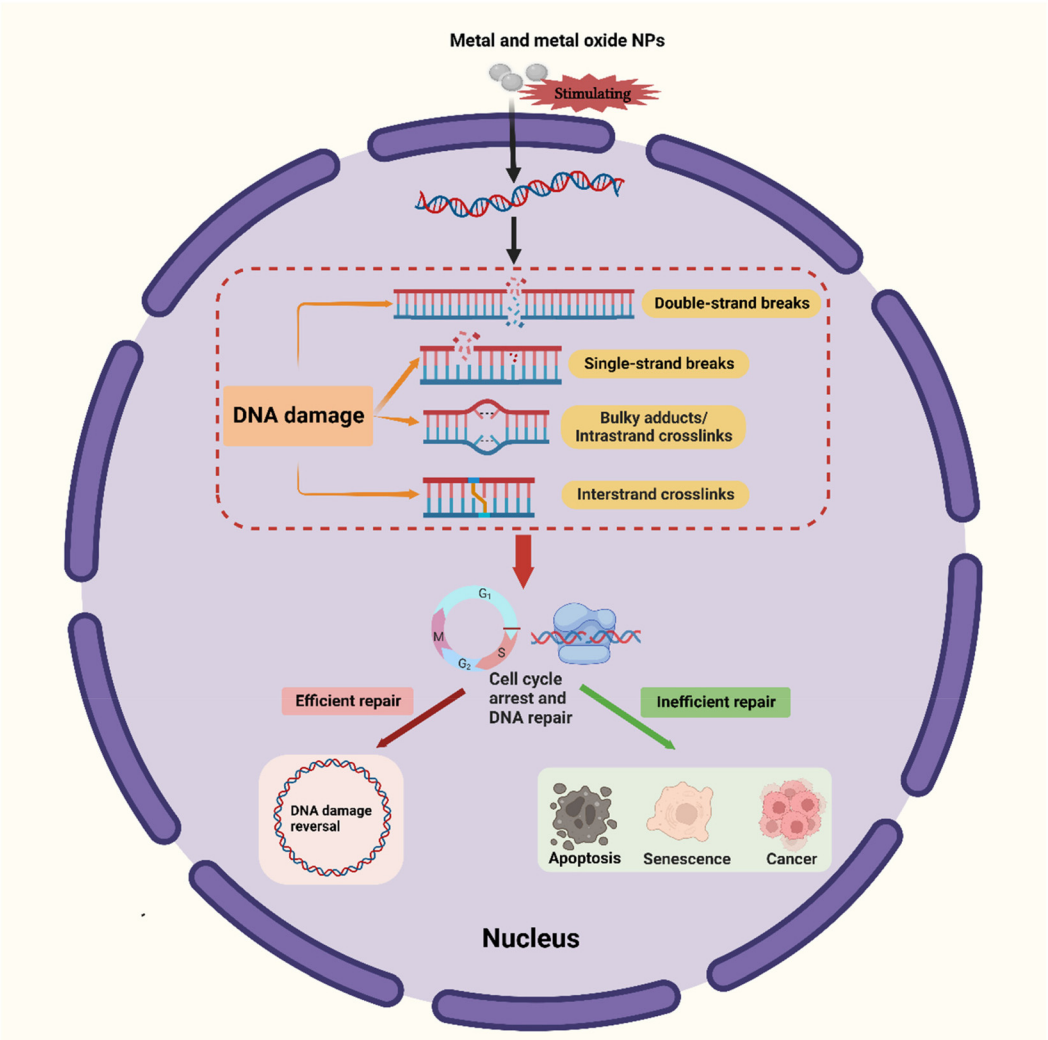

Fig. 4 Mechanisms of DNA damage induced by metal and metal oxide NPs. Metal and metal oxide NPs may cause different types of DNA damage, including DNA double/single strand breaks, DNA adducts and DNA cross-linking. DNA damage can lead to cell cycle arrest and DNA repair, while inefficient DNA repair can lead to apoptosis, senescence and cancer. Created with BioRender.com.

3.5.2 Epigenetic toxicity. Epigenetic modification leads to genomic changes without any alterations in the DNA sequence (*e.g.*, DNA methylation, histone modification, and regulation by non-coding RNAs such as miRNAs).[147] miRNA changes were reported in Jurkat cells after 24 h of treatment with 0.2 mg L^{-1} Ag NPs (<100 nm) and Ag^{+}, and this induction was associated with different epigenetic mechanisms.[148] Ag NPs up-regulated MT1F and TRIB3 (regulated by miR-219-5p), while Ag^{+} up-regulated ENDOGL1 (regulated by miR654-3p).[148] In addition, Ag NPs with a diameter of 25 nm coated with PVP significantly reduced the methylation levels of histone 3 (H3) in mouse erythroleukemia cells. In contrast, no corresponding changes in cells treated with Ag^{+} were observed.[149] This indicated that Ag NPs could modify the methylation status of histones and induce epigenetic toxicity. The increased CpG methylation of Gsr, Cdk, and Atm genes was also detected in the lungs of male BALB/c mice after intratracheal exposure to Au NPs, while the CpG methylation of Gpx, Gsr and Trp53 genes was reduced. Trp53 methylation was associated with the size of NPs.[150] The interactions between the CpG sequence and methyl-CpG binding protein were affected by DNA methylation. If chromatin remodelling occurred, the gene promoter would not be processed during transcription, leading to the alteration of gene expression levels.[151] The epigenetic toxicity of heavy metal and metal oxide NPs in immune cells has been confirmed. For instance, different miRNAs were found to be altered in THP-1 cells 6 and 24 h after exposure to subtoxic doses of ZnO, AgO, and TiO_2 NPs.[152] Furthermore, TiO_2 NPs altered the expression levels of miRNA/isomiR (miR) in THP-1 cells, and these changes were associated with potential health risks.[152] It has been reported that different concentrations of CuO NPs (58.7 nm; 0.5 and 30 μg mL^{-1}) induced changes in the DNA methylation status in LINE-1 and Alu/SINE *in vitro* and *in vivo* (THP-1, RAW 264.7 and BALB/c mice lungs [intratracheal administration, 2.5 mg kg^{-1}]).[153,154]

Thus, metal and metal oxide NPs can induce epigenetic toxicity in immune cells, leading to alterations in chromatin conformation and gene expression levels, thereby exerting toxic effects on the immune system. However, due to the influence of confounding factors such as NP concentration, particle size, surface modification, and study conditions, the relevant mechanisms are not fully understood. Thus, more rigorous and systematic studies are required to explore this further.

3.6 Immunosuppressive response

Immunosuppression refers to the inhibition of an immune response (*e.g.*, anti-inflammatory response). The immunoregulatory mechanisms of metal and metal oxide NPs are complex, and their immunostimulatory or inhibitory effects may be related to their composition, size, surface coating, and other factors. Studies have revealed that metal and metal oxide NPs can cause immunosuppression according to their structure (Fig. 5), consistent with the immunosuppressive effects of NMNs (*e.g.*, Au and Ag) in various immune cells.[155] For

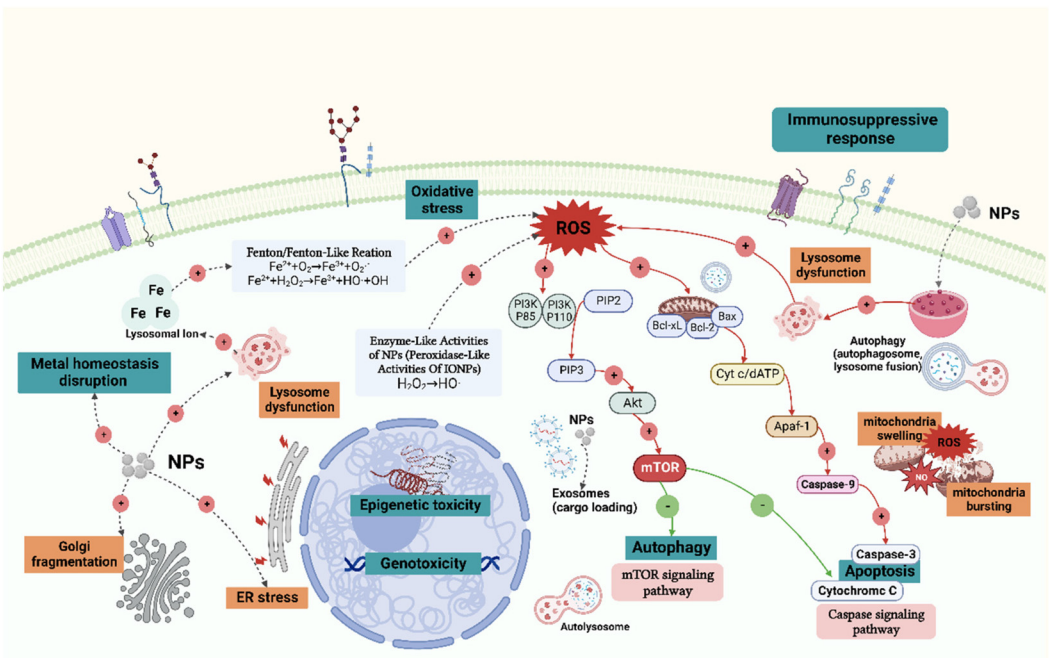

Fig. 5 Various intracellular mechanisms of immunotoxicity mediated by metal and metal oxide NPs. Metal and metal oxide NPs may cause oxidative stress, autophagy and apoptosis, which can lead to dysfunction of different organelles in immune cells, including mitochondrial and lysosomal damage, endoplasmic reticulum stress and Golgi fragmentation, and may also lead to genotoxicity, epigenetic toxicity and disruption of metal homeostasis through direct or indirect effects. In this process, many different signal transduction molecular mechanisms, including mTOR and caspase signalling pathways, are activated or inhibited. These signalling pathways are also cross-linked to varying degrees, which have toxic effects on the immune system and ultimately determine the fate and function of immune cells. Created with BioRender.com.

example, Ag-PVP NPs (10–80 nm) induced size-dependent anti-inflammatory effects in mouse macrophages infected with live *Chlamydia trachomatis*, with smaller NPs producing a more pronounced down-regulation of pro-inflammatory factors such as IL-6 and TNF.[156] Iron oxide NPs also show immunosuppressive effects on immune cells. For example, OVA-specific IgG (1) and IgG (2a) are significantly reduced in BALB/c mice after the intravenous injection of a single dose of iron oxide NPs (10–60 mg Fe per kg) over 7 days.[157] In addition, IONPs attenuated Th1 and Th2 cell-mediated immunity in OVA-sensitized mice, and inhibitory effects on IL-17, IL-6, ROR-γt, and Th17 immune responses were observed in OVA-sensitized mice after exposure to Resovist® (containing iron oxide NPs, 28 mg Fe per mL; single intravenous injection).[158] Hence, systemic exposure to a single dose of iron oxide NPs inhibited antigen-specific antibody production and T cell function, thereby weakening immune responses. Notably, CeO_2 NPs showed a scavenging effect against ROS. These NPs were found to scavenge free radicals and ROS in J774A.1 mouse macrophages and inhibit the production of inflammatory mediators, thereby exerting antioxidant and anti-inflammatory effects *in vitro*.[159]

3.7 Metal homeostasis disruption

The human body naturally contains different metallic elements. Na, K, Mg, Ca, Fe, Mn, Co, Cu, Zn, and Mo are essential elements for life processes. These metals can significantly affect a variety of cellular functions, including immune function.[160,161] Metal and metal oxide NPs can dissolve or degrade into metallic elements or ions after entering the body, destroying the metal balance *in vivo*. Iron metabolism is tightly controlled in the body. Iron regulates macrophage polarisation, neutrophil recruitment, and NK cell activity in innate immunity. In contrast, in adaptive immunity, iron affects the activation and differentiation of Th1, Th2, and Th17 cells as well as CTLs, in addition to antibody responses in B cells.[162] Thus, disturbances to iron metabolism can disrupt metal homeostasis and promote immune responses (Fig. 5). *In vitro* and *in vivo* studies have demonstrated that Zn, Cu, Fe_2O_3, and Ag NPs can disrupt metal homeostasis,[163] with Feraheme® affecting iron homeostasis in human primary T cells.[118] However, whether they can affect intracellular transport and other functions after accumulation in immune cells warrants further investigation. In addition, the dissociation of ZnO NPs can disrupt zinc homeostasis in primary macrophages.[164] Similarly, studies by Cuillel M. *et al.* showed that the disruption of Cu and Zn homeostasis, including intracellular Cu overload and interference with Cu–Zn exchange on metallothionein, occurred in hepatocytes treated with subtoxic doses of CuO NPs.[165] The metallothionein family could activate related transcription factors in the presence of excess metal, thereby regulating metal homeostasis.[166] In addition, the expression of Met-RNA was found to be higher in cells treated with Zn, Ag, and CuO NPs. However, no significant up-regulation of metal homeostasis-related genes was observed in some hepatocytes treated with Zn, Cu, or AgO NPs.[163]

Although these studies have proven that metal and metal oxide NPs can cause specific effects on metal homeostasis *in vivo*, the overall literature remains limited. At present, the specific mechanisms by which free ions released by NPs act on cells are still unclear. Therefore, the destruction of metal homeostasis as a mechanism of immunotoxicity requires validation in future studies.

4. Metabolism and fate of metal and metal oxide NPs *in vivo*

Humans are often exposed to metal and metal oxide NPs through ingestion, inhalation, and skin contact, and the emergence of therapeutic drugs based on these NPs has increased the interest in their fate after administration.[167,168] NPs are stable under colloidal, chemical, and biological conditions.[1] However, their stability can be lost under physiological conditions (*e.g.*, in blood, tissues, and cells) or during storage.[169,170] During this process, NPs may clump or gather (*e.g.*, protein corona) or disintegrate and corrode (*e.g.*, release metal from metal and metal oxide NPs)[171] (Fig. 6). The degradation, dissolution, and erosion of metal and metal oxide NPs can be divided into core erosion, surface erosion, and bulk erosion, and these processes are referred to as biodegradation, biodissolution, and bioerosion, respectively, when they occur in response to biological agents or physiological conditions[1] (Fig. 6). These physiological conditions can represent a simple simulation of the biological environment, such as lysosomal pH, or biological macromolecules such as enzymes. In addition, changes in environmental pH may alter the degradation and dissolution of metal and metal oxide NPs based on their physicochemical properties. Thus, during the interaction of NPs with tissues or cells *in vivo*, cells may encounter the biodegradation products of NPs, which may eventually cause a range of molecular alterations. After digestion in cells or tissues, NP fragments may be recognised as foreign antigens in the host, triggering different immune responses, and eventually leading to different outcome pathways.

