Do Engineered Nanomaterials Affect Immune Responses by Interacting With Gut Microbiota?

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Engineered nanomaterials (ENMs) have been widely exploited in several industrial domains as well as our daily life, raising concern over their potential adverse effects. While in general ENMs do not seem to have detrimental effects on immunity or induce severe inflammation, their indirect effects on immunity are less known. In particular, since the gut microbiota has been tightly associated with human health and immunity, it is possible that ingested ENMs could affect intestinal immunity indirectly by modulating the microbial community composition and functions. In this perspective, we provide a few pieces of evidence and discuss a possible link connecting ENM exposure, gut microbiota and host immune response. Some experimental works suggest that excessive exposure to ENMs could reshape the gut microbiota, thereby modulating the epithelium integrity and the inflammatory state in the intestine. Within such microenvironment, numerous microbiota-derived components, including but not limited to SCFAs and LPS, may serve as important effectors responsible of the ENM effect on intestinal immunity. Therefore, the gut microbiota is implicated as a crucial regulator of the intestinal immunity upon ENM exposure. This calls for including gut microbiota analysis within future work to assess ENM biocompatibility and immunosafety. This also calls for refinement of future studies that should be designed more elaborately and realistically to mimic the human exposure situation.

Keywords: engineered nanomaterials (ENMs), gut microbiota, intestinal permeability, immunomodulation, bacterial components

INTRODUCTION

Unique properties including large surface area, high catalytic properties and antimicrobial efficacy confer to engineered nanomaterials (ENMs) a significant range of applications in nanomedicine and consumer products (1, 2), raising public concerns about their biosafety. For example, nanoparticulate Ag, TiO₂, ZnO and plastics are widely used in food additives (3), components of food packaging and containers (4, 5), and toothpaste (6). Oral exposure to these ENMs in our daily
life is therefore likely through ingestion of food or water that deliberately or inadvertently contain ENMs. ENMs might therefore reach the gastro-intestinal tract (GIT) and interact with mucosal cells. Indeed, endocytosis of ENMs by intestinal epithelial cells (IECs) and various immune cells is observed using either conventional 2D in vitro models such as tumor cell lines (7, 8) or in vivo animal models (9). Moreover, it has been reported that ENMs could modulate innate/inflammatory immune responses upon direct interactions with neutrophils, macrophages, dendritic cells (DCs) and the complement system (10–13). Upon ingestion, ENMs most likely also come in contact with gut microbiota, i.e., the population of microbes residing in the intestinal lumen and mucosa. It has been long known that the gut microbiota is essential for the development of the immune system and for immune homeostasis (14). Recent observations suggest that the ENM effects on innate/inflammatory responses largely depend on the co-presence of bacterial agents such as lipopolysaccharide (LPS) (15, 16). Thus, it is a logical assumption that ENMs could affect immunity by altering gut microbiota, a concept that is currently unexplored.

Herein, we provide an overview of the current state-of-the-art, and discuss a hypothetical scenario in which ingested EMNs may affect host immunity by modulating the gut microbiota. From published in vivo studies in different models and with different ENMs, a high level of variability is found regarding the ENM effects on gut microbiota and local/systemic immunity (Table 1).

LPS AND SCFAS: TWO REPRESENTATIVE MICROBIAL MOLECULES BRIDGING GUT MICROBIOTA AND INTESTINAL IMMUNITY

Mounting evidence has highlighted the tremendous contribution of gut microbiota to human physiology (30–35). Within this microbiota-immune system interaction, a large amount of microbial metabolites and components serve as potent effectors to orchestrate their communication (36, 37). We will specifically discuss hereafter the immunomodulatory effects of short-chain fatty acids (SCFAs) and LPS. More comprehensive information is shown in Figure 1 and extensively discussed in other excellent reviews (32, 33, 36–39). SCFAs are generated from indigestible oligosaccharides by gut commensals, including Lactobacillus, Bacteroides, Bifidobacterium, Faecalibacterium, etc. (40). LPS is the major membrane component of Gram-negative bacteria and has profound immunostimulatory and inflammatory capacity (41). The immunological effects of these microbiota-derived molecules are manifold, covering innate and adaptive immunity.

