MICROBIOTA AND HOST

Microbiota and Host (2024) **2** e230015
https://doi.org/10.1530/MAH-23-0015

Received 16 August 2023
Accepted 12 December 2023
Available online 12 December 2023
Version of Record published 11 January 2024

REVIEW

Effects of heavy metals on gut barrier integrity and gut microbiota

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Abstract

Exposure to environmental pollutants such as heavy metals lead to significant damage in intestinal epithelial barrier, loss of microbial and immune homeostasis. The intestinal epithelial barrier protects and regulates the responses against several endogenous and exogenous factors including inflammatory cytokines, pathogens, toxins, and pollutants. Intestinal epithelial barrier dysfunction, immune dysregulation and microbial dysbiosis are associated with several gastrointestinal (GI)-related disorders including inflammatory bowel disease (IBD). The mechanisms and consequences of exposure to environmental toxins on gut barrier function and mucosal immune system are not fully understood. This review explores some of the recent findings of heavy metals and their effect on intestinal barrier function, microbiota, and their contributions to human health and pathogenesis of GI-related disorders such as IBD.

Keywords: heavy metal toxicity; pollution; intestinal barrier dysfunction; tight junctional proteins; gut microbiota; microbial metabolites

Introduction

The industrial revolution caused a significant increase in the release of several toxic chemicals into environment that became part of our food cycle. Epidemiological studies have suggested an association between metal pollution and worst health outcomes and increased risk for numerous disorders (Tchounwou *et al.* 2012, Chowdhury *et al.* 2018). Exposure to heavy metals through water, air, and foods affects different organs and causes nervous system disorders, skin lesions, vascular damage, immune system dysfunction, birth defects, cancer, and gastrointestinal (GI) and kidney dysfunction. Additionally, exposure to multiple metals may exert cumulative adverse effects on overall health.

The effects of metals on target organs vary from each other based on their route of exposure, amounts of metals (low vs high), duration of exposure as well as species. For instance, mice develop intestinal adenomas and carcinomas upon chronic exposure to high concentration of hexavalent chromium in drinking water, but not in rats (Thompson *et al.* 2013). Similarly, acute exposure to high-dose mercury and lead may induce kidney failure, abdominal colic pain, and bloody diarrhea. In contrast, chronic exposure at low doses regularly can cause complications such as neuropsychiatric disorders including fatigue, anxiety, and detrimental impacts on intelligence quotient (IQ) and intellectual function in children (Balali-Mood *et al.* 2021). Mechanistically, high-dose exposure to heavy metals leads to DNA damage, disrupts proteins and mechanisms involved in DNA synthesis and repair. Commonly, heavy metal-induced reactive oxygen species, suppression of oxidative stress and inactivation of critical metabolic enzymes are responsible for heavy metal-mediated adverse effects. However, the

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mechanism of actions of each metal may vary based on organ, tissue, and the cell types involved. Current review focuses on how heavy metal(s) exposure show impact on gut microbiota and gut barrier functions leading to GI-related disorders.

Recent studies have highlighted that the exposure to environmental pollutants leads to significant changes in the composition of gut microbiota (Breton *et al.* 2013*b*, Shao & Zhu 2020), epithelial barrier dysfunction, and increased intestinal inflammation (Celebi Sozener *et al.* 2020, Mitamura *et al.* 2021, Lindell *et al.* 2022). Microbial dysbiosis, gut barrier dysfunction, and immune dysregulation are associated with GI-related disorders including inflammatory bowel disease (IBD) (Martini *et al.* 2017, Guan, 2019), which comprises ulcerative colitis (UC) and Crohn's disease (CD). Gut barrier consists of single layer of intestinal epithelial cells (IECs) that selectively allows transmigration of nutrients and protects from external challenges and pathogenic bacteria (Ghosh *et al.* 2021). Additionally, gut barrier mediates the cross talk between commensal microbes and immune system, and provides the first line of defense against pathogens, toxins, and environmental pollutants. Gut barrier dysfunction results in leakiness of bacteria and toxins, leading to immune imbalance at gut mucosal sites, which potentially promotes pathogenesis of GI-related disorders including IBD. The mechanisms and long-term impact of environmental toxins on gut barrier functions and related disorders are not fully understood. Gut microbiota and their metabolites can play an important role in regulation of the environmental toxins-mediated etiology and pathogenesis of GI disorders (Claus *et al.* 2016, Bist & Choudhary 2022). The use of probiotics has become as an attractive strategy to reduce the adverse effects of toxic metals (Duan *et al.* 2020). In this article, we review the structure of intestinal barrier and its impact upon exposure to heavy metals. The review also explores some of the possible mechanisms through which heavy metals interact and affect gut barrier function, microbiome and microbial metabolites.