4.1 Immune recognition, metabolism, and clearance of NPs

The *in vivo* recognition of metal and metal oxide NPs can also have an important effect on their metabolism and clearance. In the body, most metal and metal oxide NPs are recognised by immune cells as foreign antigens, triggering immune responses. Although some proteins from the biological microenvironment may get adsorbed onto the surface of these particles and cause poor immune cell recognition, most metal NPs cannot escape immune recognition.[172] Biodistribution studies showed that the biodegradation and removal of IONPs within 2 weeks of distribution in the liver and spleen depended mainly on their size and surface coating.[173] Metal ions released *in vivo* from metal and metal oxide NPs (*e.g.*, Ag^+, Au^+, Cd^+, Zn^{2+}, and Fe^{2+}) may be toxic even at low concentrations, participate in different cellular pathways, or induce

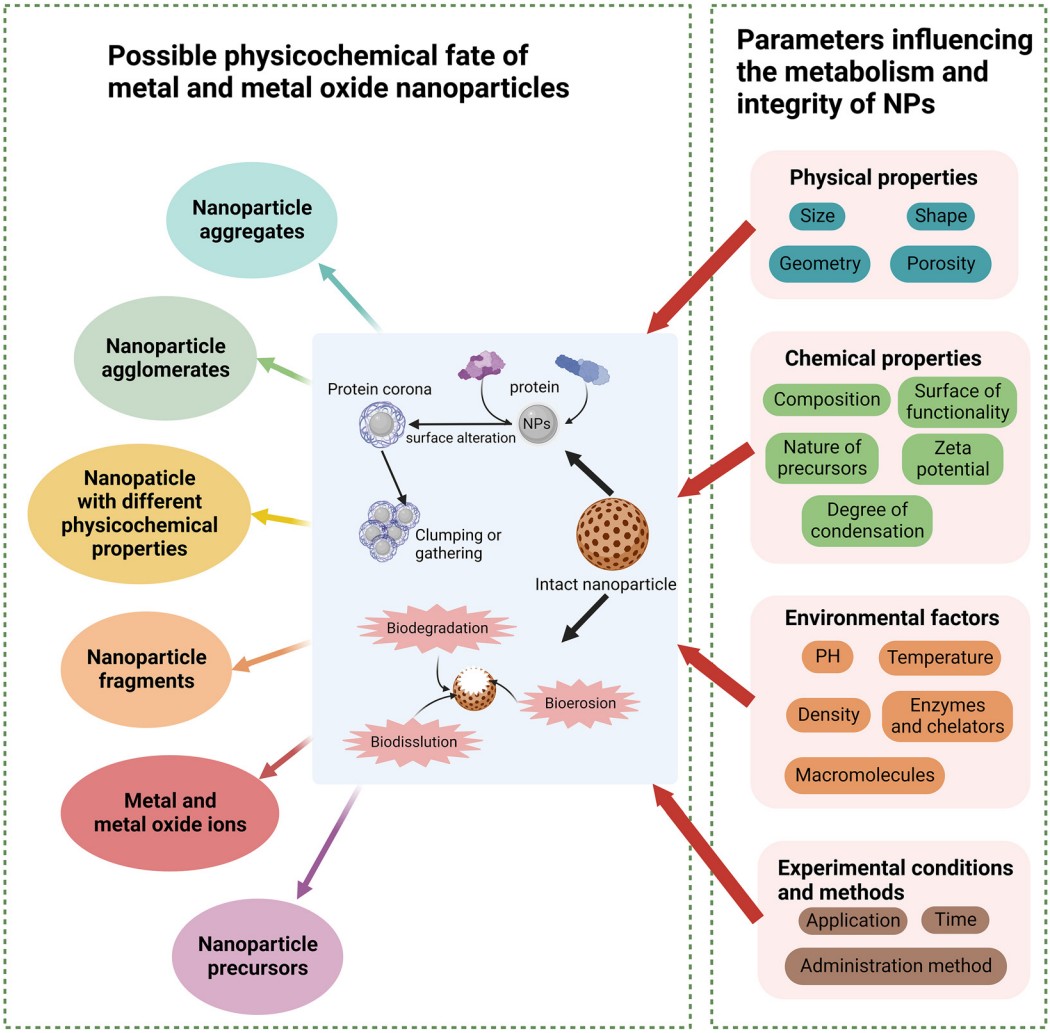

Fig. 6 Possible physicochemical fates of metal and metal oxide NPs and parameters influencing their metabolism and integrity. Metal and metal oxide NPs may lose their stability under physiological conditions in the body, form protein coronas and clump or gather, or undergo degradation, dissolution, and erosion to form precursors, debris, or metal and metal ions. In this process, a variety of physical and chemical properties, environmental factors and experimental conditions and methods will affect their integrity and metabolic outcomes to varying degrees. Created with BioRender.com.

changes in ROS and intracellular metal homeostasis.[170] Some ultra-small metal and metal oxide NPs can be transported across the epithelial barrier, penetrate the bloodstream, and then be swallowed by immune cells. These NPs interact with immune-related tissues and organs during accumulation in the body, resulting in far-reaching effects, which may manifest as the activation or inhibition of immune function.[174,175]

Studies have shown that metal and metal oxide NPs accumulate in tissues and organs to varying degrees after entering the body, and are eventually metabolized into substances that cells use or excreted through the urine and faeces[1] (Fig. 7). For example, the size-dependent distribution of Ag NPs (20, 80, and 110 nm) was analysed after intravenous administration in rats.[176] The 20 nm particles were mainly distributed in the liver, kidney, and spleen. In contrast, the larger particles were primarily distributed in the spleen, followed by the liver and lungs.[176] Renal clearance is the most effective pathway for excreting metal and metal oxide NPs. After entering the blood circulation, NPs can be excreted effectively *via* the kidneys in the urine. In this excretory pathway, the NPs have the least interaction with the body, which minimises their possible toxic effects.[177] However, larger metal and metal oxide NPs cannot be effectively removed *via* the kidneys and may be excreted *via* bile and the gastrointestinal tract[167] (Fig. 7), which is the main route for removing NPs that cannot be directly cleared by the kidneys. In general, NPs or degradation products smaller than 5.5 nm are rapidly cleared primarily through the urinary system, while that larger than 6 nm are often removed by the hepatobiliary system.[178] Therefore, the liver and kidneys show higher levels of NP accumulation than other organs, and the excretion of these NPs may be size-dependent. In addition, NPs can easily enter the human diges-

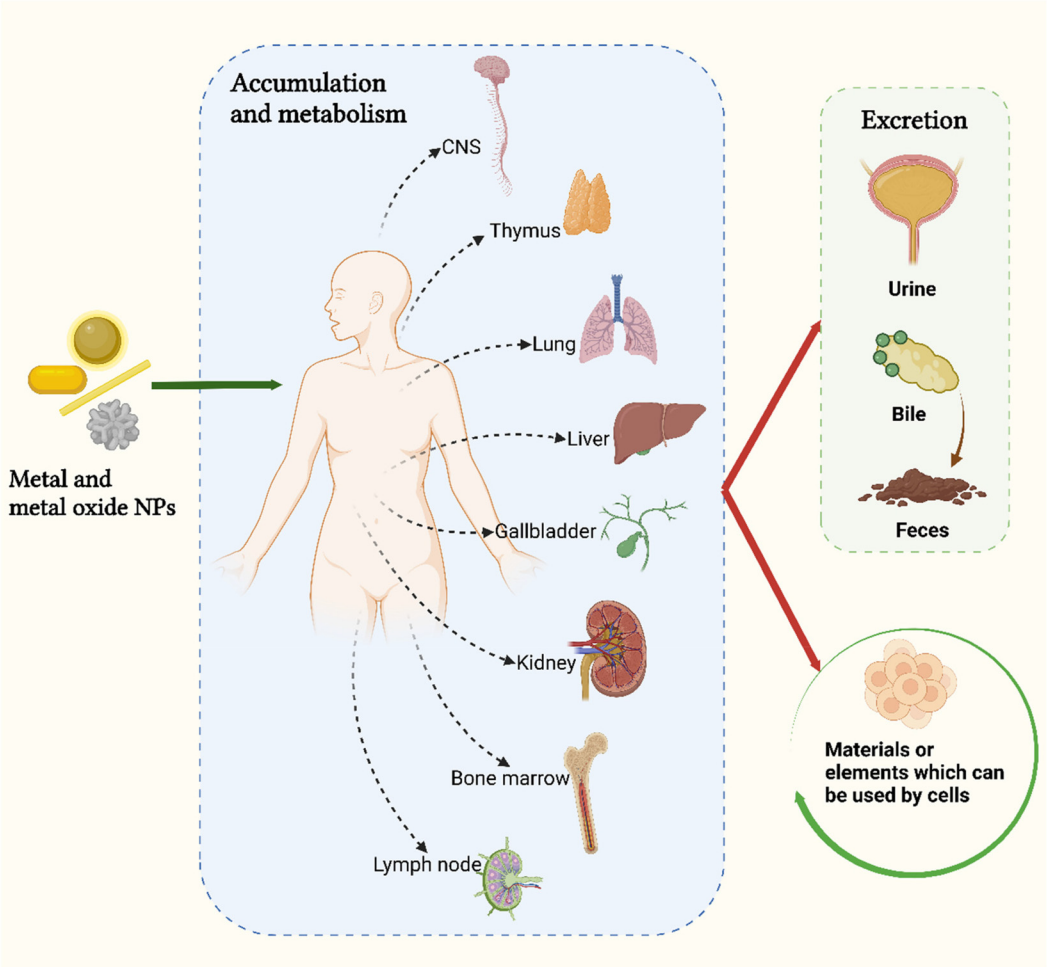

Fig. 7 Accumulation, metabolism and excretion of metal and metal oxide NPs *in vivo*. Metal and metal oxide NPs enter the body through different pathways and accumulate to varying degrees in tissues and organs related to metabolism, immunity, and consciousness such as the lung, spleen, kidney, and central nervous system, and are eventually metabolized into materials or elements that cells can use, or excreted through urine, feces, or liver. Created with BioRender.com.

tive system and accumulate in the gastrointestinal tract (due to its direct contact with the external environment), while smaller-sized NPs are more likely to pass through the gastrointestinal tract.[179]

In summary, metal and metal oxide NPs can be distributed from the exposure sites (*e.g.*, blood and intestines) to secondary organs (liver and kidney), and ultimately undergo different outcomes. The clearance period of these NPs is significant given that internalised metal and metal oxide NPs can persist in the body for a long duration, being trapped in the kidneys, liver, and reticuloendothelial system and having a significant impact on these metabolism- and immunity-related tissues and organs.

4.2 The effects of physicochemical properties and experimental methods on the immunotoxicity of NPs

Metal and metal oxide NPs are transported through the circulatory system and reach different organs and tissues after entering the body *via* different routes.[180] Their transport mainly depends on their physical and chemical properties (size, shape, charge, surface coating, stability, crystallinity, and agglomeration state). These factors affect the function and activity of NPs, including their transfer from epithelial cells to organs, intracellular localization, action on receptors, and ROS-enhancing effects.[181] For example, biodistribution and toxicity studies of gold nanoclusters (Au NCs) with different charges (5.9 mg kg^{-1}; administered for 1, 7, 30, 60, and 90 days) showed that negative Au NCs were more likely to accumulate in the liver and spleen in male C57 mice, while positive Au NCs could damage the peripheral blood system,[182] suggesting that surface charge is a decisive factor affecting the location of NP accumulation *in vivo*.

In general, the blood, liver, spleen, and kidneys are the primary hosts for NPs. After intravenous injection, AuNPs of different sizes (10, 50, 100, and 250 nm) showed size-dependent toxicity and accumulation in rats. The larger particles were detected only in the blood, liver, and spleen, while the smallest NPs could accumulate in all organs, including the brain.[183] Based on the above evidence, we speculate that the

dispersion of NPs in the body is negatively correlated with their size, that is, the smaller the size of NPs, the more extensive their distribution and accumulation *in vivo*. It is worth noting that surface coating may be an effective strategy for altering the stability and toxicity of NPs *in vivo*.[184] However, a study compared the adverse effects of PAA or citrate-coated gold nanospheres and PAA or PEG-coated gold nanorods on human dermal fibroblasts (HDFs). The results showed that gold nanorods altered gene expression, where in this group, IL-6 expression was 12-fold higher than that in control cells,[185] suggesting that the surface chemistry of PEG is not as insignificant as commonly believed and may enhance the immunotoxicity of NPs.

The toxic effects induced by metal and metal oxide NPs in different animal models and cell lines are usually different, and NP concentrations, exposure duration, exposure modes, and temperatures also affect their toxicity. In general, the toxicity of NPs increases with an increase in their concentration and exposure duration.[186,187] It is worth noting that the exposure pathway of NPs is directly related to their immunotoxic effects. For example, single or multiple intravenous injections of Ag NPs and $AgNO_3$ with different sizes (25 μg Ag per dose of Ag NPs and 2.5 μg Ag per dose of $AgNO_3$: 1, 4, and 10 days) led to biodistribution in the liver, lungs, and kidneys in female BALB/c mice. In this model, toxicity was caused by endothelial barrier disruption.[188] After the intravenous injection of ZnO NPs, high amounts of ZnO NPs were detected in the blood of rats. However, the oral administration of these NPs (30 mg kg^{-1}) led to obvious gastrointestinal under-adsorption.[189] In addition, after the intraperitoneal injection of NPs of different sizes (micro-TiO_2 and 5, 10, 60, and 90 nm anatase TiO_2) and concentrations (5, 10, 50, 100, 150, and 200 mg kg^{-1}; once a day for 14 days), mice (22 ± 3 g, half male and half female) showed Ti accumulation in the brain, spleen, lungs, and kidneys. Further, the accumulation was concentration-dependent.[190] Notably, the liver was found to be severely damaged owing to mitochondrial destruction and the induction of hepatocyte apoptosis, with smaller NPs being more toxic than micro-NPs.[190]

The above-mentioned results demonstrate that different structural characteristics (surface charge, size, coating, *etc.*) and administration methods (intravenous injection, intraperitoneal injection, oral administration, *etc.*) of metal and metal oxide NPs affect their immunotoxic effects. These nanoparticles cause inflammatory responses and lead to chronic toxicity over time. The size-dependent toxicity and excretion of metal and metal oxide NPs have been clear, that is, smaller NPs have stronger toxic effects on the immune system and metabolic tissues because they are internalised more easily by immune cells and cross biological barriers *in vivo*, thereby expanding the scope of their toxicological effects.