Regulation of Innate Immunity

As a physical barrier at the intestinal surface, IECs are equipped with an array of immune receptors to sense and integrate microbiota-derived metabolites and components for maintaining immune homeostasis. By activating G-protein-coupled-receptors (GPR41, GPR43, GPR109A) on IECs, SCFAs can promote the activation of the NOD-like-receptor-protein 3 (NLRP3) inflammasome, inducing production of the homeostatic cytokine interleukin-18 (IL-18) (42). SCFAs can also stimulate goblet cell differentiation, mucin gene transcription and mucus secretion (43). Pattern recognition receptors (PRRs) on the IEC surface, such as Toll-like receptors (TLRs), can sense microbial antigens. Notably, a number of homeostatic mechanisms ensure immune tolerance towards commensals, such as the basolateral location of the LPS receptor TLR4 that allows binding and activation only to invading bacteria (44) and the constitutive expression of the anti-inflammatory IL-1R8, which binds to and inhibits TLR and IL-1 receptors (45).

Intriguingly, the commensal gut microbiota also interacts with IECs to maintain an effective gut barrier. SCFAs, particularly butyrate, have crucial roles in regulating tight junction (TJ) proteins via multifaceted signaling pathways (46), such as HIF-1 stabilization (47), and histone deacetylase (HDAC) inhibition (48). By contrast pathogenic E. coli Shiga-toxins and LPS (49) could compromise the epithelial barrier by disrupting TJ. LPS increases intestinal epithelium permeability through the TLR4/MyD88/TGF-β activated kinase 1 (TAK1)/nuclear-factor-κB (NF-κB) cascade in both in vitro and in vivo models (50).

Immunoregulation of gut microbiota also covers innate lymphoid cells (ILCs), a subpopulation of innate cells (natural killer cells, ILC1, ILC2, ILC3) specialized in recognizing and reacting to infectious challenges. SCFAs can modulate ILC3 proliferation and stimulate IL-22 production in an AKT/STAT3-dependent manner. IL-22 promotes antimicrobial peptide (AMP) production, mucin secretion and colonization of commensal microbes (51).

Intestinal resident macrophages maintain the tissue homeostasis by removing senescent and anomalous cells, and contribute to tissue defense by eliminating invading pathogens and foreign objects. Upon binding to TLR4, LPS can promote inflammatory macrophage activation (M1 polarization), with the production of an array of inflammatory cytokines, IL-1β, IL-6, IL-12 and tumor necrosis factor-α (TNF-α) (52). Conversely, SCFA butyrate facilitates the anti-inflammatory/tissue-healing macrophage polarization, probably by activation of the H3K9/STAT6 signaling pathway (53).

Regulation of Adaptive Immunity

The impact of gut microbiota goes beyond the innate immunity, through its ability to affect the activation of antigen-presenting cells (APCs), which are the link between innate and adaptive immunity. APCs in the gut encompass resident DCs and tissue macrophages, which are involved in antigen presentation to naive and primed T cells. Activation, maturation and functionality of DCs and macrophages can be influenced by LPS and SCFAs. As the major APCs in the intestine (54), macrophages can be regulated by microbial niacin and butyrate via activating GPR109A, which in turn increases production of anti-inflammatory IL-10 and Aldehyde-Dehydrogenase-1-Family-Member-A1 (ALDH1A1), and induces differentiation of T cells (55). LPS is a potent elicitor of DC
Engineered NanoMaterials Animal model Exposure dose Exposure way and duration Analysis methods of gut microbiota Gut microbiota changes by ENM treatment Immune markers Clinical effect/Immune response References

Silver nanoparticles with a diameter of 55 ± 3 nm 3 mo-old C57BL/6 female mice 0, 11.4, 114 and 1140 μg/kg bw Oral gavage for 28 days 16S RNA Sequencing of Bacterial DNA from Fecal Samples Odonobacteraceae, Bacteroidaceae and 524-7 family decreased while Lactobacillaceae and Lachnospiraceae increased Firmicutes/Bacteroides ratio reduced. Akkermansia, Bacteroides and Prevotella increased, while Lactobacillus decreased Serum C-reactive protein level; histology of ileum villi, intestinal goblet cells, glycoplyx and colon Blood cell level, serum lymphocyte level, colon length, disease activity index (DAI), histology of colon; intestinal permeability; IL-1β, IL-6 and TNF-α in small bowel and colon No overt effect on body weight gain, the intestinal histology as well as the serum C-reactive protein level. (17)

Silver nanoparticles with a diameter of 12 ± 3 nm 7 wk-old CD-1 (ICR) male mice 2.5 mg/kg bw/ Dy Oral gavage for 7 days Pyrosequencing of 16S RNA genes in fecal samples Escherichia coli, Bacteroides and Prevotella increased, while Lactobacillus decreased Stool consistency; colon length; weight; colon histopathology; myeloperoxidase activity in the colon. Colon smooth muscle thickness; Presence of ulcers, hemorrhage, fecal blood, and diarrhea. Histology of liver, kidney, ileum and myocardium. Twenty-four-hour urine and feces. Not studied. (18)

