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

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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.

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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 CeO₂ 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 occur³ (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

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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 Fe₃O₄ 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 $\rho > 4.5 \text{ g cm}^{-3}$): 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 $\rho < 4.5 \text{ g cm}^{-3}$): 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 poten-

tial 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 ferri-ferrous 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

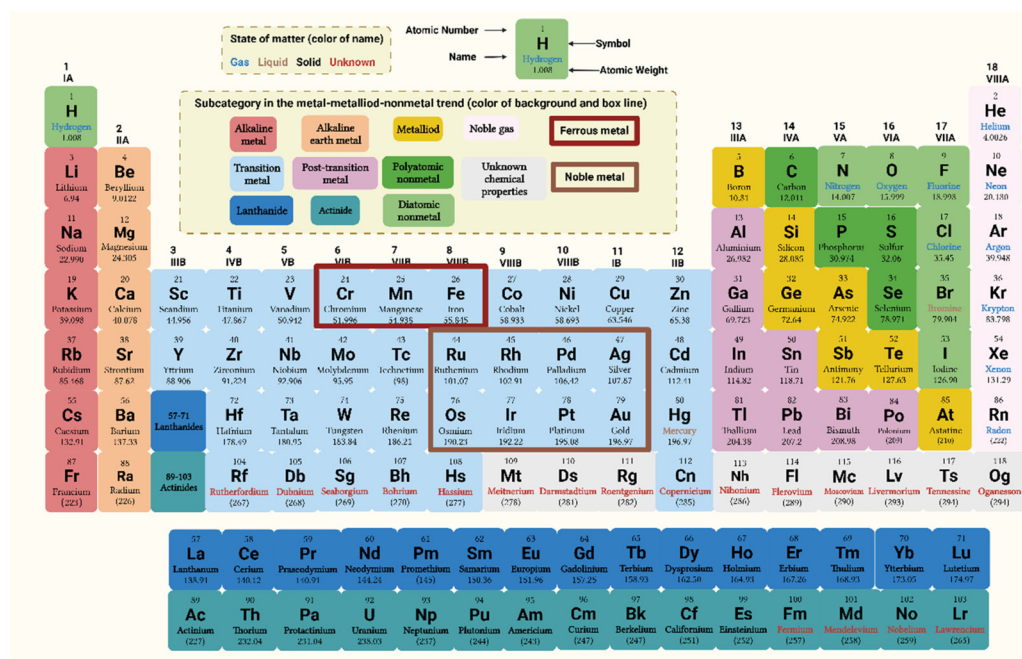


Fig. 1 Location of different types of metal elements in the periodic table. The positions shown in the red and green boxes are ferrous metals and precious metals, respectively. Except for iron, manganese and chromium, all other metal elements belong to non-ferrous metals. Created with BioRender.com.



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, CeO₃ and TiO₂ 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, TiO₂, and Fe₂O₃ 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 TiO₂ NPs.⁴⁰ Moreover, TiO₂ 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

Type	Cell subtypes	Main ways of energy metabolism				Ref.
		Glycolysis	OXPPOS	FAO	Glutaminolysis	
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 ₂ O ₃ NPs	Magnetic, traceability, imaging	Cell labeling, tumor therapy, MRI	226–228
	Fe ₃ O 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 ₃ O ₄ /Mn ₂ O ₃ /MnO ₂ NPs	Antioxidant activity	ROS scavengers	234–236
	Cr ₂ O 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 ₂ O ₃ /Co ₃ O ₄ /CoO NPs	Antibacterial activity	Antibacterial agents	237
Light metal	Al ₂ O 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
Scarce metal	AgO NPs	Antiviral	Fighting the drug-resistant types of viruses	271
	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, <i>in vivo</i> treatment of transplanted vascular lesions	65, 278 and 279

different sizes and charges can cause immunotoxicity by inducing inflammation⁴² 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-1 β , 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 ₂ O 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 <i>in vivo</i> 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 <i>in vitro</i> .	42
	Co ₃ O ₄	\leq 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-1 β .	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, IKK α / 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-1 β , 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, Fe₂O₃) 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 be 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 ₂ O 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 ₃ O 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 nitrate 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
	ZnO	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
		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 <i>in vivo</i> ; 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. Significantly increase the levels of various inflammatory factors and chemokines, while decreasing NKG2D, Nkp46 and 2B4.	Lymphocyte subsets and immune capacity decreased, spleen damage. Significantly increase the spleen and thymus indices, spleen damage.	40
				Activation of NF-κB-mediated MAPKs pathway.	Exert toxic effects on lymphoid organs and T cells and innate immune cell homeostasis.	297
		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.	76 298



Table 4 (Contd.)