4.3 Effects of metal and metal oxide NPs on metabolism-related tissues and organs

Metal and metal oxide NPs may affect the function and histopathology of metabolically relevant organs that interact with sub-organ cells (Fig. 8). For example, Ag NPs (3–20 nm; 5, 10, 15, and 20 mg kg^{-1} for 21 days) damaged epithelial microvilli and intestinal glands, and the loss of microvilli reduced the absorption capacity of the intestinal epithelium. The body weight of mice decreased significantly in all the Ag NP treatment groups.[191] Intravenous administration of small-sized (10 nm) Ag NPs led to enhanced tissue distribution and significant hepatobiliary toxicity, while surface coatings (citrate and PVP) showed no related effects.[192] Interestingly, a single intravenous injection of Pt NPs sized less than 1 nm had no significant toxic effect on the lungs, spleen, and heart of mice. However, tubular epithelial cell necrosis and urinary casts increased, and the mice showed a dose-dependent increase in blood urea nitrogen (an indicator of renal injury).[193]

The liver is the main detoxification organ in the human body, and hepatic storage can reduce the systemic toxicity of NPs to some extent. These NPs tend to be digested or metabolized in the liver, and then neutralised and stored in the body to reduce toxicity.[2] Therefore, the accumulation of metal and metal oxide NPs in metabolic organs can also be considered a protective mechanism. The degradation of NPs mainly depends on the phagocytic activity of Kupffer cells in the liver. One day after injection, Au NPs were found in almost all Kupffer cells. Transmission electron microscopy showed that they accumulated in the vesicular lysosomal/endosomal structures of macrophages.[194] Similar results were obtained by Dragoni S. *et al.* in their study assessing the uptake and cytotoxicity of PVP-coated 5 nm Au NPs.[195] The results indicated that although the Au NPs were rapidly distributed in the liver, they were not assimilated in hepatocytes but rather digested and accumulated in the lysosomes of macrophages through enzymatic digestion, which reduced their systemic toxicity. Furthermore, although Au NPs were rapidly internalised in the liver, they induced a reduction in lactate dehydrogenase release and MTT and glutathione levels in rat hepatocytes, with no apparent cytotoxicity. This confirmed that Au NPs have a certain degree of biocompatibility with rat hepatocytes.[195] Therefore, morphological and functional alterations in metabolism-related tissues and organs can increase the body's tolerance to metal and metal oxide NPs (Table 5).

5. Discussion

Numerous studies have confirmed that the metabolic pathways associated with immune effects and the energy required to produce these effects can regulate the activation of immune responses.[196,197] Usually, resting leukocytes show basal activity in all major metabolic pathways. Upon activation, they undergo metabolic reprogramming, which alters the structure and function of their mitochondria and energy consumption patterns, leading to the early use of specific metabolic pathways and metabolite fluxes.[196–198] There is substantial evidence showing that immune cell polarisation is associated with metabolism, and regulating metabolism is considered an

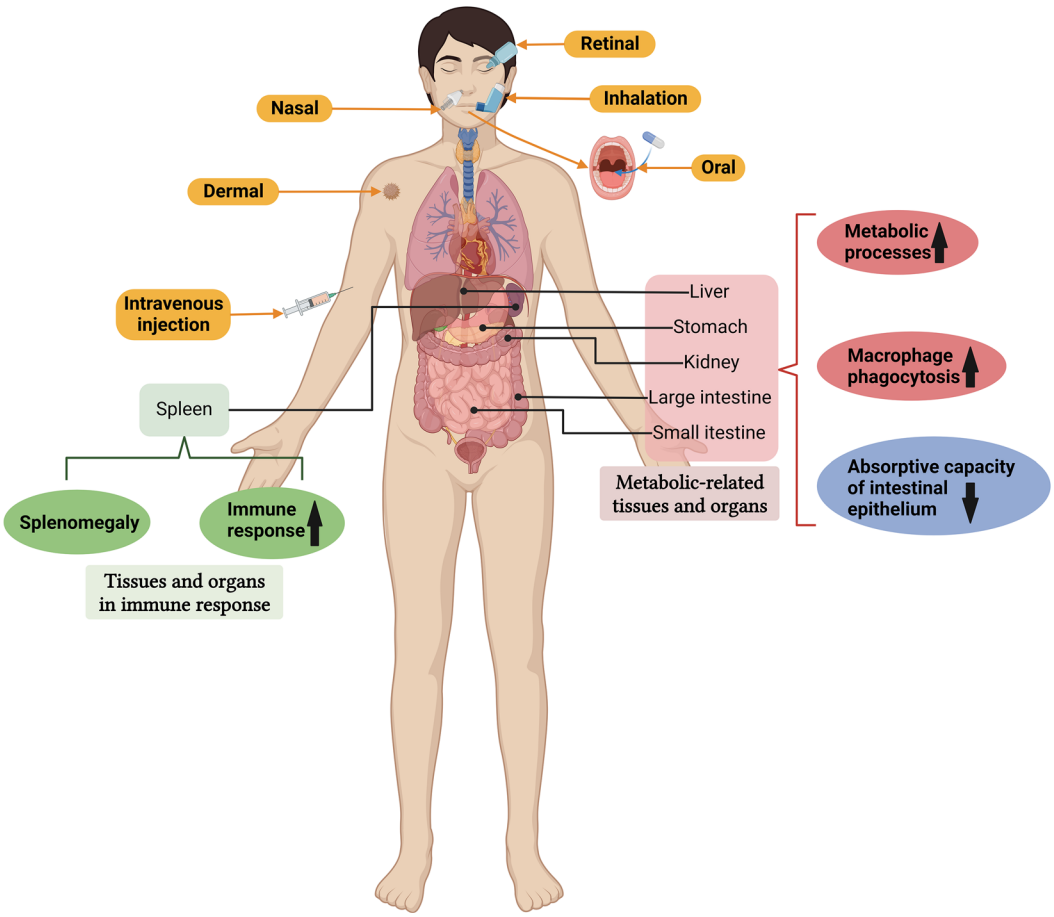

Fig. 8 Common exposure pathways for the administration of metal and metal oxide NPs and their physiological and pathological effects on metabolic and immune-related tissues and organs. Metal and metal oxide NPs can enter the body through many different ways, such as oral, inhalation, skin contact or intravenous injection, and may have significant physiological and pathological effects on metabolism and immune-related tissues and organs. Created with BioRender.com.

effective means to guide immune cells to a pathway that promotes infection clearance, *i.e.*, metabolic reprogramming[198] (Fig. 9 and Table 1). The mTOR pathway has been confirmed to be associated with metabolism.[199] It is worth noting that with the emergence of critical metabolic nodes, various methods that rely on drugs, cytokines, lipid messengers, and microRNAs appear to be effective metabolic regulators.[200] Therefore, understanding the regulatory mechanism of metabolic pathways on immune function is conducive to the development of NPs that can target immune metabolism to reshape the function of immune cells and provide a new direction for the treatment of anti-tumor function of metabolically activated immune cells.

Iron metabolism is closely linked to the metabolic characteristics of different types of macrophages during differentiation and their differentiation outcomes.[201] In addition, targeting iron metabolism can reprogram tumour-associated macrophages (TAMs) into M1-like macrophages, thus playing an important role in anticancer therapy.[202] Therefore, in recent years, iron oxide NPs have been increasingly used to induce the metabolic reprogramming of macrophages owing to their good biocompatibility and ability to regulate macrophage activation.[203] For example, spherical Au/Fe_3O_4 NPs could regulate the pro-inflammatory state of RAW 264.7 cells, which manifested as a significant increase in the level of pro-inflammatory factors.[204] In addition, the use of IONPs as cancer therapy has shown great potential in modulating macrophages, given that they promote M1 polarisation (pro-inflammatory), thereby inhibiting tumour growth. IONPs can also serve as carriers for other immunotherapy agents and ameliorate inflammatory responses.[202]

Other types of metal nanoparticles may also have adverse effects on the body by regulating immunometabolism. Ag NPs are widely used due to their unique antibacterial properties. However, exposure to Ag NPs can also cause adverse effects, including inflammation, accumulation, and cell damage in various organs. It is worth noting that the study by Tiwari R. *et al.* showed that perinatal exposure to Ag NPs may reprogram immunometabolism and promote pancreatic β-cell death and renal damage in mice.[205] This study also found that exposure

Table 5 The effects of metal and metal oxide NPs on immune and metabolic-related tissues and organs

Tissues/ organs	NP types	Models	Physiological and pathological effects	Ref.
Lung	Au	Adult male CD-1 mice	The size and the shape greatly influence the kinetics of accumulation and excretion. Only star-like GNPs can accumulate in the lung.	301
	Ag	Sprague-Dawley rats	Yellow discolouration of the lung, which is not dose-dependent. No haematological and histopathological change.	302
			Lung inflammation at day 1, disappearing by day 21.	303
			Increased alveolar inflammation and small granulomatous lesions.	304
		Female Wistar rats	Low dose deposition in lungs of adult healthy rats to avoid nasopharyngeal deposition.	305
	TiO_2	C57BL/6JRj mice	Large aggregates induce higher lung response.	286
Liver	Au	BALB/c mice	Significant genetic changes, but histological analysis showed no pathological changes, and the two sizes of NPs exhibited similar biological effects.	306
		Female C57BL mice	No obvious pathological changes.	194
		Male Wistar albino rats	Lactate dehydrogenase release and glucuronidase induction, proinflammatory effects.	195
	Ag	Male Sprague-Dawley rats	Hepatic cytoplasmic vacuolation, no significant changes in hematology and blood biochemistry.	307
		F344 rats	Significant dose-dependent changes in alkaline phosphatase and cholesterol, mild liver damage.	308
	Cu	Male Sprague-Dawley rats	Induce liver damage and profibrotic changes.	292
	TiO_2	SD rats	No significant adverse toxicological effects.	309
		C57BL/6JRj mice	Blood DNA damage.	286
Gallbladder	Ag	F344 rats	High incidence of bile duct hyperplasia with or without necrosis, fibrosis and/or pigmentation.	308
Spleen	Au	Adult female Swiss albino mice	Distorted lymphoid structure, reduced lymphoid follicles, diffuse white pulp.	310
		Male Wistar rats	Significant effects on detoxification, lipid metabolism, cell cycle, defense response, and circadian rhythm-related genes.	311
	Ag	Wistar rats	Immune cells and antibody levels in the spleen increase dramatically, spleen weight increased.	295
			Increased spleen weight, number of splenocytes and splenic cell subsets.	312
	Cu	Male Sprague-Dawley rats	The number of macrophages in the red pulp area increased, splenic trabecular artery muscle cell degeneration, inflammatory cell infiltration; change spleen lymphocyte subsets.	291
	ZnO	Male Wistar albino rats	Degenerative changes in the spleen, decreased number of cells expressing anti-PCNA positive reaction, increased number of cells expressing anti-p53 positive reaction.	48
Thymus	ZnO	Male Wistar albino rats	Thymic degeneration.	48
	TiO_2	Female ICR mice	Thymus weight increased, lymphocyte subsets decreased; cortical starry appearance in the thymus due to macrophages, hemorrhage, severe hemolysis or congestion, steatosis and apoptosis or necrosis.	76
Stomach	Ag	Human gastric epithelial cells	Form a complex with *Helicobacter pylori* to weaken *Helicobacter pylori* infection.	313
Intestine	Ag	Female Swiss albino mice	Reduced microvilli, intestinal epithelial absorption, weight loss.	191
		Sprague-Dawley rats	Diffuse brown pigmentation, female accumulation more than male.	314
	TiO_2	Female Kunming mice	Decreased villus height, increased crypt depth, ileal cell apoptosis.	299
Kidney	Au	NRK cells and female BALB/c mice	Early renal fibrosis.	315
	Ag	Sprague-Dawley rats	No treatment-related histopathological changes; diffuse brown pigmentation, significantly higher accumulation in female rats.	314
			Dose-dependent effects on alkaline phosphatase and cholesterol; higher accumulation in female rats.	316
	Pt	Male BALB/c and C57BL/6 mice	Necrosis of renal tubular epithelia and urinary cast; dose-dependent increase of blood urea nitrogen (renal injury index).	193

to low doses of Ag NPs during pregnancy enhanced immune adaptation and could protect mouse offspring against STZ-induced diabetic nephropathy by altering immunometabolism.[206] In addition, NPs have been shown to improve the activity of NK cells, enhancing their anti-tumour and anti-viral functions, by promoting the metabolic reprogramming of immune cells to effectively modulate their responses to immunotherapies.[207] Therefore, by utilising the unique functional properties of NPs to promote the metabolic reprogramming of cells, the therapeutic efficacy can be enhanced and toxic effects can be attenuated. This provides an exciting therapeutic opportunity. However, the lack of standards for preclinical studies and the varying experimental conditions have created obstacles for further human trials and hindered the development of this field.[202] Currently, several issues need to be addressed before the clinical transformation of NPs for immune metabolic reprogramming, including their physicochemical properties, safety and efficacy, route of administration, timing of administration, pharmacokinetics, and biodistribution. Strategies for the large-scale production of these NPs are also required.[208] Overall, the use of