Silver nanoparticles with a diameter of 294 nm 6 wk-old BALB/c male mice 5 mg/dy Oral gavage for 4 days A few specific bacteria from the colon mucosa were isolated and colonized by selective plates Lactobacillus sp. decreased, while Clostridium perfringens and Escherichia coli increased but not significantly Lactobacillus sp. increased while Clostridium perfringens and Bacteroides decreased Stool consistency; colon length; weight; colon histopathology; myeloperoxidase activity in the colon. Colon smooth muscle thickness; Presence of ulcers, hemorrhage, fecal blood, and diarrhea. Histology of liver, kidney, ileum and myocardium. Twenty-four-hour urine and feces. Not studied. (19)

Silver nanoparticles with a diameter of 122 nm PVP-stabilized silver nanoparticle with a diameter of 14 nm 4 wk-old Wistar Hannover Galas rats 2.25, 4.5 or 9 mg/kg twenty 16S rRNA sequencing of contents in the cecal tips Bacterial phyla in caecum content were quantified by qPCR 16S rRNA sequencing of contents in the cecal tips Lactobacillus sp. increased while Clostridium perfringens and Bacteroides decreased No significant change No significant change Blood cell level, serum lymphocyte level, colon length, histology of colon; intestinal permeability; IL-1β, IL-6 and TNF-α in small bowel and colon No overt effect on body weight gain, organ weight, organ histology and leucocyte infiltration Not studied. (20)

PVP- or citrate-coated silver nanoparticles with a diameter of 20 and 110 nm 10-12 wk-old C57BL/6NCrl male mice 15 mg/kg/bw Oral gavage for 28 days Bacterial phyla in caecum content were quantified by qPCR 16S rRNA sequencing of contents in the cecal tips Bacterial phyla in caecum content were quantified by qPCR Bacteroides decreased No significant change No significant change Blood cell level, serum lymphocyte level, colon length, histology of colon; intestinal permeability; IL-1β, IL-6 and TNF-α in small bowel and colon No overt effect on body weight or histology of key organs No effect on body weight or histology of key organs. (21)

TiO2 nanoparticles with a diameter of 17 ± 2 nm 7 wk-old CD-1 (ICR) male mice 2.5 mg/kg/bw Oral gavage for 7 days Pyrosequencing of 16S RNA genes in fecal samples Bacteroides and Akkermansia increased Escherichia-Shigella and Rhodococcus increased, while Bacteroidetes and Firmicutes decreased Histology of liver, spleen, kidney, lung, heart, brain, jejunum and colon. NP deposition in these organs mentioned. No effect on body weight or histology of key organs. (22)

Spherical anatase TiO2 nanoparticles with a diameter of 20 nm in water, of 134 ± 22 nm in gastric fluid, of 420 ± 25 nm in intestinal fluid Edged corner rutile TiO2 nanoparticles with a diameter of 16 nm in water, of 148 ± 30 in gastric fluid, of 301 ± 8 nm in intestinal fluid 8 wk-old C57BL/6 male mice 100 mg/kg bw/ Dy Oral gavage for 28 days 16S RNA Sequencing of Bacterial DNA from Fecal Samples Bacteroides and Akkermansia increased Escherichia-Shigella and Rhodococcus increased, while Bacteroidetes and Firmicutes decreased Histology of liver, spleen, kidney, lung, heart, brain, jejunum and colon. NP deposition in these organs mentioned. No effect on body weight or histology of key organs. (22)

Spherical anatase TiO2 nanoparticles with a diameter of 29 ± 9 nm 3 wk-old Sprague-Dawley rats 0, 2, 10, 50 mg/kg ten Oral gavage for 30 days 16S RNA Sequencing of Bacterial DNA from Fecal Samples Increased abundance of L. gasseri, Tunicibacter, and L. NKAA136_group and decreased abundance of Veillonella Body weight; LPS and short-chain fatty acids content in the feces; colon histology; fecal metabolites; presence of glutathione, glutathione peroxidase, lipid peroxidation products, superoxide dismutase, and sulfhydryl groups in tissue homogenates; Inflammatory cytokines in serum Histology of the jejunum, duodenum and ileum; serum cytokines and immunoglobins Accumulation of malondialdehyde and decreased activity of superoxide dismutase were detected in colon tissues; Increased concentration of IL-6 in the serum. The number of goblet cells decreased and inflammatory cells infiltrated in colon epithelium. (23)