Classification of metal materials	NP types	NP properties	Models	Mechanisms	Results	Ref.
Light metal	AlO	17, 117 nm	C57BL/6Jrj mice	Glutathione depletion, increased IL-8 and IL-1 β , DNA damage and cytotoxicity.	Large agglomerates of 117 nm TiO ₂ induced higher pulmonary responses and blood DNA damage compared to small agglomerates. Induced ileal physical barrier dysfunction (dose-dependent).	286
		22.75 \pm 7.04 nm	Female Kunming mice	Th1/Th2 imbalance.		299
		Aspect ratios (6.2 \pm 0.6, 2.1 \pm 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 ₂ O ₃	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-1 β , 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 TiO₂ NPs can also induce pro-inflammatory mediators such as MIP-1 α/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 production⁵⁰ (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 Al₂O₃ 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- κ B 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-1 β 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 CTSE in J774A.1 macrophages. Further, it promoted an IL-1 β -mediated inflammatory response through the MyD88-dependent TLR4 and NF- κ B signalling pathways.⁵⁹ In addition, the released Cu²⁺ ions could further activate the NLRP3 inflammasome and cause oxidative stress⁵⁹ (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-1 β 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



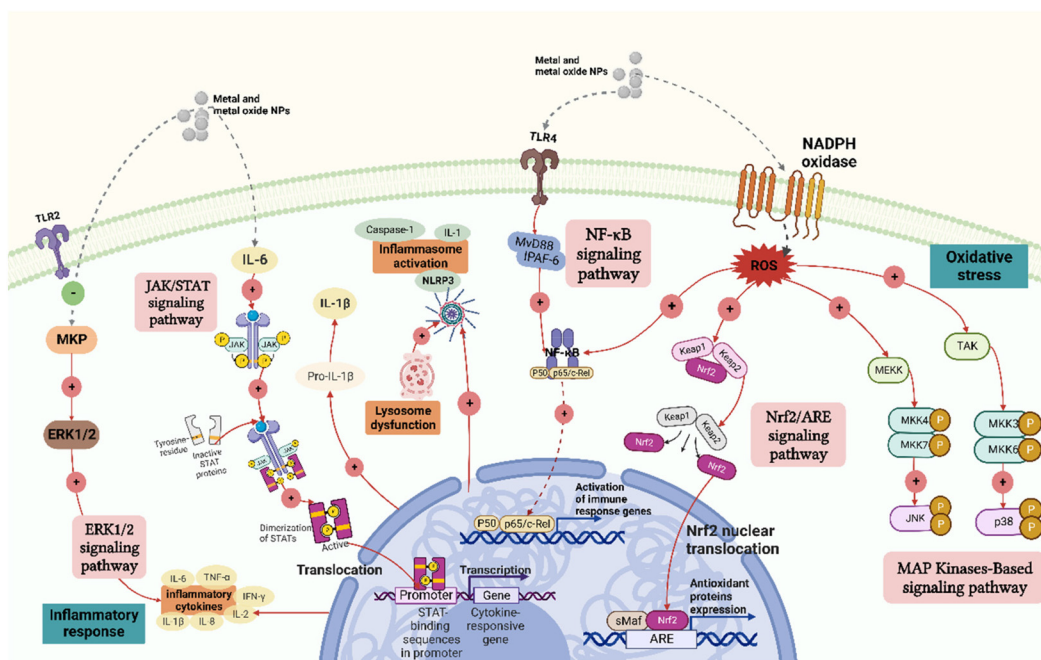


Fig. 2 Mechanisms of metal and metal oxide NP-induced inflammatory response and oxidative stress in immunotoxicity. Metal and metal oxide NPs can cause the activation of inflammasomes and the release of inflammatory factors such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α and induce inflammation and oxidative stress through a variety of different signal transduction molecular mechanisms, including the ERK1/2, JAK/STAT, NF- κ B, 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-1 β . Meanwhile, Ag NPs greater than 10 nm in size activated the NF- κ B signalling pathway.⁶² *In vivo* experiments also confirmed the activation of the inflammasome by TiO₂ NPs. For example, Kim B. *et al.* investigated the effect of TiO₂ 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-1 β , IL-18, NLRP3, and caspase-1.⁶³ Hence, targeting inflammasomes may help in controlling the airway inflammation and hyperresponsiveness induced by TiO₂ 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-1 β , 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 response⁶⁶ (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-1 β levels by activating the NF- κ B and MAPK signalling pathways to promote inflammation.⁷⁴ Current evidence suggests that TiO₂ NPs can induce oxidative stress through p38. A study exploring the *in vitro* immunotoxicity of TiO₂ 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 TiO₂ 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- κ B-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 TiO₂ 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 Gd₂O₃:Eu³⁺ 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- κ B pathways⁸⁸ (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 Gd₂O₃:Eu³⁺ 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 Gd₂O₃:Eu³⁺ 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,