Gut barrier dysfunction and systemic responses

Intestinal epithelial cells

The GI barrier consists of three key components: the mucus layer, the IEC layer, and the immunological barrier. Goblet cells (GC) are responsible for secretion of mucins, which create protective mucus layer (Kim & Ho 2010, Johansson *et al.* 2013). This mucus layer, composed of an outer and an inner layer, serves as the primary barrier against luminal microorganisms and foreign antigens (Thornton & Sheehan 2004, Bansil & Turner 2006, Leal *et al.* 2017). The mucus layer consists of glycoprotein sheets, featuring a densely packed inner layer and a less dense outer layer, that serve as a niche for various intestinal bacteria by acting as a carbon source for microbial metabolism (Johansson *et al.* 2013, Pelaseyed *et al.* 2014). The mucus functions as a barrier for hydrophilic solutes, which can only be transported via specific transporters (Turner 2009, Li *et al.* 2020). Mucins and their glycosylation status play a critical role in regulating gut barrier functions. Deficiencies in mucin production lead to defective gut barrier activities and promote GI-related disorders, including IBD, irritable bowel syndrome (IBS), and cancer.

The intestinal epithelium is composed of various cellular subtypes, such as enterocytes, Paneth cells, M cells, endocrine cells, and tuft cells, which collectively play essential roles in digestion, nutrient absorption, and protection against pathogens, among other functions (reviewed elsewhere (Clevers 2013, Peterson & Artis 2014, Okumura & Takeda 2017)). IEC junctions including tight junctions (TJs), adherens junctions (AJs), desmosomes, and gap junctions (GJ) are specialized structures present in the epithelial cell membranes. They establish contacts between IECs and regulate the transport of molecules based on their size and charge through the paracellular space (González-Mariscal *et al.* 2003, Van Itallie & Anderson 2014, Zihni *et al.* 2016). Defects in paracellular permeability are associated with several GI-related disorders (Odenwald & Turner 2013, Bischoff *et al.* 2014, Vermette *et al.* 2018, Vanuytsel *et al.* 2021).

Enterocytes, comprising over 80% of IECs, are polarized cells with microvilli expanding the absorptive surface. These cells are interconnected via several proteins that enable cell-to-cell adherence through formation of junctions. Enterocytes undergo apoptosis and are replaced by crypt-derived stem cells. It is known that the enterocytes regulate water and nutrient absorption, contribute to intestinal layer formation, macromolecular transportation, and digestion (Snoeck *et al.* 2005). They are involved in macromolecular transport via receptor-mediated endocytosis (Stern & Walker 1984, Snoeck *et al.* 2005). Increased inflammation, elicited by elevated TNF-α, accelerates enterocyte turnover and proliferation, leading to heightened shedding and apoptosis, potentially compromising the intestinal barrier and promoting bacterial translocation, particularly in conditions like IBD.

Paneth cells are another subset of differentiated secretory cells located at the base of the crypts of Lieberkuhn. Paneth cells are essential for enteric immune homeostasis and actively secrete antimicrobial peptides such as alpha defensins, lysozyme, and phospholipases A2 that limit bacterial numbers. Infants with necrotizing enterocolitis (NEC) have significantly decreased levels of Paneth cells compared to age-matched controls (Underwood 2012, McElroy *et al.* 2013). It was shown that depletion or dysfunction of Paneth cells in mouse models results in an NEC-like phenotype, indicating the importance of Paneth cell function in immature intestine (Sampath *et al.* 2017,

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Lueschow *et al.* 2018, Lueschow & McElroy 2020). Paneth cells can directly sense gut commensals and control intestinal barrier penetration in a myeloid differentiation marker 88 (MyD88)-dependent manner at the intestinal host–microbial interface (Vaishnava *et al.* 2008). Paneth cells depend on autophagy to control their secretion capacity of antimicrobial peptides. Mutations in genes like *Atg16L1* disrupt this process, leading to reduced Paneth cell function, imbalanced gut microbiota, compromised barrier integrity, and an elevated risk of diseases such as Crohn's disease in humans (Cray *et al.* 2021).