Spherical anatase TiO2 nanoparticles with a diameter of 20 nm 4 wk-old Sprague-Dawley rats 0, 2, 10, 50 mg/kg ten Oral gavage for 30 days 16S RNA Sequencing of Bacterial DNA from Fecal Samples Increased abundance of L. gasseri, Tunicibacter, and L. NKAA136_group and decreased abundance of Veillonella Body weight; LPS and short-chain fatty acids content in the feces; colon histology; fecal metabolites; presence of glutathione, glutathione peroxidase, lipid peroxidation products, superoxide dismutase, and sulfhydryl groups in tissue homogenates; Inflammatory cytokines in serum Histology of the jejunum, duodenum and ileum; serum cytokines and immunoglobins Accumulation of malondialdehyde and decreased activity of superoxide dismutase were detected in colon tissues; Increased concentration of IL-6 in the serum. The number of goblet cells decreased and inflammatory cells infiltrated in colon epithelium. (23)

ZnO nanoparticles with a diameter of average 71.61 nm 26 dy-old weaned pigs 150, 300, or 450 mg/kg in diet Dietary exposure for 21 days The cecal, colonic and rectal contents were spread on selective plates to assess E. coli, Salmonella, Lactobacillus, and Bifidobacterium E. coli decreased Histology of the jejunum, duodenum and ileum; serum cytokines and immunoglobins Signiﬁcant improvements in average daily weight gain, average daily feed intake and gain to feed ratio were observed. The diarrhea rate was reduced. The villus height in the jejunum, duodenum and ileum was increased. The blood concentration of IgA, serum concentrations of IL-6 and TNF-α was increased; while the blood concentration of IgM was decreased. (24)