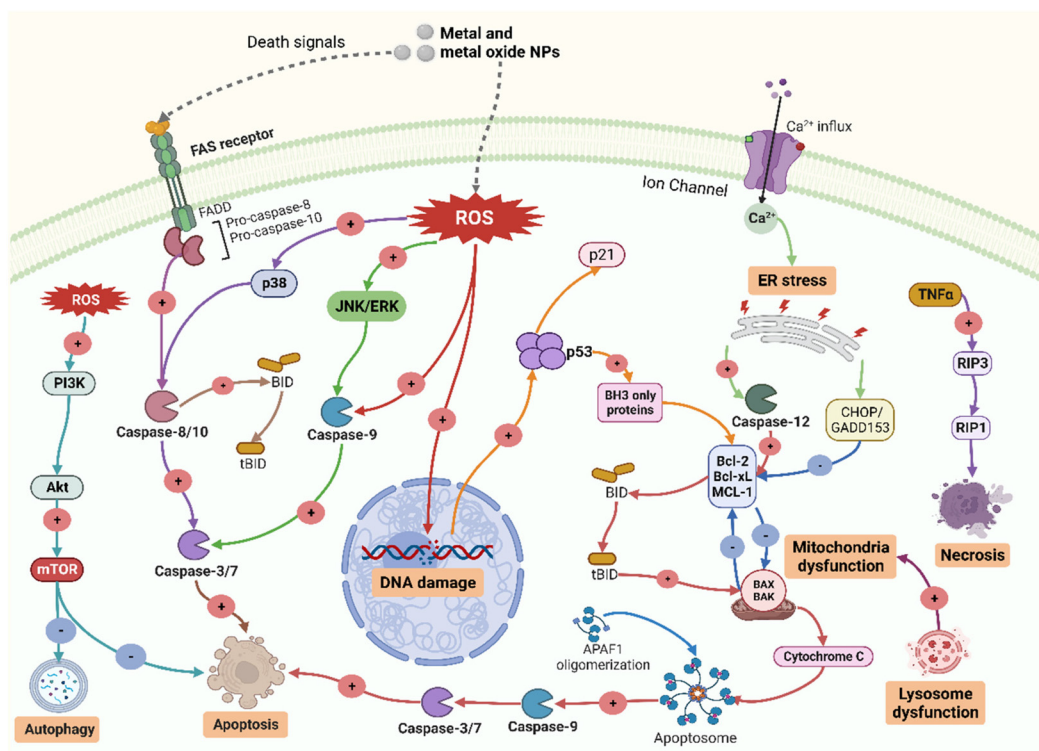


Fig. 3 Mechanisms of metal and metal oxide NP-induced apoptosis and autophagy in immunotoxicity. Apoptosis is caspase-dependent cell death involving three main pathways including death receptor pathway, mitochondrial pathway and endoplasmic reticulum stress pathway. The PI3K-Akt-mTOR pathway may have 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 Zn²⁺ ions under the acidic conditions in human THP-1 cell lysosomes, resulting in the loss of lysosome integrity and stability. However, TiO₂ NPs did not produce these effects.⁹⁸ Chen *et al.*⁹⁹ studied the effect of TiO₂ nanotubes loaded with silver NPs (Ag@TiO₂-NTs) on macrophage polarisation. They found that Ag@TiO₂-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-1 β , IL-2, IL-12p40/70, TNF- α , and IL-10, as well as MCP-1 and SDF-1 α).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 Zn²⁺ from ZnO NPs.⁹⁵ Similarly, ZnO NP-induced autophagy could promote the transfer of NPs to lysosomes and promote NP degradation and continuous Zn²⁺ release under acidic conditions.¹⁰⁵ These Zn²⁺ 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 other¹⁰⁷ (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} TiO₂ 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, TiO₂ 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 pathways¹¹³ (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 functions² (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 dysfunction¹¹⁵ (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 TiO₂ 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 TiO₂ 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-Fe₃O₄ NPs could impair mitochondrial dynamics by activating the PGC-1α pathway and inducing a loss of mitochondrial stability in DCs.¹¹⁹ PEG-Fe₃O₄ NPs also reduced autophagy to inhibit mitochondrial degradation and promote mitochondrial rupture, altering the immature state of DC function¹¹⁹ (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 stress¹²² (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 ($25\ \mu\text{g mL}^{-1}$) 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 over-expression of superoxide dismutase 2 (SOD2), but not cytoplasmic SOD, was detected in primordial macrophages exposed to M-FeNPs ($50\ \mu\text{g mL}^{-1}$); 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 ($19.3 \pm 5.4\ \text{nm}$) 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 ($20\ \mu\text{g mL}^{-1}$).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 ($\geq 12.5\ \text{ppm}$) 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 $100 \mu\text{g mL}^{-1}$, 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 ($75 \mu\text{g cm}^{-2}$ after 4 h of exposure, $75 \mu\text{g cm}^{-2}$ after 24 h of exposure; 15 and $75 \mu\text{g cm}^{-2}$).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).