M cells are specialized epithelial cells found in the gut-associated lymphoid tissue (GALT) of Peyer's patches of the small intestine, isolated lymphoid follicles, colonic patches, and nasopharyngeal-associated lymphoid tissues (a.k.a. NALT) (Dillon & Lo 2019). These cells are involved in immune responses, especially antigen sampling, uptake of microorganisms and interact with dendritic cells or lymphocytes to initiate adaptive immunity. M cells deliver samples of foreign material from the lumen to organized mucosal lymphoid tissues. They interact closely with immune cells of Peyer's patches and play an important role in the initiation of immunological response and tolerance. During chronic inflammation, increased levels of M cells were observed along with selective apoptosis of M cells, leading to elevated uptake of microorganisms and inflammation (Kucharzik *et al.* 2000*b*). Increased apoptosis of M cells during ileitis conditions leads to breakdown of intestinal barrier, resulting in translocation of bacteria and enhanced inflammation (Kucharzik *et al.* 2000*a*).

Enteroendocrine cells representing 1% of the intestinal epithelium are also a type of intestinal secretary cells that mediate hormone release and are critical for digestion. Enteroendocrine cells are a major component of a specialized chemosensory system that can sense the intestinal microbiota and their metabolites. These cells secrete peptide hormones and classical cytokines to the surrounding immune cells and modulate both innate and adaptive immune systems. Enteroendocrine cells possess cytoplasmic processes in close proximity to the enteric nerve terminals and mediate several physiological functions including visceral hyperalgesia, intestinal motility, and synaptic transmission. Importantly, enteroendocrine hormones can modulate the intestinal epithelial barrier function through both transcellular and paracellular pathways (Yu *et al.* 2019).

Tuft cells are chemosensory sentinel cells that sense signals from the local milieu and communicate to immune cells within the intestine. These cells have been recently described to be critical in exerting an immune response against helminths (Allaire *et al.* 2018). The underlying mechanisms of these sensory signals are yet to be established. It is known that Tuft cells secrete IL-25 continuously to maintain type 2 innate lymphoid cells (ILC2) homeostasis. Helminth infection leads to increased levels of IL-25 production by tuft cells, which directly acts on ILC2 to release IL-13, which in turn acts on tuft cells and goblet cells to promote hyperplasia in an IL-13Rα1/IL-4αR-dependent manner. These series of activities lead to an increase in mucin levels to expel the parasite from the GI tract and protect the gut barrier (von Moltke *et al.* 2016, Ting & von Moltke 2019).

Tight junction proteins in gut epithelium

Claudins are 20–27 kDa tetraspan membrane proteins that contain four hydrophobic transmembrane domains with two extracellular loops and N- and C-terminal cytoplasmic domains (Van Itallie & Anderson 2006). The extracellular loops are responsible for homophilic and/or heterophilic TJ protein–protein interactions and the formation of ion-selective channels. The intracellular C-terminal domain anchors claudin to the cytoskeleton through interactions with PDZ-binding domain proteins including zonula occludens 1 (ZO-1), ZO-2, and ZO-3 (Morita *et al.* 1999). Thus far, at least 24 members of the claudin family have been reported. Claudins play an important role in 'pore formation' and 'sealing (barrier forming)' of the epithelium in the GI tract and classified as 'pore' vs 'barrier-forming' TJ proteins (Günzel & Yu 2013). The 'pore' forming claudins specifically increase paracellular permeability either for molecules of a certain size or of a certain charge (or both), and decrease transepithelial resistance but leave the epithelial barrier function against macromolecules intact (Günzel & Yu 2013). Similarly, 'barrier-forming' claudins decrease paracellular permeability and increase transepithelial resistance. It was also demonstrated that treatment with IL-17A protected against TNF-α-mediated barrier disruption in Caco-2 cells through regulating occludin, suggesting a direct role for IL-17 and IL-17R signaling on epithelial cells (Lee *et al.* 2015). In an independent study, Dr Rao's group reported that the deficiency of occludin increased the susceptibility to ethanol-induced colonic mucosal barrier permeability and liver damage in mice (Mir *et al.* 2016). Moreover, inflammatory cytokines like TNF-α and IFN-γ are reported to downregulate intestinal epithelial barrier function by reorganizing several TJ proteins such as ZO-1, claudin 1, claudin 4, occludin, and JAM-A (Zolotarevsky *et al.* 2002). Th2 cytokines like IL-4 and IL-13 also cause an increase in intestinal permeability through induction of epithelial apoptosis and expression of the pore-forming TJ protein claudin 2 (Berin *et al.* 1999, Ceponis *et al.* 2000, Madden *et al.* 2002).