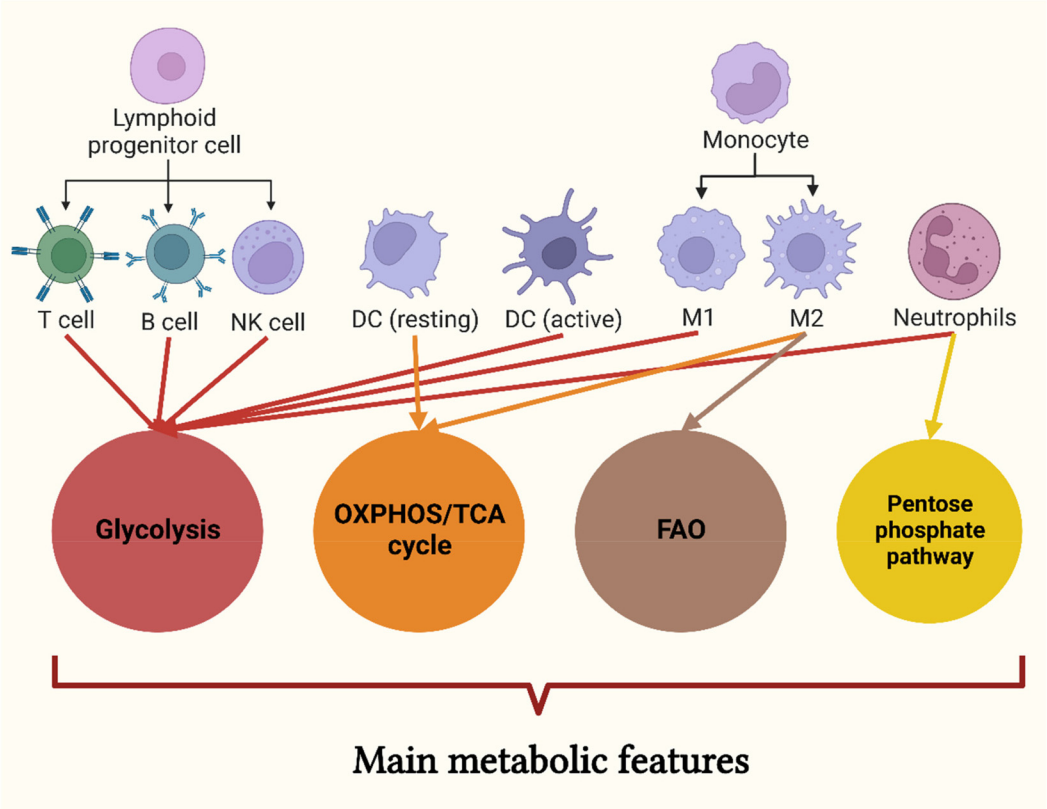

Fig. 9 Main metabolic features of innate and adaptive immune cells. Adaptive immune cells, NK cells, activated dendritic cells, M1 macrophages and neutrophils are mainly metabolized by the glycolysis pathway, in which neutrophils can also experience pentose phosphate metabolic pathway. The main metabolic pathways of M2 macrophages are oxidative phosphorylation and fatty acid β-oxidation. In addition, oxidative phosphorylation is also the main metabolic pathway of quiescent dendritic cells. Created with BioRender.com.

NPs as immunomodulators to regulate immune responses requires more targeted studies.

Although current research has revealed that many key metabolites in the process of immunometabolism can affect the function of immune cells, the research in this field is still in its infancy, and thus more comprehensive exploration may be needed in the future bases on the following two aspects. Firstly, in terms of mechanism exploration, the mechanisms of action of many metabolites on other cells have been reported. Do these metabolites also play a role in immune cells and play different roles in different immune cells? In addition, as the intermediate bridge between metabolic characteristics and immune function, there are still many gaps in the understanding of molecular mechanisms. For example, fatty acid oxidation is a metabolic feature of M2 macrophage polarization, but the specific molecular mechanism of fatty acid as a metabolic substrate for fatty acid oxidation to regulate M2 polarization is not clear. Secondly, how to apply these new mechanisms to treatment is also a matter of concern. For example, the diversity of innate immune cells leads to the possibility that the same metabolic pattern may play different immune regulatory roles in different innate immune cells. Therefore, in the tumor microenvironment where multiple cells coexist, interfering with glycolysis may simultaneously affect the survival of tumor cells and the immunosuppressive function of TAM, and may also affect the anti-tumor function of DC cells and NK cells. Whether this two-way effect will affect the treatment, there is no reasonable assessment. It is worth noting that the regulatory mechanism between the metabolic characteristics of immune cells and immune responses is highly dependent on the environment in which the cells are located.[209] Therefore, the metabolic characteristics and immune response regulation mechanisms should be accurately analysed in a specific environment, which can help promote the precise application of NPs as metabolic regulators of immune cells.

6. Conclusion, limitations, and prospects

We comprehensively reviewed the ability of metal and metal oxide NPs to induce inflammation, oxidative stress, DNA damage, and autophagy. After entering the body through different pathways, these NPs can activate various pathways that work independently or interact with each other to modu-

late the immune system. In this process, the NPs can undergo different degrees of degradation and dissolution and eventually be excreted through the metabolism-related organs of the body. However, as described in this review, there remain several unresolved issues in understanding the physicochemical properties of metal and metal oxide NPs and the effects of their degradation products and administration routes on immunotoxicity, as follows: (I) the physical and chemical stability of NPs can vary after reaching target cells or tissues. However, it is still difficult to fully track the changes in NP characteristics during this process, even though the changes can alter the immune properties of the host. (II) Our understanding of the effects of these alterations and degradation processes on immunotoxic effects is still limited, and better animal or cellular models and more accurate assays are needed to carefully examine these alterations and their effects.[1] (III) The immune response induced by nanoparticles depends on the interaction between nanoparticles and immune cells. Therefore, current research also focuses on the relationship between different types of immune cells and nanoparticles in the immune system. However, due to the lack of research on immunotoxicity, we should also pay attention to the immunological properties of nanomaterials themselves, and it is particularly important to understand their complete immunological properties. Therefore, the formulation and design of metal and metal oxide NPs must be considered during their development. Many of the considerations involved have always been complex problems in this field. Future challenges will include the classification of metal and metal oxide NPs based on the results of toxicological studies. Based on studies *in vitro*, considering the complexity of the immune system *in vivo*, more experimental studies should be carried out *in vivo* to further clarify the immunoregulatory mechanisms of metal and metal oxide NPs, which is also lacking in current research and needs to be studied. In addition, more consideration should be given to using metal and metal oxide NPs as tools for reprogramming the metabolism of immune cells, and more mechanistic studies should be conducted to elucidate the underlying mechanisms to minimize the toxic effects of NPs themselves. This can endow NPs with superior and longer-lasting therapeutic effects.

Author contributions

Conceptualization, J. B. and C. M.; writing – original draft preparation, J. B., C. M., S. L., Y. L. and P. Y.; writing – review and editing, Z. L., B. J. and S. X. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

Authors do not have any conflicts of interest to declare.

Acknowledgements

J.B. and C.M. contributed equally to this work. The work was supported by the Start-up project research of Stomatological Hospital, School of Stomatology, Southern Medical University (Grant no., PY2021018).

References

1 R. Mohammapdour and H. Ghandehari, Mechanisms of immune response to inorganic nanoparticles and their degradation products, *Adv. Drug Delivery Rev.*, 2022, **180**, 114022.
2 Q. Huang, *et al.*, Adaptive changes induced by noble-metal nanostructures in vitro and in vivo, *Theranostics*, 2020, **10**(13), 5649–5670.
3 A. N. Ilinskaya and M. A. Dobrovolskaia, Understanding the immunogenicity and antigenicity of nanomaterials: Past, present and future, *Toxicol. Appl. Pharmacol.*, 2016, **299**, 70–77.
4 P. J. Murray, J. Rathmell and E. Pearce, SnapShot: Immunometabolism, *Cell Metab.*, 2015, **22**(1), 190–190.e1.
5 N. Golbamaki, *et al.*, Genotoxicity of metal oxide nanomaterials: review of recent data and discussion of possible mechanisms, *Nanoscale*, 2015, **7**(6), 2154–2198.
6 P. Makvandi, *et al.*, Gum polysaccharide/nanometal hybrid biocomposites in cancer diagnosis and therapy, *Biotechnol. Adv.*, 2021, **48**, 107711.
7 M. Sethurajan, E. D. van Hullebusch and Y. V. Nancharaiah, Biotechnology in the management and resource recovery from metal bearing solid wastes: Recent advances, *J. Environ. Manage.*, 2018, **211**, 138–153.
8 J. Singh and S. P. Singh, Geopolymerization of solid waste of non-ferrous metallurgy - A review, *J. Environ. Manage.*, 2019, **251**, 109571.
9 Z. Jiang, *et al.*, Heavy metals in soils around non-ferrous smelteries in China: Status, health risks and control measures, *Environ. Pollut.*, 2021, **282**, 117038.
10 Z. Fu and S. Xi, The effects of heavy metals on human metabolism, *Toxicol. Mech. Methods*, 2020, **30**(3), 167–176.
11 S. K. Sahu, *Metallurgy of Light Metals*, Minerals & Mining, 2008.
12 M. Azharuddin, *et al.*, A repertoire of biomedical applications of noble metal nanoparticles, *Chem. Commun.*, 2019, **55**(49), 6964–6996.
13 R. R. Arvizo, *et al.*, Intrinsic therapeutic applications of noble metal nanoparticles: past, present and future, *Chem. Soc. Rev.*, 2012, **41**(7), 2943–2970.
14 Y. V. Nancharaiah, S. V. Mohan and P. N. L. Lens, Biological and Bioelectrochemical Recovery of Critical and Scarce Metals, *Trends Biotechnol.*, 2016, **34**(2), 137–155.
15 R. U. Ayres and L. T. Peiró, Material efficiency: rare and critical metals, *Philos. Trans. R. Soc., A*, 2013, **371**(1986), 20110563.

16 L. T. Peiró, G. V. Méndez and R. U. Ayres, Material flow analysis of scarce metals: sources, functions, end-uses and aspects for future supply, *Environ. Sci. Technol.*, 2013, **47**(6), 2939–2947.
17 S. Bhattacharyya, *et al.*, Inorganic nanoparticles in cancer therapy, *Pharm. Res.*, 2011, **28**(2), 237–259.
18 C. M. Santoro, N. L. Duchsherer and D. W. Grainger, Antimicrobial efficacy and ocular cell toxicity from silver nanoparticles, *Nanobiotechnology*, 2007, **3**(2), 55–65.
19 D. A. Giljohann and C. A. Mirkin, Drivers of biodiagnostic development, *Nature*, 2009, **462**(7272), 461–464.
20 R. Xu, *et al.*, Ag nanoparticles sensitize IR-induced killing of cancer cells, *Cell Res.*, 2009, **19**(8), 1031–1034.
21 Y. F. Li and C. Chen, Fate and toxicity of metallic and metal-containing nanoparticles for biomedical applications, *Small*, 2011, **7**(21), 2965–2980.
22 M. F. Kircher, *et al.*, A brain tumor molecular imaging strategy using a new triple-modality MRI-photoacoustic-Raman nanoparticle, *Nat. Med.*, 2012, **18**(5), 829–834.
23 C. N. Loynachan, *et al.*, Renal clearable catalytic gold nanoclusters for in vivo disease monitoring, *Nat. Nanotechnol.*, 2019, **14**(9), 883–890.
24 S. Sheikpranbabu, *et al.*, Silver nanoparticles inhibit VEGF-and IL-1beta-induced vascular permeability via Src dependent pathway in porcine retinal endothelial cells, *J. Nanobiotechnol.*, 2009, **7**, 8.
25 F. Sang, *et al.*, Recyclable colorimetric sensor of Cr(3+) and Pb(2+) ions simultaneously using a zwitterionic amino acid modified gold nanoparticles, *Spectrochim. Acta, Part A*, 2018, **193**, 109–116.
26 I. H. El-Sayed, X. Huang and M. A. El-Sayed, Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer, *Nano Lett.*, 2005, **5**(5), 829–834.
27 A. T. Haine and T. Niidome, Gold Nanorods as Nanodevices for Bioimaging, Photothermal Therapeutics, and Drug Delivery, *Chem. Pharm. Bull.*, 2017, **65**(7), 625–628.
28 V. S. Marangoni, J. Cancino-Bernardi and V. Zucolotto, Synthesis, Physico-Chemical Properties, and Biomedical Applications of Gold Nanorods–A Review, *J. Biomed. Nanotechnol.*, 2016, **12**(6), 1136–1158.
29 S. Saha, *et al.*, Gold Nanoparticle Reprograms Pancreatic Tumor Microenvironment and Inhibits Tumor Growth, *ACS Nano*, 2016, **10**(12), 10636–10651.
30 R. Baghban, *et al.*, Were magnetic materials useful in cancer therapy?, *Biomed. Pharmacother.*, 2021, **144**, 112321.
31 H. Wei, *et al.*, Superparamagnetic Iron Oxide Nanoparticles: Cytotoxicity, Metabolism, and Cellular Behavior in Biomedicine Applications, *Int. J. Nanomed.*, 2021, **16**, 6097–6113.
32 S. Hemmati, *et al.*, Application of copper nanoparticles containing natural compounds in the treatment of bacterial and fungal diseases, *Appl. Organomet. Chem.*, 2020, **34**(4), DOI: **10.1002/aoc.5465**.
33 X. Ge, Z. Cao and L. Chu, The Antioxidant Effect of the Metal and Metal-Oxide Nanoparticles, *Antioxidants*, 2022, **11**(4), 791.
34 L. A. Dykman and N. G. Khlebtsov, Immunological properties of gold nanoparticles, *Chem. Sci.*, 2017, **8**(3), 1719–1735.
35 V. Galbiati, *et al.*, In vitro assessment of silver nanoparticles immunotoxicity, *Food Chem. Toxicol.*, 2018, **112**, 363–374.
36 N. Ninan, N. Goswami and K. Vasilev, The Impact of Engineered Silver Nanomaterials on the Immune System, *Nanomaterials*, 2020, **10**(5), 967.
37 C. M. Lappas, The immunomodulatory effects of titanium dioxide and silver nanoparticles, *Food Chem. Toxicol.*, 2015, **85**, 78–83.
38 A. Shah and M. A. Dobrovolskaia, Immunological effects of iron oxide nanoparticles and iron-based complex drug formulations: Therapeutic benefits, toxicity, mechanistic insights, and translational considerations, *Nanomedicine*, 2018, **14**(3), 977–990.
39 J. K. Tee, *et al.*, Oxidative stress by inorganic nanoparticles, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2016, **8**(3), 414–438.
40 X. Sang, *et al.*, The chronic spleen injury of mice following long-term exposure to titanium dioxide nanoparticles, *J. Biomed. Mater. Res., Part A*, 2012, **100**(4), 894–902.
41 M. Winter, *et al.*, Activation of the inflammasome by amorphous silica and TiO2 nanoparticles in murine dendritic cells, *Nanotoxicology*, 2011, **5**(3), 326–340.
42 C. S. Kim, *et al.*, Immunotoxicity of zinc oxide nanoparticles with different size and electrostatic charge, *Int. J. Nanomed.*, 2014, **9**(Suppl 2), 195–205.
43 H. Li, *et al.*, Toxicity of alumina nanoparticles in the immune system of mice, *Nanomedicine*, 2020, **15**(9), 927–946.
44 J. Małaczewska, The splenocyte proliferative response and cytokine secretion in mice after oral administration of commercial gold nanocolloid, *Pol. J. Vet. Sci.*, 2015, **18**(1), 181–189.
45 M. Zhu, *et al.*, Nanoparticle-induced exosomes target antigen-presenting cells to initiate Th1-type immune activation, *Small*, 2012, **8**(18), 2841–2848.
46 E. J. Park, *et al.*, Chronic pulmonary accumulation of iron oxide nanoparticles induced Th1-type immune response stimulating the function of antigen-presenting cells, *Environ. Res.*, 2015, **143**(Pt A), 138–147.
47 C. C. Shen, *et al.*, A role of cellular glutathione in the differential effects of iron oxide nanoparticles on antigen-specific T cell cytokine expression, *Int. J. Nanomed.*, 2011, **6**, 2791–2798.
48 M. A. Abass, *et al.*, Effect of orally administered zinc oxide nanoparticles on albino rat thymus and spleen, *IUBMB Life*, 2017, **69**(7), 528–539.
49 D. M. Gonçalves, S. Chiasson and D. Girard, Activation of human neutrophils by titanium dioxide (TiO2) nanoparticles, *Toxicol. in Vitro*, 2010, **24**(3), 1002–1008.