(Continued)
### TABLE 1 | Continued

| Engineered NanoMaterials | Animal model | Exposure dose | Exposure way and duration | Analysis methods of gut microbiota | Gut microbiota changes by ENM treatment | Immune markers | Clinical effect/Immune response | References |
|--------------------------|--------------|---------------|---------------------------|-----------------------------------|----------------------------------------|---------------|-------------------------------|------------|
| ZnO nanoparticles with a diameter of 23-25 nm | 27 dy-old weaned piglets | 600 mg/kg in diet | Dietary exposure for 14 days | 16S rRNA sequencing of the intestinal contents | Lactobacillus increased while Prevotella and Oscillibactera decreased in the colon | Histology of jejunal tissue; gene expression of pro-inflammatory cytokines, cell proliferation markers, antioxidant markers, tight junction proteins and cell death markers in the jejunal tissue | The diarrhea incidence was reduced; average daily gain and feed intake were unaltered; villus height as well as the ratio of villus height to crypt depth was increased; the expression of antioxidant enzymes and tight junction in the jejunal tissues was increased significantly; the expression of cell proliferation markers was increased; the expression of pro-inflammatory markers was reduced. Ulceration, crypt damage, and inflammatory cell infiltration were observed in the duodenum and colon. The intestinal permeability was significantly increased. IL-1β, IL-6, and TNF-α increased in the duodenum and the colon. White blood cell, lymphocytes, and intermediate cell counts significantly elevated in the serum. Slight microvilli damage and inflammatory cell infiltration in duodenum and a few inflammatory cell infiltrations in colon. Significant increase of intestinal permeability and the elevated levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α in duodenum and colon were observed. Slightly pathological changes of epithelium loss and inflammatory cell infiltration in duodenum. Significant increase of intestinal permeability and the elevated levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α in duodenum and colon were observed. | (25) |
| SWCNT with a diameter of 1 nm and a length of 1-5 μm | 7 wk-old CD-1 (ICR) male mice | 0.05, 0.5, and 2.5 mg/kg bw/dy | Oral gavage daily for 7 days | 16S rRNA sequencing of fecal samples | Bacteroides, Prevotella, and Akkermansia increased, whereas Bacteroides, Lachnospiraceae and Lactobacillus decreased | Intestinal histology, intestinal epithelium permeability, cytokine production in both duodenum and colon and lymphocyte abundance in the serum. | | | |
| MWCNT with a diameter of 8 ± 1 nm and a length of 0.5-2 μm | 7 wk-old CD-1 (ICR) male mice | 2.5 mg/kg bw/dy | Oral gavage daily for 7 days | 16S rRNA sequencing of fecal samples | Bacteroides, Prevotella, Akkermansia, and Ruminococcaceae increased, whereas Bacteroides, Lachnospiraceae and Lactobacillus decreased | Intestinal histology, intestinal epithelium permeability, cytokine production in both duodenum and colon and lymphocyte abundance in the serum. | | | |
| Graphene oxide nanoparticles with a thickness of 1-2 μm and a dimension area of 1-14 μm² | 23-30 dy-old BALB/c mice | 2.5 mg/kg bw/dy | Oral gavage or intraperitoneal dosing weekly for 7 or 8 weeks | 16S rRNA sequencing of fecal samples | Lachnospiraceae, Lactobacillus, Ruminococcaceae, Akkermansia, Oscillibacter, and Prevotella increased, while Bacteroides and Bacteroides decreased | Intestinal histology, intestinal epithelium permeability, cytokine production in both duodenum and colon and lymphocyte abundance in the serum. | | | |
| Lysine-modified SWCNT with a length of 400 nm and a diameter of 2-3 nm | 23-30 dy-old BALB/c mice | 4.25 mg/wk | Oral gavage or intraperitoneal dosing weekly for 7 or 8 weeks | 16S rRNA sequencing of fecal samples | The α- and β-diversity of the mouse microbiota reduced in the duodenum and not in colon or jejunum. | Body weight, liver and kidney weight. | | No overt effect on body weight as well as liver and kidney weights | (27) |
| Polyethylene microplastics with a diameter of 10-150 μm | C57BL/6 mice | 6, 60, and 600 μg/dy | Dietary exposure for 5 weeks | 16S rRNA sequencing of fecal samples | The α- and β-diversity of the mouse microbiota increased, Staphylococcus increased, while Panbacteroides decreased | Serum cytokine; T cells in the spleen; TLR4, AP-1, and IRF5 expression; intestinal histology. | Serum concentrations of IL-1α increased; the percentage of Th17 and Treg cells among CD4+ cells decreased; edema occurred and lymphocyte and plasma cell infiltration was observed in the lamina propria of the colon and duodenum; TLR4, AP-1, and IRF5 expression significantly increased in the colon and duodenum. | (28) |
| Cuboid CuO nanoparticles with a dimension area of 20 nm by 50 nm | Eisenia fetida with a weight range between 300 and 600 mg | 160 mg/kg soil | Exposure to soil containing ENMs for 28 days | 16S rRNA sequencing of microbiota in gut tissue | Candidatus Lumbria limicola and Luteibacter decreased | Histology of the gut epithelium and longitudinal muscle tissue; expression of coelomic cytolytic factor, lysozyme. | No overt effect on tissue integrity, and immune responses | (29) |

Doses relevant for human exposure level are marked using underline. AP-1, activating protein-1; Bw, body weight; CuO, copper oxide; DSS, dextran sulfate sodium; Dy, day; GIT, gastrointestinal tract; IL, interleukin; Ig, immunoglobin; IRF5, interferon regulatory factor 5; LPS, lipopolysaccharide; M0, month; MWCNT, multi-walled carbon nanotubes; PVP, polyvinyl pyrrolidone; SWCNT, single-walled carbon nanotubes; Th17, T helper type 17; TNBS, trinitrobenzene sulfonic acid; TLR4, Toll-like receptor 4; TNF, tumour necrosis factor; Wk, week; ZnO, zinc oxide.
migration and maturation by activating mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways (56). SCFAs can block the DC generation from bone marrow stem cells (57), and down-regulate expression of the T cell-stimulatory proteins CD80, CD83 and major-histocompatibility-complex class II (MHCII) (58).

Through its effects on APCs that produce several cytokines necessary for T cell activation, the gut microbiota is also involved in differentiation of naïve CD4+ T cells into defined subsets, including T helper (Th1, Th2 and Th17) and regulatory T cells (Tregs). Inhibition of HDAC by SCFAs can regulate the mTOR–S6K pathway required for generation of Th17, Th1, and IL-10+ T cells (59). Tregs have important anti-inflammatory roles, allowing the immune system to tolerate antigens derived from gut microbiota and diet. Through binding to GPR43, SCFAs can stimulate Treg proliferation (60). Additionally, SCFAs control the expression of genes necessary for plasma B cell differentiation and Immunoglobulin A (IgA) production (61). As the largest class of immunoglobulins in the intestinal mucosa, IgA targets microbial antigens and preferentially coats colitogenic bacteria, therefore preventing inflammation and perturbation of intestinal homeostasis (62).