Adherens junction proteins in gut epithelium

AJs, also known as zonula adherens, are located on the lateral membrane of epithelial cells and maintain cell-to-cell contact, cell polarity, motility, and proliferation

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(Perez-Moreno *et al.* 2003, Hartsock & Nelson 2008). Interconnections between transmembrane proteins, intracellular adaptor proteins, and the cytoskeleton form AJ protein complexes. AJs are reported to present beneath TJs and are required for the assembly of TJs. TJs along with AJs are linked to the peri-junctional ring of cellular actin and myosin, leading to junction regulation via the cytoskeleton. AJs primarily consist of cadherin and catenin proteins that are linked to several intracellular cytoskeletal protein domains (Perez-Moreno *et al.* 2003). One of the most characterized AJ protein, E-cadherin binds to repeat regions of β-catenin, α-catenin, or γ-catenin (plakoglobin) at varying affinities based on phosphorylation states (Gumbiner 1996, Halbleib & Nelson 2006, Perez-Moreno & Fuchs 2006). Moreover, the functions of AJ and TJ proteins are regulated by myosin and actin proteins that form a dense ring, which encircles the cell. Activation of actomyosin contraction is regulated by phosphorylation of myosin II regulatory light chain (MLC) by MLC kinase (MLCK) and is implicated in AJ and TJ protein regulation. It has been demonstrated that MLCK-driven MLC phosphorylation is involved in signal transduction pathways that influence gut barrier function in response to diverse stimuli (Turner 2000, Shen *et al.* 2006, Cunningham & Turner 2012). In the case of IBD, decreased E-cadherin–catenin complexes lead to a loss of cell-to-cell adhesions along with impairment of the integrity of the mucosal barrier. Exposure of the mucosal immune system to luminal substances leads to inflammation and further increased gut barrier permeability (Mehta *et al.* 2015). Furthermore, modulation of intestinal permeability by oxidative stress leads to redistribution of E-cadherin and β-catenin (Rao *et al.* 2002, Rao 2008).

Desmosomes in gut epithelium

Desmosomes are located on the basolateral membrane and are known to provide mechanical support due to connections to the intracellular cytoskeletal framework (Gumbiner 1996, Green & Simpson 2007, Nekrasova & Green 2013). Desmosomes form links between adjacent cells and provide connection between intermediate filaments of the cell cytoskeletons. Desmosomes consist of the cadherin family of proteins, desmoglein and desmocollins (Gumbiner 1996, Nekrasova & Green 2013). Together with AJs, desmosomes maintain the integrity of the epithelium by imparting strong adhesive bonds. Studies revealed that desmoglein 2 (Dsg2), which is expressed in enterocytes, potentially contributes to the pathogenesis of CD (Spindler *et al.* 2015). It was demonstrated that Dsg2 regulates intestinal epithelial barrier function in enterocytes by modulating the p38MAPK signaling cascade (Ungewiss *et al.* 2017).

Gap junctions in gut epithelium

Gap junctions (GJ) form tiny connections between adjacent cells allowing passage of small molecules, ions and electrical signals (Goodenough & Paul 2009). Small molecules and ions diffuse through GJ channels that span the bilayers of both cells and the extracellular space that separate them. GJ are formed by head-to-head docking of proteins called connexins. Connexins are hexameric protein transmembrane complex that spans between membranes of adjacent cells (Goodenough *et al.* 1996). The gap inside the hexameric GJ protein passes the respective signal or molecule to the next cells. Ey *et al.* showed that during IEC injury, activated Toll-like receptor 2 drives connexin 43 protein synthesis and subsequently increases gap junctional intercellular communication, leading to increased epithelial permeability and related diseases (Ey *et al.* 2009).

In general, the dysfunction of gut epithelial cells can have far-reaching consequences, primarily stemming from disruptions in the mucus layer and junctional protein complexes within the gut epithelium. This dysfunction ultimately leads to an increase in intestinal permeability, allowing bacteria and bacterial endotoxins to leak through. This, in turn, triggers an elevation in the levels of inflammatory cytokines and recruitment of inflammatory cells. These interconnected events culminate in the development of microbial dysbiosis, a substantial rise in mucosal and systemic inflammation. These conditions, in turn, play a pivotal role in the onset and progression of various disorders, such as IBD, diabetes, neurological disorders, liver diseases, and various types of cancers, among others.