50 C. Parnsamut and S. Brimson, Effects of silver nanoparticles and gold nanoparticles on IL-2, IL-6, and TNF-α production via MAPK pathway in leukemic cell lines, *Genet. Mol. Res.*, 2015, **14**(2), 3650–3668.
51 G. Côté-Maurais and J. Bernier, Silver and fullerene nanoparticles' effect on interleukin-2-dependent proliferation of CD4 (+) T cells, *Toxicol. in Vitro*, 2014, **28**(8), 1474–1481.
52 K. L. Owen, N. K. Brockwell and B. S. Parker, JAK-STAT Signaling: A Double-Edged Sword of Immune Regulation and Cancer Progression, *Cancers*, 2019, **11**(12), 2002.
53 S. Banerjee, *et al.*, JAK-STAT Signaling as a Target for Inflammatory and Autoimmune Diseases: Current and Future Prospects, *Drugs*, 2017, **77**(5), 521–546.
54 L. Xu, *et al.*, Genotoxicity and molecular response of silver nanoparticle (NP)-based hydrogel, *J. Nanobiotechnol.*, 2012, **10**, 16.
55 D. J. You, *et al.*, Sex differences in the acute and subchronic lung inflammatory responses of mice to nickel nanoparticles, *Nanotoxicology*, 2020, **14**(8), 1058–1081.
56 X. Li, *et al.*, Suppression of PTPN6 exacerbates aluminum oxide nanoparticle-induced COPD-like lesions in mice through activation of STAT pathway, *Part. Fibre Toxicol.*, 2017, **14**(1), 53.
57 F. Zeng, *et al.*, A drug-free nanozyme for mitigating oxidative stress and inflammatory bowel disease, *J. Nanobiotechnol.*, 2022, **20**(1), 107.
58 P. Broz and V. M. Dixit, Inflammasomes: mechanism of assembly, regulation and signalling, *Nat. Rev. Immunol.*, 2016, **16**(7), 407–420.
59 X. Tao, *et al.*, A tandem activation of NLRP3 inflammasome induced by copper oxide nanoparticles and dissolved copper ion in J774A.1 macrophage, *J. Hazard. Mater.*, 2021, **411**, 125134.
60 J. C. Simard, *et al.*, Silver nanoparticles induce degradation of the endoplasmic reticulum stress sensor activating transcription factor-6 leading to activation of the NLRP-3 inflammasome, *J. Biol. Chem.*, 2015, **290**(9), 5926–5939.
61 A. Murphy, *et al.*, Silver nanoparticles induce pro-inflammatory gene expression and inflammasome activation in human monocytes, *J. Appl. Toxicol.*, 2016, **36**(10), 1311–1320.
62 M. Zhu, *et al.*, Cell-Penetrating Nanoparticles Activate the Inflammasome to Enhance Antibody Production by Targeting Microtubule-Associated Protein 1-Light Chain 3 for Degradation, *ACS Nano*, 2020, **14**(3), 3703–3717.
63 B. G. Kim, *et al.*, Effect of TiO_2 Nanoparticles on Inflammasome-Mediated Airway Inflammation and Responsiveness, *Allergy, Asthma Immunol. Res.*, 2017, **9**(3), 257–264.
64 E. J. Park, *et al.*, A higher aspect ratio enhanced bioaccumulation and altered immune responses due to intravenously-injected aluminum oxide nanoparticles, *J. Immunotoxicol.*, 2016, **13**(4), 439–448.
65 L. Zhang, *et al.*, Investigation of Cytotoxicity, Oxidative Stress, and Inflammatory Responses of Tantalum Nanoparticles in THP-1-Derived Macrophages, *Mediators Inflammation*, 2020, **2020**, 3824593.
66 J. Lugrin, *et al.*, The role of oxidative stress during inflammatory processes, *Biol. Chem.*, 2014, **395**(2), 203–230.
67 A. Nel, *et al.*, Toxic potential of materials at the nanolevel, *Science*, 2006, **311**(5761), 622–627.
68 G. Huang, L. Z. Shi and H. Chi, Regulation of JNK and p38 MAPK in the immune system: signal integration, propagation and termination, *Cytokine*, 2009, **48**(3), 161–169.
69 Z. Yuan, *et al.*, Koumine Promotes ROS Production to Suppress Hepatocellular Carcinoma Cell Proliferation Via NF-κB and ERK/p38 MAPK Signaling, *Biomolecules*, 2019, **9**(10), 559.
70 K. Ito, *et al.*, Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells, *Nat. Med.*, 2006, **12**(4), 446–451.
71 Y. Yang, *et al.*, Gold nanoparticles synergize with bacterial lipopolysaccharide to enhance class A scavenger receptor dependent particle uptake in neutrophils and augment neutrophil extracellular traps formation, *Ecotoxicol. Environ. Saf.*, 2021, **211**, 111900.
72 V. Mulens-Arias, *et al.*, Polyethylenimine-coated SPIONs trigger macrophage activation through TLR-4 signaling and ROS production and modulate podosome dynamics, *Biomaterials*, 2015, **52**, 494–506.
73 L. H. Fell, *et al.*, Impact of individual intravenous iron preparations on the differentiation of monocytes towards macrophages and dendritic cells, *Nephrol., Dial., Transplant.*, 2016, **31**(11), 1835–1845.
74 V. A. Senapati, *et al.*, ZnO nanoparticles induced inflammatory response and genotoxicity in human blood cells: A mechanistic approach, *Food Chem. Toxicol.*, 2015, **85**, 61–70.
75 M. Dhupal, *et al.*, Immunotoxicity of titanium dioxide nanoparticles via simultaneous induction of apoptosis and multiple toll-like receptors signaling through ROS-dependent SAPK/JNK and p38 MAPK activation, *Int. J. Nanomed.*, 2018, **13**, 6735–6750.
76 F. Hong, *et al.*, Immunotoxic effects of thymus in mice following exposure to nanoparticulate TiO(2), *Environ. Toxicol.*, 2017, **32**(10), 2234–2243.
77 F. He, X. Ru and T. Wen, NRF2, a Transcription Factor for Stress Response and Beyond, *Int. J. Mol. Sci.*, 2020, **21**(13), 4777.
78 I. Buendia, *et al.*, Nrf2-ARE pathway: An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases, *Pharmacol. Ther.*, 2016, **157**, 84–104.
79 L. Böhmert, *et al.*, Molecular mechanism of silver nanoparticles in human intestinal cells, *Nanotoxicology*, 2015, **9**(7), 852–860.
80 A. Goldstein, *et al.*, The bright side of plasmonic gold nanoparticles; activation of Nrf2, the cellular protective pathway, *Nanoscale*, 2016, **8**(22), 11748–11759.
81 L. Zhang, *et al.*, Stabilization of Nrf2 leading to HO-1 activation protects against zinc oxide nanoparticles-induced

endothelial cell death, *Nanotoxicology*, 2021, **15**(6), 779–797.
82 A. Loboda, *et al.*, Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism, *Cell. Mol. Life Sci.*, 2016, **73**(17), 3221–3247.
83 J. Liu, *et al.*, Sub-10 nm monoclinic Gd_2O3:Eu3+ nanoparticles as dual-modal nanoprobes for magnetic resonance and fluorescence imaging, *Langmuir*, 2014, **30**(43), 13005–13013.
84 J. Wang, *et al.*, Nano-titanium nitride causes developmental toxicity in zebrafish through oxidative stress, *Drug Chem. Toxicol.*, 2022, **45**(4), 1660–1669.
85 S. Gui, *et al.*, Renal injury and Nrf2 modulation in mouse kidney following chronic exposure to TiO_2 nanoparticles, *J. Agric. Food Chem.*, 2013, **61**(37), 8959–8968.
86 E. E. Khayal, *et al.*, Combined lead and zinc oxide-nanoparticles induced thyroid toxicity through 8-OHdG oxidative stress-mediated inflammation, apoptosis, and Nrf2 activation in rats, *Environ. Toxicol.*, 2021, **36**(12), 2589–2604.
87 R. Sehsah, *et al.*, Protective role of Nrf2 in zinc oxide nanoparticles-induced lung inflammation in female mice and sexual dimorphism in susceptibility, *Toxicol. Lett.*, 2022, **370**, 24–34.
88 Z. Gholinejad, M. H. Khadem Ansari and Y. Rasmi, Titanium dioxide nanoparticles induce endothelial cell apoptosis via cell membrane oxidative damage and p38, PI3K/Akt, NF-κB signaling pathways modulation, *J. Trace Elem. Med. Biol.*, 2019, **54**, 27–35.
89 M. D. Mauricio, *et al.*, Nanoparticles in Medicine: A Focus on Vascular Oxidative Stress, *Oxid. Med. Cell. Longevity*, 2018, **2018**, 6231482.
90 G. Ciofani, *et al.*, Effects of cerium oxide nanoparticles on PC12 neuronal-like cells: proliferation, differentiation, and dopamine secretion, *Pharm. Res.*, 2013, **30**(8), 2133–2145.
91 C. Zheng, *et al.*, In vivo immunotoxicity of Gd(2) O(3) :Eu (3+) nanoparticles and the associated molecular mechanism, *J. Biochem. Mol. Toxicol.*, 2020, **34**(11), e22562.
92 Z. Xie and D. J. Klionsky, Autophagosome formation: core machinery and adaptations, *Nat. Cell Biol.*, 2007, **9**(10), 1102–1109.
93 A. G. Renehan, C. Booth and C. S. Potten, What is apoptosis, and why is it important?, *Br. Med. J.*, 2001, **322**(7301), 1536–1538.
94 S. Chatterjee, S. Sarkar and S. Bhattacharya, Toxic metals and autophagy, *Chem. Res. Toxicol.*, 2014, **27**(11), 1887–1900.
95 B. M. Johnson, *et al.*, Acute exposure to ZnO nanoparticles induces autophagic immune cell death, *Nanotoxicology*, 2015, **9**(6), 737–748.
96 C. H. Jung, *et al.*, mTOR regulation of autophagy, *FEBS Lett.*, 2010, **584**(7), 1287–1295.
97 W. J. Song, *et al.*, Zinc Oxide Nanoparticles Induce Autophagy and Apoptosis via Oxidative Injury and Pro-Inflammatory Cytokines in Primary Astrocyte Cultures, *Nanomaterials*, 2019, **9**(7), 1043.
98 W. S. Cho, *et al.*, Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn2+ dissolution inside lysosomes, *Part. Fibre Toxicol.*, 2011, **8**, 27.
99 Y. Chen, *et al.*, Improved Immunoregulation of Ultra-Low-Dose Silver Nanoparticle-Loaded TiO(2) Nanotubes via M2 Macrophage Polarization by Regulating GLUT1 and Autophagy, *Int. J. Nanomed.*, 2020, **15**, 2011–2026.
100 X. Ma, *et al.*, Gold nanoparticles induce autophagosome accumulation through size-dependent nanoparticle uptake and lysosome impairment, *ACS Nano*, 2011, **5**(11), 8629–8639.
101 H. Zhou, *et al.*, Gold nanoparticles impair autophagy flux through shape-dependent endocytosis and lysosomal dysfunction, *J. Mater. Chem. B*, 2018, **6**(48), 8127–8136.
102 R. Jin, *et al.*, Iron oxide nanoparticles promote macrophage autophagy and inflammatory response through activation of toll-like Receptor-4 signaling, *Biomaterials*, 2019, **203**, 23–30.
103 J. Du, *et al.*, Reduction of polyethylenimine-coated iron oxide nanoparticles induced autophagy and cytotoxicity by lactosylation, *Regener. Biomater.*, 2016, **3**(4), 223–229.
104 T. Shen, *et al.*, Lactosylated N-Alkyl polyethylenimine coated iron oxide nanoparticles induced autophagy in mouse dendritic cells, *Regener. Biomater.*, 2018, **5**(3), 141–149.
105 J. Zhang, *et al.*, Zinc oxide nanoparticles harness autophagy to induce cell death in lung epithelial cells, *Cell Death Dis.*, 2017, **8**(7), e2954.
106 Y. R. Lin, *et al.*, Remote Magnetic Control of Autophagy in Mouse B-Lymphoma Cells with Iron Oxide Nanoparticles, *Nanomaterials*, 2019, **9**(4), 551.
107 S. Elmore, Apoptosis: a review of programmed cell death, *Toxicol. Pathol.*, 2007, **35**(4), 495–516.
108 S. M. Man and T. D. Kanneganti, Converging roles of caspases in inflammasome activation, cell death and innate immunity, *Nat. Rev. Immunol.*, 2016, **16**(1), 7–21.
109 L. Wang, *et al.*, Zinc oxide nanoparticles induce human tenon fibroblast apoptosis through reactive oxygen species and caspase signaling pathway, *Arch. Biochem. Biophys.*, 2020, **683**, 108324.
110 H. Attia, H. Nounou and M. Shalaby, Zinc Oxide Nanoparticles Induced Oxidative DNA Damage, Inflammation and Apoptosis in Rat's Brain after Oral Exposure, *Toxics*, 2018, **6**(2), 29.
111 L. Zhang, *et al.*, Gestational exposure to titanium dioxide nanoparticles impairs the placentation through dysregulation of vascularization, proliferation and apoptosis in mice, *Int. J. Nanomed.*, 2018, **13**, 777–789.
112 Q. He, *et al.*, Titanium dioxide nanoparticles induce mouse hippocampal neuron apoptosis via oxidative stress- and calcium imbalance-mediated endoplasmic reticulum stress, *Environ. Toxicol. Pharmacol.*, 2018, **63**, 6–15.
113 H. Liu, *et al.*, Exposure to copper oxide nanoparticles triggers oxidative stress and endoplasmic reticulum (ER)-