**NUMEROUS ENMs COULD RESHAPE THE GUT MICROBIOTA SIGNATURE BUT IT IS NOT A GENERAL EFFECT**

ENMs might interact with gut microbes in different manners (Figure 2). Of special concern is the intrinsic antimicrobial potency of some ENMs. Nanoparticulate Au, Ag, TiO₂ and...
ZnO can exert bactericidal activity by disrupting the bacterial membrane (63, 64), inducing intracellular reactive oxygen species (64, 65) and causing direct genotoxicity (66). Conversely, iron oxide and graphene ENMs can promote the growth of some bacterial species, with mechanisms still largely unknown (67, 68). Adding to the complexity is that many gut microbes could rapidly develop strategies to resist ENM bactericidal actions (69). Gram-negative bacteria are thought to be more tolerant to ENMs, either impairing the bacterial membrane, or causing intracellular oxidative stress, or generating genotoxicity. As responses to the ENM bactericidal effects, members of gut microbiota may rapidly develop resistance, but the associated molecular strategies and efficacy often differ among distinct members. Many in vitro and in vivo assays showed that ENMs can alter the gut microbiota profile, enrich the relative abundance of pathogens or decrease that of gut commensals. This effect often associates with intestinal inflammation and tissue injury. While some ENMs could increase gut commensals, which in turn exert anti-inflammatory effects. Conversely, a few works show that the gut microbiota remains resilient following oral exposure to ENMs, indicating that the ENM effect on gut microbiota/mucosal immunity is not general.

**FIGURE 2** | ENMs could not only modulate several components of the mucosal immune systems directly, but also reshape the gut microbiota, which may potentially act as an alternative but important regulator to mediate the immuno-modulatory effects of ENMs. ENMs could accumulate and directly interact with neutrophils, macrophages, dendritic cells (DCs) and the complement system to modulate innate/inflammatory immune responses. On the other hand, several metallic and non-metallic ENMs are proved to be bactericidal, either impairing the bacterial membrane, or causing intracellular oxidative stress, or generating genotoxicity. As responses to the ENM bactericidal effects, members of gut microbiota may rapidly develop resistance, but the associated molecular strategies and efficacy often differ among distinct members. Many in vitro and in vivo assays showed that ENMs can alter the gut microbiota profile, enrich the relative abundance of pathogens or decrease that of gut commensals. This effect often associates with intestinal inflammation and tissue injury. While some ENMs could increase gut commensals, which in turn exert anti-inflammatory effects. Conversely, a few works show that the gut microbiota remains resilient following oral exposure to ENMs, indicating that the ENM effect on gut microbiota/mucosal immunity is not general.

**Akkermansia** increased (22). Oral administration of non-metallic single-walled carbon nanotubes (SWCNTs) modestly altered the α- and β-diversity of the mouse microbiome (27). The modulation of animal gut microbiota by other ENMs is systemically summarized in recent reviews, which include nanoparticulate plastics, graphene oxide, multi-walled carbon nanotubes (MWCNT), SWCNT, Ag, ZnO, MoO₃, MoS₂, TiO₂, CuO and SiO₂ (5, 71, 72). Numerous in vitro assays also validate the ENM modulatory effect on gut microbiota samples (68, 73–75). Importantly, there appears to be no consensus effect, as multiple ENM-related factors (dose, physicochemical nature, particle size, surface charge, shape and stability) might dictate their modulatory mechanisms and efficacy (64, 76). In addition, the gut microbiota signature varies among individuals, and even within the same subject it changes with time, food intake and health conditions (77–79).
ENMs could accumulate in the intestine, favor inflammatory responses and impair the barrier function, including IEC apoptosis, tight junction opening, decreased AMP production, Th1/Th2 imbalance, aberrant IgA secretion and inflammatory activation of macrophages (72). In this situation, the gut microbiota can be in turn altered by the mucosal immunity. But, a considerable number of ENMs (Ag, SWCNT, CuO, TiO2) are shown to alter the gut microbiota without inducing any detectable changes in intestinal immunity (17, 22, 27, 29). These data suggest that ENMs may cooperate with the mucosal immunity to modulate the gut microbiota.