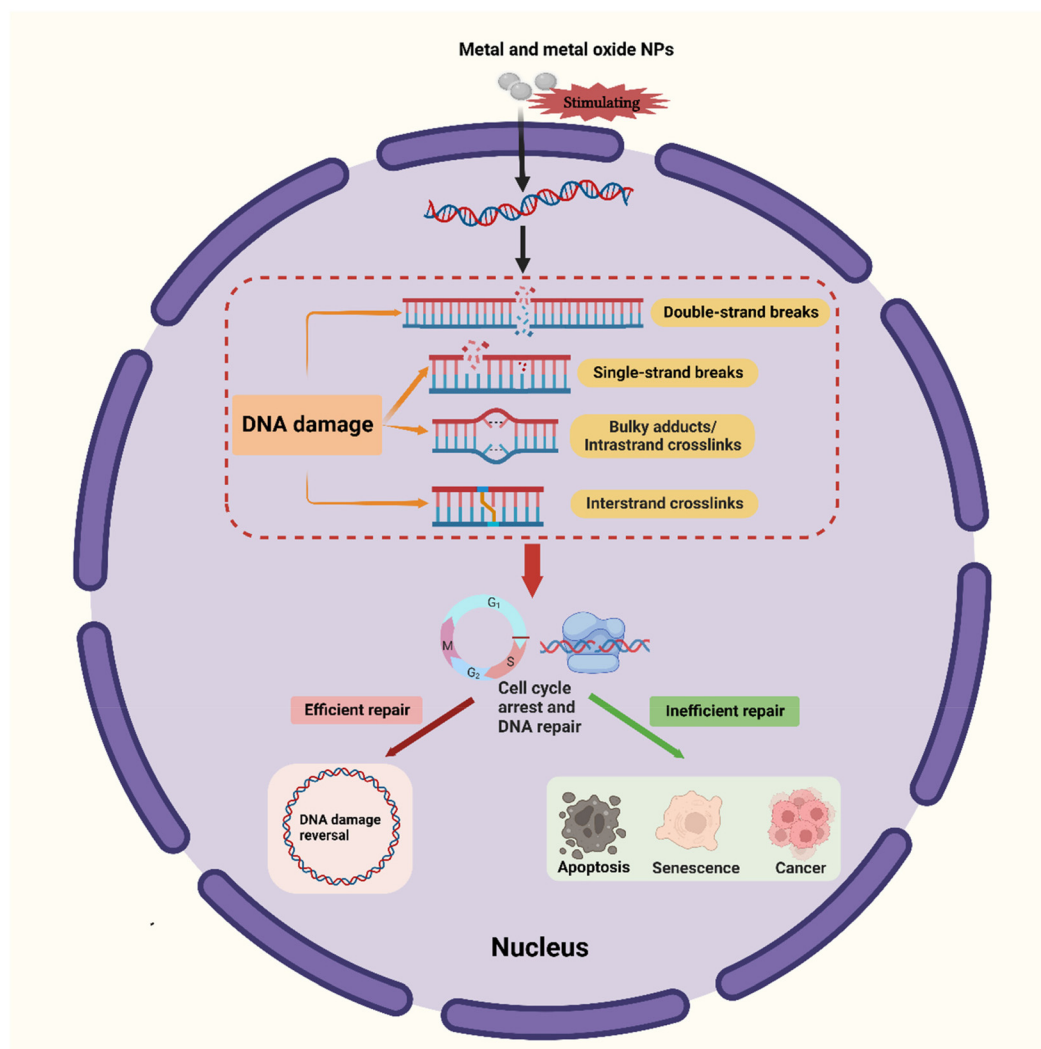


Fig. 4 Mechanisms of DNA damage induced by metal and metal oxide NPs. Metal and metal oxide NPs may cause different types of DNA damage, including DNA double/single strand breaks, DNA adducts and DNA cross-linking. DNA damage can lead to cell cycle arrest and DNA repair, while inefficient DNA repair 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 TiO₂ NPs.¹⁵² Furthermore, TiO₂ 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