stress induced toxicology and apoptosis in male rat liver and BRL-3A cell, *J. Hazard. Mater.*, 2021, **401**, 123349.
114 Q. Wu, *et al.*, Iron oxide nanoparticles and induced autophagy in human monocytes, *Int. J. Nanomed.*, 2017, **12**, 3993–4005.
115 D. Maysinger, *et al.*, Gold nanoclusters elicit homeostatic perturbations in glioblastoma cells and adaptive changes of lysosomes, *Theranostics*, 2020, **10**(4), 1633–1648.
116 Y. Xu, *et al.*, Silver nanoparticles impede phorbol myristate acetate-induced monocyte-macrophage differentiation and autophagy, *Nanoscale*, 2015, **7**(38), 16100–16109.
117 R. F. Hamilton, *et al.*, Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity, *Part. Fibre Toxicol.*, 2009, **6**, 35.
118 A. Shah, *et al.*, Feraheme® suppresses immune function of human T lymphocytes through mitochondrial damage and mitoROS production, *Toxicol. Appl. Pharmacol.*, 2018, **350**, 52–63.
119 T. G. Zhang, *et al.*, Impairment of mitochondrial dynamics involved in iron oxide nanoparticle-induced dysfunction of dendritic cells was alleviated by autophagy inhibitor 3-methyladenine, *J. Appl. Toxicol.*, 2020, **40**(5), 631–642.
120 P. Orlowski, *et al.*, Tannic Acid-Modified Silver and Gold Nanoparticles as Novel Stimulators of Dendritic Cells Activation, *Front. Immunol.*, 2018, **9**, 1115.
121 A. K. Dey, *et al.*, Impact of Gold Nanoparticles on the Functions of Macrophages and Dendritic Cells, *Cells*, 2021, **10**(1), 96.
122 A. A. Khan, *et al.*, Endoplasmic Reticulum Stress Provocation by Different Nanoparticles: An Innovative Approach to Manage the Cancer and Other Common Diseases, *Molecules*, 2020, **25**(22), 5336.
123 H. Yasui, *et al.*, Radiosensitization of tumor cells through endoplasmic reticulum stress induced by PEGylated nanogel containing gold nanoparticles, *Cancer Lett.*, 2014, **347**(1), 151–158.
124 E. J. Park, *et al.*, Magnetic iron oxide nanoparticles induce autophagy preceding apoptosis through mitochondrial damage and ER stress in RAW264.7 cells, *Toxicol. in Vitro*, 2014, **28**(8), 1402–1412.
125 B. Ghaemi, *et al.*, Supramolecular Insights into Domino Effects of Ag@ZnO-Induced Oxidative Stress in Melanoma Cancer Cells, *ACS Appl. Mater. Interfaces*, 2019, **11**(50), 46408–46418.
126 X. Ma, *et al.*, Evaluation of Turning-Sized Gold Nanoparticles on Cellular Adhesion by Golgi Disruption in Vitro and in Vivo, *Nano Lett.*, 2019, **19**(12), 8476–8487.
127 D. M. Pegtel and S. J. Gould, Exosomes, *Annu. Rev. Biochem.*, 2019, **88**, 487–514.
128 M. Zhu, *et al.*, Exosomes as extrapulmonary signaling conveyors for nanoparticle-induced systemic immune activation, *Small*, 2012, **8**(3), 404–412.
129 M. Kumari, A. Mukherjee and N. Chandrasekaran, Genotoxicity of silver nanoparticles in Allium cepa, *Sci. Total Environ.*, 2009, **407**(19), 5243–5246.
130 J. Yu, *et al.*, Insights into the epigenetic effects of nanomaterials on cells, *Biomater. Sci.*, 2020, **8**(3), 763–775.
131 M. Ghosh, L. Godderis and P. Hoet, Epigenetic Mechanisms in Understanding Nanomaterial-Induced Toxicity, *Adv. Exp. Med. Biol.*, 2022, **1357**, 195–223.
132 Z. Magdolenova, *et al.*, Mechanisms of genotoxicity. A review of in vitro and in vivo studies with engineered nanoparticles, *Nanotoxicology*, 2014, **8**(3), 233–278.
133 M. Dusinska, *et al.*, Immunotoxicity, genotoxicity and epigenetic toxicity of nanomaterials: New strategies for toxicity testing?, *Food Chem. Toxicol.*, 2017, **109**(Pt 1), 797–811.
134 W. Zhang, *et al.*, Engineered nanoparticle-induced epigenetic changes: An important consideration in nanomedicine, *Acta Biomater.*, 2020, **117**, 93–107.
135 K. S. Butler, *et al.*, Silver nanoparticles: correlating nanoparticle size and cellular uptake with genotoxicity, *Mutagenesis*, 2015, **30**(4), 577–591.
136 Y. Li, *et al.*, Differential genotoxicity mechanisms of silver nanoparticles and silver ions, *Arch. Toxicol.*, 2017, **91**(1), 509–519.
137 Q. Xia, *et al.*, The effect of particle size on the genotoxicity of gold nanoparticles, *J. Biomed. Mater. Res., Part A*, 2017, **105**(3), 710–719.
138 Z. Magdolenova, *et al.*, Coating-dependent induction of cytotoxicity and genotoxicity of iron oxide nanoparticles, *Nanotoxicology*, 2015, **9**(Suppl 1), 44–56.
139 S. Ghosh, *et al.*, Genotoxicity and biocompatibility of superparamagnetic iron oxide nanoparticles: Influence of surface modification on biodistribution, retention, DNA damage and oxidative stress, *Food Chem. Toxicol.*, 2020, **136**, 110989.
140 D. Couto, *et al.*, Polyacrylic acid coated and non-coated iron oxide nanoparticles are not genotoxic to human T lymphocytes, *Toxicol. Lett.*, 2015, **234**(2), 67–73.
141 H. Jiang, *et al.*, Effects of cobalt nanoparticles on human T cells in vitro, *Biol. Trace Elem. Res.*, 2012, **146**(1), 23–29.
142 S. Rajiv, *et al.*, Comparative cytotoxicity and genotoxicity of cobalt (II, III) oxide, iron(III) oxide, silicon dioxide, and aluminum oxide nanoparticles on human lymphocytes in vitro, *Hum. Exp. Toxicol.*, 2016, **35**(2), 170–183.
143 T. Chen, J. Yan and Y. Li, Genotoxicity of titanium dioxide nanoparticles, *J. Food Drug Anal.*, 2014, **22**(1), 95–104.
144 A. Kazimirova, *et al.*, Titanium dioxide nanoparticles tested for genotoxicity with the comet and micronucleus assays in vitro, ex vivo and in vivo, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, 2019, **843**, 57–65.
145 A. Sliwinska, *et al.*, Genotoxicity and cytotoxicity of ZnO and Al_2O_3 nanoparticles, *Toxicol. Mech. Methods*, 2015, **25**(3), 176–183.
146 Y. Teow, *et al.*, Health impact and safety of engineered nanomaterials, *Chem. Commun.*, 2011, **47**(25), 7025–7038.
147 C. Dupont, D. R. Armant and C. A. Brenner, Epigenetics: definition, mechanisms and clinical perspective, *Semin. Reprod. Med.*, 2009, **27**(5), 351–357.
148 H. J. Eom, *et al.*, Integrated mRNA and micro RNA profiling reveals epigenetic mechanism of differential sensi-

tivity of Jurkat T cells to AgNPs and Ag ions, *Toxicol. Lett.*, 2014, **229**(1), 311–318.
149 Y. Qian, *et al.*, Silver nanoparticle-induced hemoglobin decrease involves alteration of histone 3 methylation status, *Biomaterials*, 2015, **70**, 12–22.
150 A. M. Tabish, *et al.*, Changes in DNA Methylation in Mouse Lungs after a Single Intra-Tracheal Administration of Nanomaterials, *PLoS One*, 2017, **12**(1), e0169886.
151 A. Stoccoro, *et al.*, Epigenetic effects of nano-sized materials, *Toxicology*, 2013, **313**(1), 3–14.
152 J. Ndika, *et al.*, Silver, titanium dioxide, and zinc oxide nanoparticles trigger miRNA/isomiR expression changes in THP-1 cells that are proportional to their health hazard potential, *Nanotoxicology*, 2019, **13**(10), 1380–1395.
153 X. Lu, *et al.*, Short-term exposure to engineered nanomaterials affects cellular epigenome, *Nanotoxicology*, 2016, **10**(2), 140–150.
154 X. Lu, *et al.*, In vivo epigenetic effects induced by engineered nanomaterials: A case study of copper oxide and laser printer-emitted engineered nanoparticles, *Nanotoxicology*, 2016, **10**(5), 629–639.
155 T. A. Ngobili and M. A. Daniele, Nanoparticles and direct immunosuppression, *Exp. Biol. Med.*, 2016, **241**(10), 1064–1073.
156 A. N. Yilma, *et al.*, Anti-inflammatory effects of silver-polyvinyl pyrrolidone (Ag-PVP) nanoparticles in mouse macrophages infected with live Chlamydia trachomatis, *Int. J. Nanomed.*, 2013, **8**, 2421–2432.
157 C. C. Shen, *et al.*, A single exposure to iron oxide nanoparticles attenuates antigen-specific antibody production and T-cell reactivity in ovalbumin-sensitized BALB/c mice, *Int. J. Nanomed.*, 2011, **6**, 1229–1235.
158 Y. P. Hsiao, *et al.*, Iron oxide nanoparticles attenuate T helper 17 cell responses in vitro and in vivo, *Int. Immunopharmacol.*, 2018, **58**, 32–39.
159 S. M. Hirst, *et al.*, Anti-inflammatory properties of cerium oxide nanoparticles, *Small*, 2009, **5**(24), 2848–2856.
160 A. E. Palmer and K. J. Franz, Introduction to "cellular metal homeostasis and trafficking", *Chem. Rev.*, 2009, **109**(10), 4533–4535.
161 M. A. Zoroddu, *et al.*, The essential metals for humans: a brief overview, *J. Inorg. Biochem.*, 2019, **195**, 120–129.
162 S. Ni, *et al.*, Iron Metabolism and Immune Regulation, *Front. Immunol.*, 2022, **13**, 816282.
163 M. Chevallet, *et al.*, Impact of labile metal nanoparticles on cellular homeostasis. Current developments in imaging, synthesis and applications, *Biochim. Biophys. Acta, Gen. Subj.*, 2017, **1861**(6), 1566–1577.
164 T. Xia, *et al.*, Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties, *ACS Nano*, 2008, **2**(10), 2121–2134.
165 M. Cuillel, *et al.*, Interference of CuO nanoparticles with metal homeostasis in hepatocytes under sub-toxic conditions, *Nanoscale*, 2014, **6**(3), 1707–1715.
166 G. K. Andrews, Cellular zinc sensors: MTF-1 regulation of gene expression, *BioMetals*, 2001, **14**(3–4), 223–237.
167 J. Bourquin, *et al.*, Biodistribution, Clearance, and Long-Term Fate of Clinically Relevant Nanomaterials, *Adv. Mater.*, 2018, **30**(19), e1704307.
168 A. Zamborlin, *et al.*, The Fate of Intranasally Instilled Silver Nanoarchitectures, *Nano Lett.*, 2022, **22**(13), 5269–5276.
169 M. Chanana, *et al.*, Physicochemical properties of protein-coated gold nanoparticles in biological fluids and cells before and after proteolytic digestion, *Angew. Chem., Int. Ed.*, 2013, **52**(15), 4179–4183.
170 S. J. Soenen, *et al.*, (Intra)cellular stability of inorganic nanoparticles: effects on cytotoxicity, particle functionality, and biomedical applications, *Chem. Rev.*, 2015, **115**(5), 2109–2135.
171 G. Nichols, *et al.*, A review of the terms agglomerate and aggregate with a recommendation for nomenclature used in powder and particle characterization, *J. Pharm. Sci.*, 2002, **91**(10), 2103–2109.
172 S. Keshavan, *et al.*, Nano-bio interactions: a neutrophil-centric view, *Cell Death Dis.*, 2019, **10**(8), 569.
173 Q. Feng, *et al.*, Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings, *Sci. Rep.*, 2018, **8**(1), 2082.
174 J. P. Almeida, *et al.*, In vivo immune cell distribution of gold nanoparticles in naïve and tumor bearing mice, *Small*, 2014, **10**(4), 812–819.
175 D. M. Smith, J. K. Simon and J. R. Baker Jr., Applications of nanotechnology for immunology, *Nat. Rev. Immunol.*, 2013, **13**(8), 592–605.
176 D. P. Lankveld, *et al.*, The kinetics of the tissue distribution of silver nanoparticles of different sizes, *Biomaterials*, 2010, **31**(32), 8350–8361.
177 M. Yu and J. Zheng, Clearance Pathways and Tumor Targeting of Imaging Nanoparticles, *ACS Nano*, 2015, **9**(7), 6655–6674.
178 G. Yang, *et al.*, Degradability and Clearance of Inorganic Nanoparticles for Biomedical Applications, *Adv. Mater.*, 2019, **31**(10), e1805730.
179 D. J. McClements, H. Xiao and P. Demokritou, Physicochemical and colloidal aspects of food matrix effects on gastrointestinal fate of ingested inorganic nanoparticles, *Adv. Colloid Interface Sci.*, 2017, **246**, 165–180.
180 J. Kolosnjaj-Tabi, J. Volatron and F. Gazeau, *Basic Principles of In Vivo Distribution, Toxicity, and Degradation of Prospective Inorganic Nanoparticles for Imaging*, Springer International Publishing, 2017.
181 Z. Cai, *et al.*, Hyaluronan-Inorganic Nanohybrid Materials for Biomedical Applications, *Biomacromolecules*, 2017, **18**(6), 1677–1696.
182 J. Y. Wang, *et al.*, Effects of surface charges of gold nanoclusters on long-term in vivo biodistribution, toxicity, and cancer radiation therapy, *Int. J. Nanomed.*, 2016, **11**, 3475–3485.