THE POTENTIAL EFFECT OF ENM-ALTERED MICROBIOTA ON INTESTINAL INFLAMMATION

As discussed above, microbiota-derived metabolites such as SCFAs have important roles in the regulation of gut immunity (Figure 1), while ENM exposure that reduces SCFA-producing bacteria may perturb the immune homeostasis and cause inflammation (Table 1). Indeed, gut microbiota dysbiosis appears to tightly associate with inflammatory bowel disease (IBD), a chronic and relapsing inflammatory disorder of the intestine (80). This link has been observed in several in vivo assays that model GIT exposure to ENMs. Oral administration of Ag ENM (2.5 mg/kg body weight daily) in mice profoundly reduced the Firmicutes to Bacteroidetes ratio, specifically due to an increase in Alistipes, Bacteroides and Prevotella, and a significant decrease in SCFA-producing Lactobacillus. The altered microbiota could cause some IBD-like symptoms, including disrupted epithelium structure, increased intestinal permeability and upregulation of inflammatory cytokines (IL-1β, IL-6 and TNF-α) (18). In the same study, oral gavage of TiO2 ENMs (2.5 mg/kg body weight daily) significantly decreased the probiotic Bacteroides and triggered a low-grade colonic inflammation (18). Likewise, administration of SWCNT, MWCNT and graphene oxide ENMs (2.5 mg/kg body weight daily) in mice disrupted the gut microbiota signature, with commensal Lactobacillus and Bacteroides decreased. The exposed mice displayed tissue injury, increased intestinal permeability and elevated production of inflammatory IL-1β, IL-6, and TNF-α (26).

Moreover, enrichment of pathogens and associated virulence factors following ENM administration could also cause intestinal inflammation (81). The work of Chen et al. showed that oral administration of TiO2 ENMs (50 mg/kg body weight) in rats decreased the number of goblet cells, elicited immune cell infiltration and mitochondrial abnormalities in the colon tissues, suggesting redox imbalance and inflammation. TiO2 ENM treatment remarkably affected the fecal metabolite profile, and particularly enriched the LPS content (23). In another work, oral gavage of TiO2 ENM (100 mg/kg body weight daily) in mice impaired the intestinal microvilli structure, and increased Escherichia and Shigella, two potential pathogens for elicitation of intestinal inflammation (22). Dietary nanoplastics (600 μg daily) for mice significantly increased pathogenic Staphylococcus abundance alongside a decrease in Parabacteroides (28). The ENM-feeding group displayed a chronic intestinal inflammation, such as increased serum IL-1α, abnormal ratio of Th17 and Treg cells among CD4+ cells, infiltration of lymphocytes and plasma B cells in the lamina propria, and higher expression of inflammatory markers (TLR4, AP-1, and IRF5) (28). In these cases, ENMs may enrich opportunistic pathogens or liberate the membrane-bound PAMPs from bacterial cells (82). The inflammatory antigens, such as LPS, exotoxin and flagellin, would bind to PRRs on IECs and immune cells, thus activating inflammatory pathways and promoting an excessive intestinal inflammation (83–86). However, most of these in vivo studies based on animal models rarely simulated the realistic human exposure condition. Table 1 details such shortcomings: either subjects were exposed to an excessive dose of ENMs, or ENMs were administered alone without food which is not a real-life fashion. Whether ENMs were contaminated by LPS was not checked, either.

There is no general effect of ENMs on gut microbiota and intestinal immunity. Contrary to the aforementioned adverse effects, other studies showed that ENM ingestion can increase commensal microbes and exert anti-inflammatory effects. Dietary ZnO ENMs (600 mg/kg food) for weaned piglets increased Lactobacillus, leading to upregulation of tight junction proteins and antioxidant enzymes, and decreased expression of inflammatory interferon-γ (IFN-γ), IL-1β, TNF-α and NF-κB (25). Similarly, oral gavage of Ag ENMs (5ng daily) attenuated the dextran sodium sulfate (DSS)-induced IBD symptoms in mice, probably by increasing Lactobacillus and decreasing Clostridium perfringens and Escherichia coli (19). Enrichment of Lactobacillus was found in these works, again highlighting the protective role of SCFA-producers in epithelium integrity and anti-inflammatory responses.