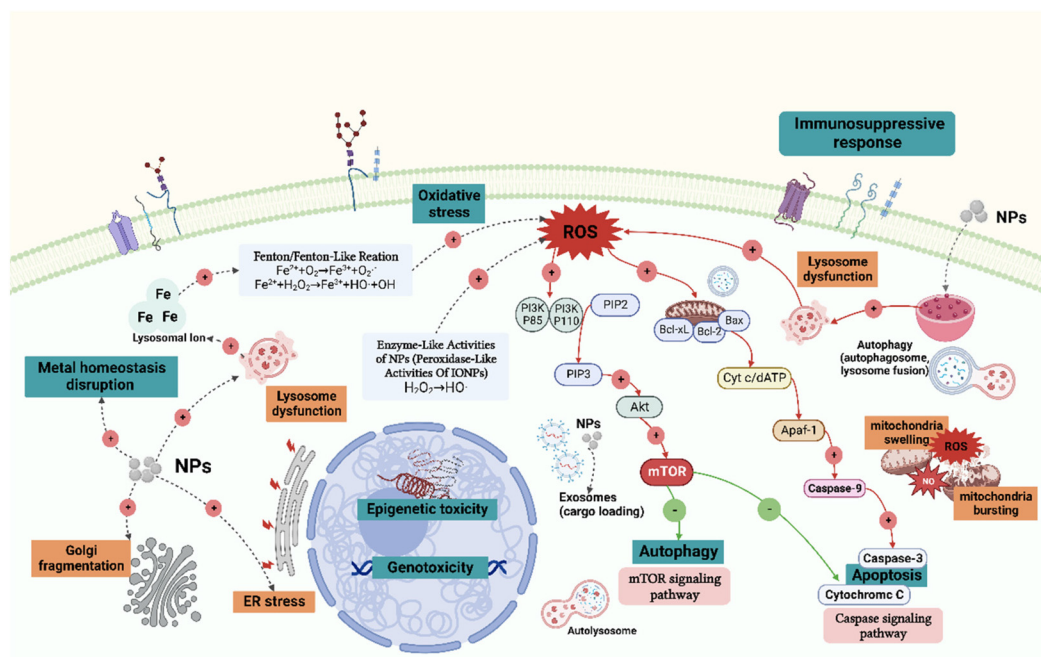


Fig. 5 Various intracellular mechanisms of immunotoxicity mediated by metal and metal oxide NPs. Metal and metal oxide NPs may cause oxidative stress, autophagy and apoptosis, which can lead to dysfunction of different organelles in immune cells, including mitochondrial and lysosomal damage, endoplasmic 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- γ t, 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, CeO₂ 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, Fe₂O₃, 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 conditions¹ (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 micro-environment 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⁺, Zn²⁺, and Fe²⁺) may be toxic even at low concentrations, participate in different cellular pathways, or induce