183 W. H. De Jong, *et al.*, Particle size-dependent organ distribution of gold nanoparticles after intravenous administration, *Biomaterials*, 2008, **29**(12), 1912–1919.
184 H. Tang, *et al.*, Blood Clearance, Distribution, Transformation, Excretion, and Toxicity of Near-Infrared Quantum Dots Ag2Se in Mice, *ACS Appl. Mater. Interfaces*, 2016, **8**(28), 17859–17869.
185 P. Falagan-Lotsch, E. M. Grzincic and C. J. Murphy, One low-dose exposure of gold nanoparticles induces long-term changes in human cells, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**(47), 13318–13323.
186 J. Unnithan, *et al.*, Aqueous synthesis and concentration-dependent dermal toxicity of TiO2 nanoparticles in Wistar rats, *Biol. Trace Elem. Res.*, 2011, **143**(3), 1682–1694.
187 A. Spengler, L. Wanninger and S. Pflugmacher, Oxidative stress mediated toxicity of TiO(2) nanoparticles after a concentration and time dependent exposure of the aquatic macrophyte Hydrilla verticillata, *Aquat. Toxicol.*, 2017, **190**, 32–39.
188 H. Guo, *et al.*, Intravenous administration of silver nanoparticles causes organ toxicity through intracellular ROS-related loss of inter-endothelial junction, *Part. Fibre Toxicol.*, 2016, **13**, 21.
189 J. Choi, *et al.*, Toxicity of zinc oxide nanoparticles in rats treated by two different routes: single intravenous injection and single oral administration, *J. Toxicol. Environ. Health, Part A*, 2015, **78**(4), 226–243.
190 X. Jia, *et al.*, The Potential Liver, Brain, and Embryo Toxicity of Titanium Dioxide Nanoparticles on Mice, *Nanoscale Res. Lett.*, 2017, **12**(1), 478.
191 B. Shahare and M. Yashpal, Toxic effects of repeated oral exposure of silver nanoparticles on small intestine mucosa of mice, *Toxicol. Mech. Methods*, 2013, **23**(3), 161–167.
192 C. Recordati, *et al.*, Tissue distribution and acute toxicity of silver after single intravenous administration in mice: nano-specific and size-dependent effects, *Part. Fibre Toxicol.*, 2016, **13**, 12.
193 Y. Yamagishi, *et al.*, Acute and chronic nephrotoxicity of platinum nanoparticles in mice, *Nanoscale Res. Lett.*, 2013, **8**(1), 395.
194 E. Sadauskas, *et al.*, Protracted elimination of gold nanoparticles from mouse liver, *Nanomedicine*, 2009, **5**(2), 162–169.
195 S. Dragoni, *et al.*, Gold nanoparticles uptake and cytotoxicity assessed on rat liver precision-cut slices, *Toxicol. Sci.*, 2012, **128**(1), 186–197.
196 E. J. Pearce and E. L. Pearce, Immunometabolism in 2017: Driving immunity: all roads lead to metabolism, *Nat. Rev. Immunol.*, 2018, **18**(2), 81–82.
197 E. L. Pearce and E. J. Pearce, Metabolic pathways in immune cell activation and quiescence, *Immunity*, 2013, **38**(4), 633–643.
198 K. Voss, *et al.*, A guide to interrogating immunometabolism, *Nat. Rev. Immunol.*, 2021, **21**(10), 637–652.
199 Y. Hu, *et al.*, mTOR-mediated metabolic reprogramming shapes distinct microglia functions in response to lipopolysaccharide and ATP, *Glia*, 2020, **68**(5), 1031–1045.
200 M. Fumagalli, *et al.*, How to reprogram microglia toward beneficial functions, *Glia*, 2018, **66**(12), 2531–2549.
201 S. K. Biswas and A. Mantovani, Orchestration of metabolism by macrophages, *Cell Metab.*, 2012, **15**(4), 432–437.
202 C. S. Nascimento, *et al.*, Immunotherapy for cancer: effects of iron oxide nanoparticles on polarization of tumor-associated macrophages, *Nanomedicine*, 2021, **16**(29), 2633–2650.
203 M. W. Hentze, *et al.*, Two to tango: regulation of Mammalian iron metabolism, *Cell*, 2010, **142**(1), 24–38.
204 L. He, *et al.*, The role of morphology, shell composition and protein corona formation in Au/Fe(3)O(4) composite nanoparticle mediated macrophage responses, *J. Mater. Chem. B*, 2021, **9**(32), 6387–6395.
205 R. Tiwari, *et al.*, Perinatal exposure to silver nanoparticles reprograms immunometabolism and promotes pancreatic beta-cell death and kidney damage in mice, *Nanotoxicology*, 2021, **15**(5), 636–660.
206 R. Tiwari, *et al.*, Gestational exposure to silver nanoparticles enhances immune adaptation and protection against streptozotocin-induced diabetic nephropathy in mice offspring, *Nanotoxicology*, 2022, **16**(4), 450–471.
207 I. Mikelez-Alonso, *et al.*, Natural killer (NK) cell-based immunotherapies and the many faces of NK cell memory: A look into how nanoparticles enhance NK cell activity, *Adv. Drug Delivery Rev.*, 2021, **176**, 113860.
208 A. C. Anselmo and S. Mitragotri, Nanoparticles in the clinic: An update, *Bioeng. Transl. Med.*, 2019, **4**(3), e10143.
209 F. Wang, *et al.*, Glycolytic Stimulation Is Not a Requirement for M2 Macrophage Differentiation, *Cell Metab.*, 2018, **28**(3), 463–475.e4.
210 N. J. MacIver, R. D. Michalek and J. C. Rathmell, Metabolic regulation of T lymphocytes, *Annu. Rev. Immunol.*, 2013, **31**, 259–283.
211 R. Wang and D. R. Green, Metabolic checkpoints in activated T cells, *Nat. Immunol.*, 2012, **13**(10), 907–915.
212 K. N. Pollizzi and J. D. Powell, Integrating canonical and metabolic signalling programmes in the regulation of T cell responses, *Nat. Rev. Immunol.*, 2014, **14**(7), 435–446.
213 H. Kojima, *et al.*, Differentiation stage-specific requirement in hypoxia-inducible factor-1alpha-regulated glycolytic pathway during murine B cell development in bone marrow, *J. Immunol.*, 2010, **184**(1), 154–163.
214 C. A. Doughty, *et al.*, Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth, *Blood*, 2006, **107**(11), 4458–4465.
215 F. J. Dufort, *et al.*, Cutting edge: IL-4-mediated protection of primary B lymphocytes from apoptosis via Stat6-dependent regulation of glycolytic metabolism, *J. Immunol.*, 2007, **179**(8), 4953–4957.
216 B. Everts, *et al.*, TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic

demands of dendritic cell activation, *Nat. Immunol.*, 2014, **15**(4), 323–332.
217 C. M. Krawczyk, *et al.*, Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation, *Blood*, 2010, **115**(23), 4742–4749.
218 B. Everts, *et al.*, Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells, *Blood*, 2012, **120**(7), 1422–1431.
219 J. I. Odegaard and A. Chawla, Alternative macrophage activation and metabolism, *Annu. Rev. Pathol.*, 2011, **6**, 275–297.
220 D. Vats, *et al.*, Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation, *Cell Metab.*, 2006, **4**(1), 13–24.
221 B. J. van Raam, A. J. Verhoeven and T. W. Kuijpers, Mitochondria in neutrophil apoptosis, *Int. J. Hematol.*, 2006, **84**(3), 199–204.
222 D. C. Dale, L. Boxer and W. C. Liles, The phagocytes: neutrophils and monocytes, *Blood*, 2008, **112**(4), 935–945.
223 H. Park, *et al.*, Metabolic regulator Fnip1 is crucial for iNKT lymphocyte development, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**(19), 7066–7071.
224 M. Dose, *et al.*, Intrathymic proliferation wave essential for Valpha14+ natural killer T cell development depends on c-Myc, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**(21), 8641–8646.
225 J. Henao-Mejia, *et al.*, The microRNA miR-181 is a critical cellular metabolic rheostat essential for NKT cell ontogenesis and lymphocyte development and homeostasis, *Immunity*, 2013, **38**(5), 984–997.
226 N. Lee, *et al.*, Magnetosome-like ferrimagnetic iron oxide nanocubes for highly sensitive MRI of single cells and transplanted pancreatic islets, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**(7), 2662–2667.
227 J. Xie, *et al.*, Human serum albumin coated iron oxide nanoparticles for efficient cell labeling, *Chem. Commun.*, 2010, **46**(3), 433–435.
228 H. Maeda, H. Nakamura and J. Fang, The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo, *Adv. Drug Delivery Rev.*, 2013, **65**(1), 71–79.
229 A. J. Cole, V. C. Yang and A. E. David, Cancer theranostics: the rise of targeted magnetic nanoparticles, *Trends Biotechnol.*, 2011, **29**(7), 323–332.
230 Y. Hu, *et al.*, Multifunctional Fe3O4@Au core/shell nanostars: a unique platform for multimode imaging and photothermal therapy of tumors, *Sci. Rep.*, 2016, **6**, 28325.
231 Y. H. Hwang, M. J. Kim and D. Y. Lee, MRI-sensitive contrast agent with anticoagulant activity for surface camouflage of transplanted pancreatic islets, *Biomaterials*, 2017, **138**, 121–130.
232 Z. Wang and A. Cuschieri, Tumour cell labelling by magnetic nanoparticles with determination of intracellular iron content and spatial distribution of the intracellular iron, *Int. J. Mol. Sci.*, 2013, **14**(5), 9111–9125.
233 H. Xu, *et al.*, Antibody conjugated magnetic iron oxide nanoparticles for cancer cell separation in fresh whole blood, *Biomaterials*, 2011, **32**(36), 9758–9765.
234 J. Yao, *et al.*, ROS scavenging Mn(3)O(4) nanozymes for in vivo anti-inflammation, *Chem. Sci.*, 2018, **9**(11), 2927–2933.
235 X. Jiang, *et al.*, Crossover between anti- and pro-oxidant activities of different manganese oxide nanoparticles and their biological implications, *J. Mater. Chem. B*, 2020, **8**(6), 1191–1201.
236 Y. Zhang, *et al.*, Multienzymatic Antioxidant Activity of Manganese-Based Nanoparticles for Protection against Oxidative Cell Damage, *ACS Biomater. Sci. Eng.*, 2022, **8**(2), 638–648.
237 E. Hoseinzadeh, *et al.*, A Review on Nano-Antimicrobials: Metal Nanoparticles, Methods and Mechanisms, *Curr. Drug Metab.*, 2017, **18**(2), 120–128.
238 H. S. Gill, *et al.*, Molecular and immune toxicity of CoCr nanoparticles in MoM hip arthroplasty, *Trends Mol. Med.*, 2012, **18**(3), 145–155.
239 R. H. Kim, D. A. Dennis and J. T. Carothers, Metal-on-metal total hip arthroplasty, *J. Arthroplasty*, 2008, **23**(7 Suppl), 44–46.
240 G. P. Jose, *et al.*, Singlet oxygen mediated DNA degradation by copper nanoparticles: potential towards cytotoxic effect on cancer cells, *J. Nanobiotechnol.*, 2011, **9**, 9.
241 X. Ma, *et al.*, Copper-containing nanoparticles: Mechanism of antimicrobial effect and application in dentistry-a narrative review, *Front. Surg.*, 2022, **9**, 905892.
242 M. A. Shahbazi, *et al.*, The versatile biomedical applications of bismuth-based nanoparticles and composites: therapeutic, diagnostic, biosensing, and regenerative properties, *Chem. Soc. Rev.*, 2020, **49**(4), 1253–1321.
243 Z. Li, *et al.*, Dual-Stimuli Responsive Bismuth Nanoraspberries for Multimodal Imaging and Combined Cancer Therapy, *Nano Lett.*, 2018, **18**(11), 6778–6788.
244 A. Naghizadeh, S. Mohammadi-Aghdam and S. Mortazavi-Derazkola, Novel CoFe(2)O(4)@ZnO-CeO(2) ternary nanocomposite: Sonochemical green synthesis using Crataegus microphylla extract, characterization and their application in catalytic and antibacterial activities, *Bioorg. Chem.*, 2020, **103**, 104194.
245 G. Tortella, *et al.*, Bactericidal and Virucidal Activities of Biogenic Metal-Based Nanoparticles: Advances and Perspectives, *Antibiotics*, 2021, **10**(7), 783.
246 A. Goel, Boydston, *et al.*: The Impact of Alternative Alkalinizing Agents on 24-Hour Urine Parameters (Urology 2020 Apr 21;S0090-4295(20)30428-3.), *Urology*, 2020, **143**, 270–271, DOI: **10.1016/j.urology.2020.04.047**.
247 S. R. Alizadeh and M. A. Ebrahimzadeh, Characterization and Anticancer Activities of Green Synthesized CuO Nanoparticles, A Review, *Anti-Cancer Agents Med. Chem.*, 2021, **21**(12), 1529–1543.
248 L. Shkodenko, I. Kassirov and E. Koshel, Metal Oxide Nanoparticles Against Bacterial Biofilms: Perspectives and Limitations, *Microorganisms*, 2020, **8**(10), 1545.