Strikingly, several studies found no significant effect of ENMs (Au, CuO, Ag and lysine-modified SWCNts) on intestinal immunity (17, 20, 21, 27, 29, 87). One possibility is that most ENMs may be rapidly excreted following ingestion, so few accumulate in the GIT and they are insufficient to modulate the immune responses. Indeed, 270-day consecutive dietary supplementation with ZnO ENMs (1600 mg/kg food) for mice revealed no detectable ENM distribution in the GIT (88). Hence, this work indicates that there is no general effect regarding the biodistribution and accumulation, it should be specific to each ENM. Additionally, the mucus layer that is mainly composed of highly-glycosylated secreted proteins overlying the intestinal epithelium could trap ENMs and minimize their contact with gut microbes and mucosal cells (8). This can explain why the modulatory effect of ENMs on gut microbes in vitro is always greater than that in vivo. When the earthworms were exposed to soil with CuO or Ag ENMs, the gut microbiota remained largely resilient, whereas both ENMs significantly changed the soil bacterial community composition (89). Moreover, though some ENMs can modify the gut microbiota, members of the core commensal consortium are not affected; or the roles of redundant symbionts affected by ENMs could be compensated by other unchanged commensals. For example, exposure of earthworms to soil
supplemented with CuO ENMs (160 mg/kg) induced substantial changes in the gut microbiota with a significant decrease in the symbiont Candidatus Lumbricincola, but it had no effect on the immune competence (29). Thereby, the gut microbiota might adapt itself in a way (which needs to be demonstrated) that ensures maintaining a proper immune homeostasis.

CONCLUSIONS AND PERSPECTIVES

To summarize, increasing observations have claimed a link between GIT exposure to ENMs, gut microbiota dysbiosis and intestinal inflammation (Figure 2). Such effects of ENMs are often dose-dependent. We acknowledge that in a few cases ENMs could induce microbiota dysbiosis characterized by a decrease in commensals (Lactobacillus, Bacteroides, Bifidobacterium, etc.) and/or an enrichment of other members (E. coli, Shigella, Listeria, etc.), which in turn cause an intestinal inflammation, compromise epithelium integrity and induce IBD-like symptoms (Figure 2). But these works suffer shortcomings and are not relevant for human exposure doses or uptake conditions. By contrast, little or no overt effect on intestinal immunity has been found in a large number of in vivo assays, where ENMs are orally administered in a more realistic dose or fashion. Notably, most in vivo studies investigate the immunotoxicity of ENMs in healthy individuals, while it might be more prominent in those with intestinal inflammation (such as IBD). Indeed, inflammatory symptoms like mucus defects (90), dysfunctional macrophages (91), etc. could increase and extend the exposure of intestinal epithelium to ENMs. Interestingly, the DSS-induced IBD symptoms in mice can be either exacerbated (92) or attenuated (19) following oral intake of ENMs, suggesting that ENM exposure do not necessarily have detrimental consequences, even for those with inflamed intestine. Future works should cover more types of ENMs, simulate the real-life ENM exposure situation, exploit both healthy and inflammatory host model, and draw cautious conclusions.

The in vivo studies on different animal models show extensive variation regarding the ENM effects on gut microbiota or intestinal immunity (Table 1). This may be due to discrepancies in the overall experimental settings (animal species, age, EMN exposure time, dose and uptake manner), the ENM physicochemical nature (size, shape, surface decoration and charge), the possible in vivo bio-transformation of ENMs and the methodology for gut microbiota analysis (Table 1). A unifying exposure model is required.

However, pitfalls of current animal models should be considered when translating gut microbiota research results to humans. The murine gut microbiota resembles the human one at phylum level, but differs at genus and species level (93). The anatomy and physiological functions of several GIT segments in the mouse are also different from those of humans (93). Therefore, a future perspective is to establish human models, necessarily in vitro, based on primary cells. To this end, micromlidic intestine-on-chips that can establish a prolonged coculture of human intestinal epithelium and gut microbes could be a promising in vitro human model to evaluate the ENM immunotoxicity (94, 95). When supplemented with immune cells, the intestine-on-a-chip could enable us to monitor the dynamics of ENM behavior in the gut tissue, gut microbiota changes, intestinal barrier function, immune cell activation and inflammation, thus providing predictive values on the ENM immunotoxicity. An additional but important point is the variability of gut microbiota, not only inter-individually but also at the intra-individual level (for instance in different health conditions). This calls for the need of a personalized profiling of the ENM effects on gut immunity, as it will depend on the individual microbiota in a given moment. Future immuno-nanosafety models, like the intestine-on-a-chip mentioned above, will therefore need to include the individual microbiota and the innate immune cells (in particular macrophages) derived from the individual subject.

AUTHOR CONTRIBUTIONS

MT, JQ, and LL devised the study and wrote the manuscript. SL, LW, and ZH contributed to literature search and gave insightful suggestions in revising this work. All authors contributed to the article and approved the submitted version.

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BioRender was used to create schematic representations.

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