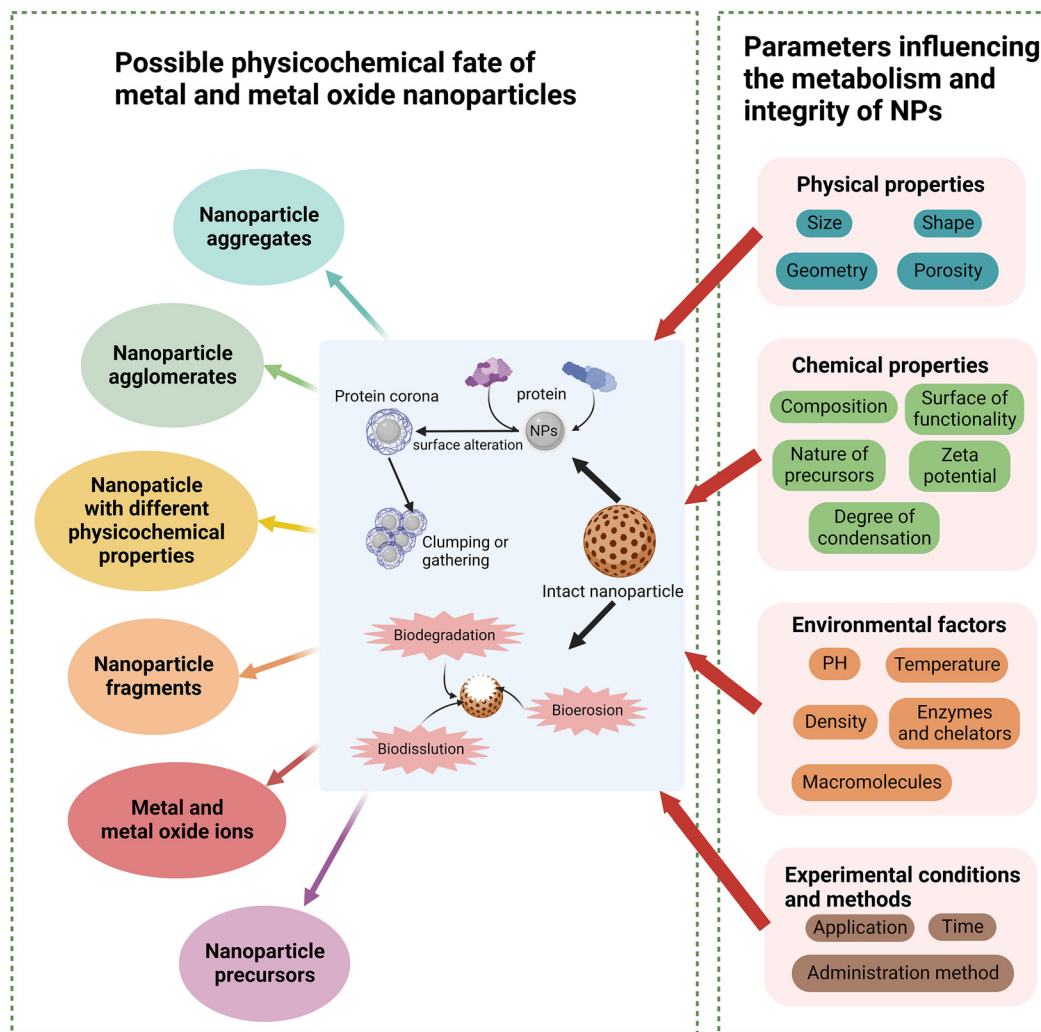


Fig. 6 Possible physicochemical fates of metal and metal oxide NPs and parameters influencing their metabolism and integrity. Metal and metal oxide NPs may lose their stability under physiological conditions in the body, form protein coronas and clump or gather, or undergo degradation, dissolution, and 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 faeces¹ (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 tract¹⁶⁷ (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-