249 B. Xu, *et al.*, Synthesis, Characterization, and Antifogging Application of Polymer/Al_2O_3 Nanocomposite Hydrogels with High Strength and Self-Healing Capacity, *Polymers*, 2018, **10**(12), 1362.
250 V. Iribarnegaray, *et al.*, Magnesium-doped zinc oxide nanoparticles alter biofilm formation of Proteus mirabilis, *Nanomedicine*, 2019, **14**(12), 1551–1564.
251 A. Aghebati-Maleki, *et al.*, Nanoparticles and cancer therapy: Perspectives for application of nanoparticles in the treatment of cancers, *J. Cell Physiol.*, 2020, **235**(3), 1962–1972.
252 K. Jadhav, *et al.*, Phytosynthesis of gold nanoparticles: Characterization, biocompatibility, and evaluation of its osteoinductive potential for application in implant dentistry, *Mater. Sci. Eng., C*, 2018, **93**, 664–670.
253 J. Noonan, *et al.*, In vivo multiplex molecular imaging of vascular inflammation using surface-enhanced Raman spectroscopy, *Theranostics*, 2018, **8**(22), 6195–6209.
254 S. C. Boca, *et al.*, Chitosan-coated triangular silver nanoparticles as a novel class of biocompatible, highly effective photothermal transducers for in vitro cancer cell therapy, *Cancer Lett.*, 2011, **311**(2), 131–140.
255 J. Liu, *et al.*, TAT-modified nanosilver for combating multidrug-resistant cancer, *Biomaterials*, 2012, **33**(26), 6155–6161.
256 L. S. Abebe, *et al.*, Point-of-Use Removal of Cryptosporidium parvum from Water: Independent Effects of Disinfection by Silver Nanoparticles and Silver Ions and by Physical Filtration in Ceramic Porous Media, *Environ. Sci. Technol.*, 2015, **49**(21), 12958–12967.
257 H. B. Dias, *et al.*, Titanium dioxide and modified titanium dioxide by silver nanoparticles as an anti biofilm filler content for composite resins, *Dent. Mater.*, 2019, **35**(2), e36–e46.
258 L. Zhang, *et al.*, Reducing stress on cells with apoferritin-encapsulated platinum nanoparticles, *Nano Lett.*, 2010, **10**(1), 219–223.
259 J. S. Lee, *et al.*, Highly Sensitive and Selective Field-Effect-Transistor NonEnzyme Dopamine Sensors Based on Pt/Conducting Polymer Hybrid Nanoparticles, *Small*, 2015, **11**(20), 2399–2406.
260 Z. Bai, *et al.*, Non-enzymatic electrochemical biosensor based on Pt NPs/RGO-CS-Fc nano-hybrids for the detection of hydrogen peroxide in living cells, *Biosens. Bioelectron.*, 2016, **82**, 185–194.
261 A. Abed, *et al.*, Platinum Nanoparticles in Biomedicine: Preparation, Anti-Cancer Activity, and Drug Delivery Vehicles, *Front. Pharmacol.*, 2022, **13**, 797804.
262 J. T. Weiss, *et al.*, Extracellular palladium-catalysed dealkylation of 5-fluoro-1-propargyl-uracil as a bioorthogonally activated prodrug approach, *Nat. Commun.*, 2014, **5**, 3277.
263 S. Tang, M. Chen and N. Zheng, Sub-10 nm Pd nanosheets with renal clearance for efficient near-infrared photothermal cancer therapy, *Small*, 2014, **10**(15), 3139–3144.
264 N. Huang, *et al.*, Ruthenium complexes/polypeptide self-assembled nanoparticles for identification of bacterial infection and targeted antibacterial research, *Biomaterials*, 2017, **141**, 296–313.
265 Y. Liu, *et al.*, Ru nanoparticles coated with γ-Fe(2)O(3) promoting and monitoring the differentiation of human mesenchymal stem cells via MRI tracking, *Colloids Surf., B*, 2018, **170**, 701–711.
266 F. Xia, *et al.*, Ultrasmall Ruthenium Nanoparticles with Boosted Antioxidant Activity Upregulate Regulatory T Cells for Highly Efficient Liver Injury Therapy, *Small*, 2022, **18**(29), e2201558.
267 S. Kang, *et al.*, Morphology-Controlled Synthesis of Rhodium Nanoparticles for Cancer Phototherapy, *ACS Nano*, 2018, **12**(7), 6997–7008.
268 L. Zhang, *et al.*, AIE Multinuclear Ir(III) Complexes for Biocompatible Organic Nanoparticles with Highly Enhanced Photodynamic Performance, *Adv. Sci.*, 2019, **6**(5), 1802050.
269 Y. Fan, *et al.*, pH-activated size reduction of large compound nanoparticles for in vivo nucleus-targeted drug delivery, *Biomaterials*, 2016, **85**, 30–39.
270 Y. Wu, *et al.*, Visible-light-excited and europium-emissive nanoparticles for highly-luminescent bioimaging in vivo, *Biomaterials*, 2014, **35**(22), 5830–5839.
271 M. M. El-Sheekh, *et al.*, Antiviral activity of algae biosynthesized silver and gold nanoparticles against Herps Simplex (HSV-1) virus in vitro using cell-line culture technique, *Int. J. Environ. Health Res.*, 2022, **32**(3), 616–627.
272 Y. Wang, *et al.*, Quercetin-Loaded Ceria Nanocomposite Potentiate Dual-Directional Immunoregulation via Macrophage Polarization against Periodontal Inflammation, *Small*, 2021, **17**(41), e2101505.
273 R. P. Senthilkumar, *et al.*, Synthesis, characterization and antibacterial activity of hybrid chitosan-cerium oxide nanoparticles: As a bionanomaterials, *Int. J. Biol. Macromol.*, 2017, **104**(Pt B), 1746–1752.
274 J. Xiang, *et al.*, Cerium Oxide Nanoparticle Modified Scaffold Interface Enhances Vascularization of Bone Grafts by Activating Calcium Channel of Mesenchymal Stem Cells, *ACS Appl. Mater. Interfaces*, 2016, **8**(7), 4489–4499.
275 O. M. David, *et al.*, The Stability and Anti-Angiogenic Properties of Titanium Dioxide Nanoparticles (TiO_2NPs) Using Caco-2 Cells, *Biomolecules*, 2022, **12**(10), 1334.
276 S. Çeşmeli and C. Biray Avci, Application of titanium dioxide (TiO(2)) nanoparticles in cancer therapies, *J. Drug Targeting*, 2019, **27**(7), 762–766.
277 T. Wang, *et al.*, Potential application of functional porous TiO2 nanoparticles in light-controlled drug release and targeted drug delivery, *Acta Biomater.*, 2015, **13**, 354–363.
278 X. Wang, *et al.*, Advances in surface modification of tantalum and porous tantalum for rapid osseointegration: A thematic review, *Front. Bioeng. Biotechnol.*, 2022, **10**, 983695.
279 A. Rafieerad, *et al.*, Fabrication of Smart Tantalum Carbide MXene Quantum Dots with Intrinsic Immunomodulatory Properties for Treatment of Allograft Vasculopathy, *Adv. Funct. Mater.*, 2021, **31**(46), 2106786.

280 U. S. Gaharwar, R. Meena and P. Rajamani, Iron oxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in lymphocytes, *J. Appl. Toxicol.*, 2017, **37**(10), 1232–1244.

281 O. M. Posada, R. J. Tate and M. H. Grant, Effects of CoCr metal wear debris generated from metal-on-metal hip implants and Co ions on human monocyte-like U937 cells, *Toxicol. in Vitro*, 2015, **29**(2), 271–280.

282 Y. M. Kwon, *et al.*, Dose-dependent cytotoxicity of clinically relevant cobalt nanoparticles and ions on macrophages in vitro, *Biomed. Mater.*, 2009, **4**(2), 025018.

283 S. L. More, *et al.*, Review and Evaluation of the Potential Health Effects of Oxidic Nickel Nanoparticles, *Nanomaterials*, 2021, **11**(3), 642.

284 X. Chen and C. Gao, Influences of size and surface coating of gold nanoparticles on inflammatory activation of macrophages, *Colloids Surf., B*, 2017, **160**, 372–380.

285 B. Vuković, *et al.*, Surface Stabilization Affects Toxicity of Silver Nanoparticles in Human Peripheral Blood Mononuclear Cells, *Nanomaterials*, 2020, **10**(7), 1390.

286 S. Murugadoss, *et al.*, Agglomeration of titanium dioxide nanoparticles increases toxicological responses in vitro and in vivo, *Part. Fibre Toxicol.*, 2020, **17**(1), 10.

287 X. Zhang, *et al.*, Iron Oxide Nanoparticles Induce Autophagosome Accumulation through Multiple Mechanisms: Lysosome Impairment, Mitochondrial Damage, and ER Stress, *Mol. Pharm.*, 2016, **13**(7), 2578–2587.

288 A. Sabareeswaran, *et al.*, Effect of surface-modified superparamagnetic iron oxide nanoparticles (SPIONS) on mast cell infiltration: An acute in vivo study, *Nanomedicine*, 2016, **12**(6), 1523–1533.

289 L. Zha, *et al.*, Chromium(III) nanoparticles affect hormone and immune responses in heat-stressed rats, *Biol. Trace Elem. Res.*, 2009, **129**(1–3), 157–169.

290 X. Chang, *et al.*, Role of NF-κB activation and Th1/Th2 imbalance in pulmonary toxicity induced by nano NiO, *Environ. Toxicol.*, 2017, **32**(4), 1354–1362.

291 X. Zhou, *et al.*, The Toxic Effects and Mechanisms of Nano-Cu on the Spleen of Rats, *Int. J. Mol. Sci.*, 2019, **20**(6), 1469.

292 I. C. Lee, *et al.*, Copper nanoparticles induce early fibrotic changes in the liver via TGF-β/Smad signaling and cause immunosuppressive effects in rats, *Nanotoxicology*, 2018, **12**(6), 637–651.

293 R. Sadiq, *et al.*, Genotoxicity of aluminium oxide, iron oxide, and copper nanoparticles in mouse bone marrow cells, *Arh. Hig. Rada Toksikol.*, 2021, **72**(4), 315–325.

294 J. Tulinska, *et al.*, Six-week inhalation of CdO nanoparticles in mice: The effects on immune response, oxidative stress, antioxidative defense, fibrotic response, and bones, *Food Chem. Toxicol.*, 2020, **136**, 110954.

295 W. H. De Jong, *et al.*, Systemic and immunotoxicity of silver nanoparticles in an intravenous 28 days repeated dose toxicity study in rats, *Biomaterials*, 2013, **34**(33), 8333–8343.

296 X. Chang, *et al.*, Effects of Th1 and Th2 cells balance in pulmonary injury induced by nano titanium dioxide, *Environ. Toxicol. Pharmacol.*, 2014, **37**(1), 275–283.

297 X. Sang, *et al.*, Immunomodulatory effects in the spleen-injured mice following exposure to titanium dioxide nanoparticles, *J. Biomed. Mater. Res., Part A*, 2014, **102**(10), 3562–3572.

298 W. Mu, *et al.*, Effect of Long-Term Intake of Dietary Titanium Dioxide Nanoparticles on Intestine Inflammation in Mice, *J. Agric. Food Chem.*, 2019, **67**(33), 9382–9389.

299 L. Yao, *et al.*, Oral exposure of titanium oxide nanoparticles induce ileum physical barrier dysfunction via Th1/Th2 imbalance, *Environ. Toxicol.*, 2020, **35**(9), 982–990.

300 P. Jalili, *et al.*, Genotoxicity of Aluminum and Aluminum Oxide Nanomaterials in Rats Following Oral Exposure, *Nanomaterials*, 2020, **10**(2), 305.

301 L. Talamini, *et al.*, Influence of Size and Shape on the Anatomical Distribution of Endotoxin-Free Gold Nanoparticles, *ACS Nano*, 2017, **11**(6), 5519–5529.

302 J. S. Hong, *et al.*, Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test, *Nanotoxicology*, 2014, **8**(4), 349–362.

303 K. F. Chung, *et al.*, Inactivation, Clearance, and Functional Effects of Lung-Instilled Short and Long Silver Nanowires in Rats, *ACS Nano*, 2017, **11**(3), 2652–2664.

304 R. M. Silva, *et al.*, Aerosolized Silver Nanoparticles in the Rat Lung and Pulmonary Responses over Time, *Toxicol. Pathol.*, 2016, **44**(5), 673–686.

305 W. G. Kreyling, *et al.*, Quantitative biokinetics over a 28 day period of freshly generated, pristine, 20 nm silver nanoparticle aerosols in healthy adult rats after a single $1\frac{1}{2}$-hour inhalation exposure, *Part. Fibre Toxicol.*, 2020, **17**(1), 21.

306 W. S. Cho, *et al.*, 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**(1), 96–102.

307 J. H. Ji, *et al.*, Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats, *Inhalation Toxicol.*, 2007, **19**(10), 857–871.

308 Y. S. Kim, *et al.*, Subchronic oral toxicity of silver nanoparticles, *Part. Fibre Toxicol.*, 2010, **7**, 20.

309 D. B. Warheit, S. C. Brown and E. M. Donner, Acute and subchronic oral toxicity studies in rats with nanoscale and pigment grade titanium dioxide particles, *Food Chem. Toxicol.*, 2015, **84**, 208–224.

310 K. E. Ibrahim, *et al.*, Histopathology of the Liver, Kidney, and Spleen of Mice Exposed to Gold Nanoparticles, *Molecules*, 2018, **23**(8), 1848.

311 S. K. Balasubramanian, *et al.*, Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats, *Biomaterials*, 2010, **31**(8), 2034–2042.

312 R. J. Vandebriel, *et al.*, Immunotoxicity of silver nanoparticles in an intravenous 28-day repeated-dose toxicity study in rats, *Part. Fibre Toxicol.*, 2014, **11**, 21.

313 D. Westmeier, *et al.*, Correction: Nanoparticle binding attenuates the pathobiology of gastric cancer-associated Helicobacter pylori, *Nanoscale*, 2020, **12**(3), 2154–2155.

314 M. D. Boudreau, *et al.*, Differential Effects of Silver Nanoparticles and Silver Ions on Tissue Accumulation, Distribution, and Toxicity in the Sprague Dawley Rat Following Daily Oral Gavage Administration for 13 Weeks, *Toxicol. Sci.*, 2016, **150**(1), 131–160.

315 X. Ma, *et al.*, Correction to Evaluation of Turning-Sized Gold Nanoparticles on Cellular Adhesion by Golgi Disruption in Vitro and in Vivo, *Nano Lett.*, 2020, **20**(1), 799.

316 Y. S. Kim, *et al.*, Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats, *Inhalation Toxicol.*, 2008, **20**(6), 575–583.