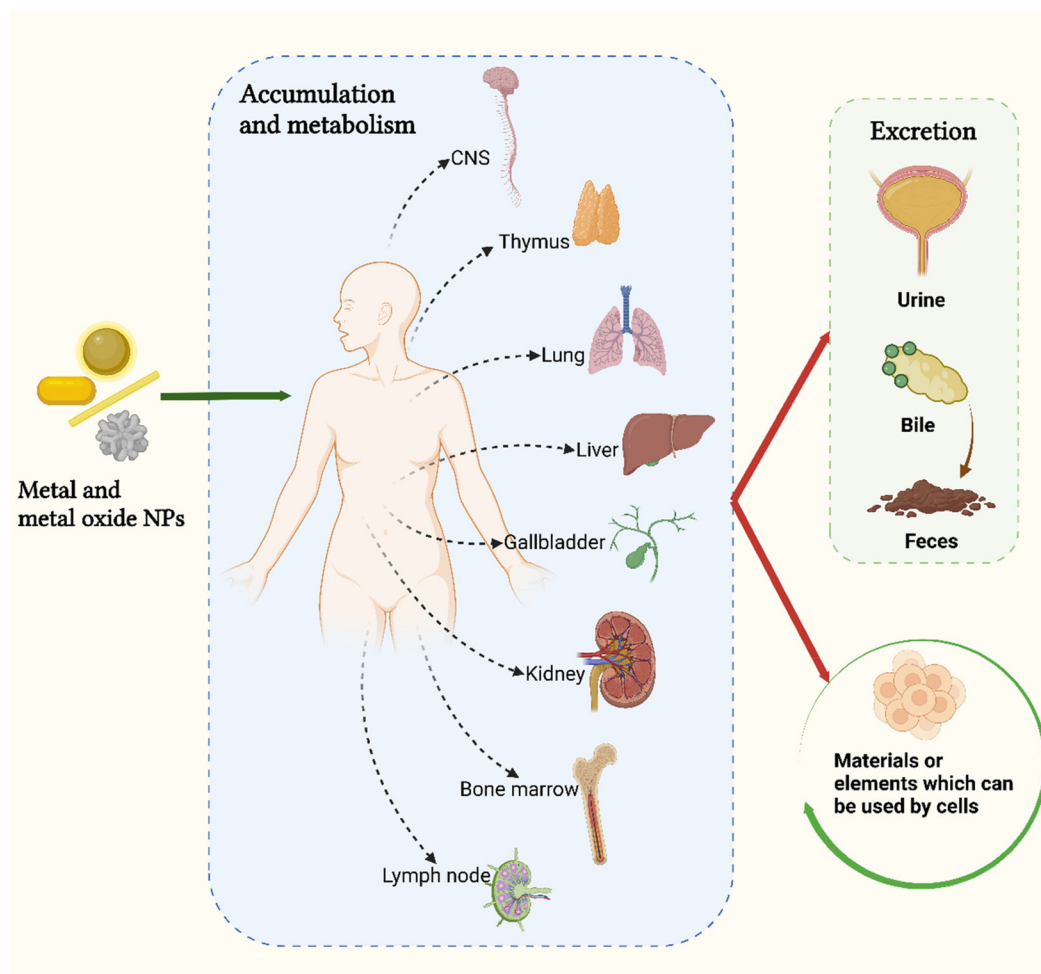


Fig. 7 Accumulation, metabolism and excretion of metal and metal oxide NPs *in vivo*. Metal and metal oxide NPs enter the body through different pathways and accumulate to varying degrees in tissues and organs related to metabolism, immunity, and consciousness such as the lung, spleen, kidney, and central 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 AgNO₃ with different sizes (25 µg Ag per dose of Ag NPs and 2.5 µg Ag per dose of AgNO₃; 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-TiO₂ and 5, 10, 60, and 90 nm anatase TiO₂) 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



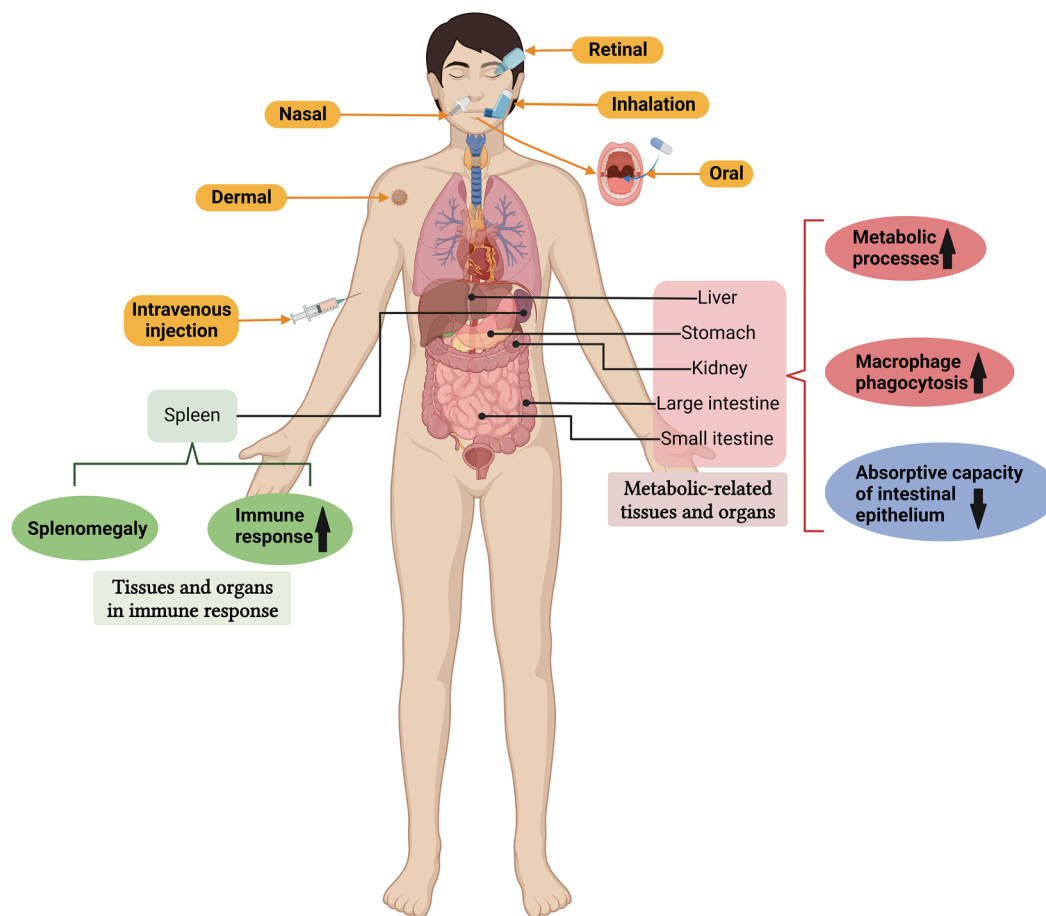


Fig. 8 Common exposure pathways for the administration of metal and metal oxide NPs and their physiological and pathological effects on metabolic and immune-related tissues and organs. Metal and metal oxide NPs can enter the body through many different ways, such as oral, inhalation, skin contact or 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 reprogramming¹⁹⁸ (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/Fe₃O₄ 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
Liver	TiO ₂	Female Wistar rats	Lung inflammation at day 1, disappearing by day 21.	303
		C57BL/6JRj mice	Increased alveolar inflammation and small granulomatous lesions.	304
		BALB/c mice	Low dose deposition in lungs of adult healthy rats to avoid nasopharyngeal deposition.	305
			Large aggregates induce higher lung response.	286
	Au		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
		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
Gallbladder	Ag	SD rats	No significant adverse toxicological effects.	309
		C57BL/6JRj mice	Blood DNA damage.	286
		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
	Cu		Increased spleen weight, number of splenocytes and splenic cell subsets.	312
		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
Thymus	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
	TiO ₂		Thymic degeneration.	48
		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 <i>Helicobacter pylori</i> to weaken <i>Helicobacter pylori</i> 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
Kidney	TiO ₂	Female Kunming mice	Decreased villus height, increased crypt depth, ileal cell apoptosis.	299
	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
	Pt		Dose-dependent effects on alkaline phosphatase and cholesterol; higher accumulation in female rats.	316
		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



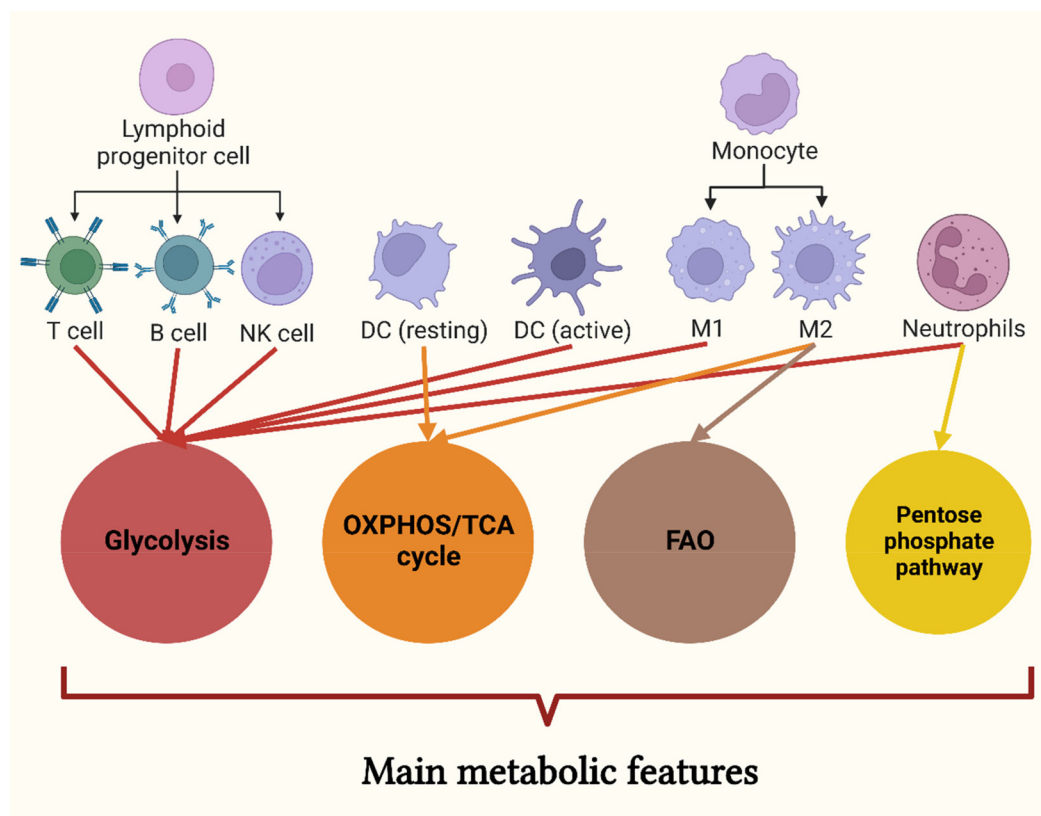


Fig. 9 Main metabolic features of innate and adaptive immune cells. Adaptive immune cells, NK cells, activated dendritic cells, M1 macrophages and neutrophils are mainly metabolized by the glycolysis pathway, in which neutrophils can also experience pentose phosphate metabolic pathway. The main metabolic 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 based 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 physico-chemical 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.

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