Microbiota is essential for gut barrier function

The gut microbiota is often referred to as an essential ‘organ’ due to the vast density and richness of microbial life that exist in the gut lumen (Eckburg *et al.* 2005). The composition of microbiota is influenced by several factors including but not limited to host genetics, age, dietary habits, drugs, environment, and lifestyles. Microbiota can be also passed from mother to child, with slight differences between delivery and postpartum birth (Palmer *et al.* 2007, Dominguez-Bello *et al.* 2010, Ursell *et al.* 2012). Germ-free (GF) mice exhibit significant deficiency in gut barrier function, suggesting the critical role of microbiota in development of gut barrier components (Parker *et al.* 2018, Wang *et al.* 2021). The inner mucus layer of GF mice is more penetrable to bacterial-sized beads compared to conventionally raised mice, suggesting defects in the mucin layer (Johansson *et al.* 2015). Introducing fecal commensal bacteria collected from conventionally raised mice into GF mice induces a normal colonic barrier structure by increasing the thickness of the colonic mucus layer and mucin glycosylation (Hayes *et al.* 2018). These studies demonstrated the importance of gut microbiota in developing gut mucus barrier and maintenance of homeostasis. It was shown that GF mice

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are more susceptible to epithelial injury as a result of an impaired intestinal barrier via downregulation of claudin 4, occludin, TFF3, and MUC3 protein expression and IL-22 secretion (Hernández-Chirlaque *et al.* 2016). It was observed that mice treated with broad-spectrum antibiotics, such as ampicillin, streptomycin, or clindamycin for 14 days showed reduced bacterial diversity and richness. These mice displayed an increase in gut permeability and decreased expression of intestinal TJ proteins such as ZO-1, occludin, and claudin 1 (Feng *et al.* 2019), reinforcing the importance of microbiota in gut barrier function. Now there is unequivocal evidence for the critical role of microbiota in the development and maintenance of gut barrier function. Hence, rebuilding a healthy gut microbiota, while maintaining the known resident commensals, is critical (Schmidt *et al.* 2018). Although the determination of 'beneficial' microbes seems to be a challenge, probiotics, prebiotics, and synbiotics may provide opportunities to restore intestinal homeostasis and enhance gut barrier function in addition to blocking of unwarranted inflammation and dysregulation (Gibson & Roberfroid 1995, Schrezenmeir & de Vrese 2001, Ghosh *et al.* 2022*c*,d). The impact of microbial metabolites on gut barrier was reviewed elsewhere (Ghosh *et al.* 2021). Members of the gut microbiota influence the host metabolic and immune status by modulating nutrient metabolism, xenobiotic and drug metabolism, and production of antimicrobial metabolites that limit numbers of competing microbes for the same niche. Gut microbes and metabolites influence the structure of the GI tract, integrity of the gut barrier, and differentiation of various immune cell subsets (Jandhyala *et al.* 2015, Thursby & Juge 2017). However, most of the functions of individual species and metabolites remain unclear.

The mucosal immune system profoundly impacts gut barrier functions both in health and disease conditions (reviewed in Mazmanian *et al.* (2005), Arpaia *et al.* (2013), Konieczna *et al.* (2013), Allaire *et al.* (2018), Kayama & Takeda (2020), Zuo *et al.* (2020)). Microbial metabolites regulate host immunity by exploiting metabolite-specific immune cell receptors such as aryl hydrocarbon receptor (AhR), farnesoid X receptor, pregnane X receptor, membrane bile acid receptor (M-BAR/TGR5), purinergic receptor, and G-protein coupled receptors (GPR 41, GPR43, GPR109A) (Liu *et al.* 2022*a*,c). These receptors play crucial roles in host–microbiota interactions and are expressed at various levels in different cell types such as IECs, innate lymphoid cells, macrophages, T cells, and dendritic cells (Furusawa *et al.* 2013). Functional immune responses often modulate gut barrier integrity; hence, the fine-tuning of gut microbiota to evoke necessary immune responses to alter intestinal barrier function is critical. The major confounding factors that disturb the composition of gut microbiota and gut barrier functions include exposure to heavy metals and associated disorders. The following sections review the current understanding of heavy metal exposure and their impact on gut barrier functions.

The role of heavy metals in regulation of gut microbiota and gut barrier function

Humans are exposed daily to numerous environmental chemicals, heavy metals, pesticides, organic pollutants, mycotoxins, food additives, and other contaminants (Celebi Sozener *et al.* 2020). Exposure to the heavy metals such as arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), and chromium (Cr) results in adverse health effects in humans and animals (Balali-Mood *et al.* 2021). Around 60% of ingested metals are absorbed by the intestine, resulting in severe oxidative stress and gut barrier damage, leading to increased intestinal inflammation (Feng *et al.* 2018, Assefa & Kohler 2020, Shao & Zhu 2020) (Fig. 1). The functional consequences of exposure to these environmental contaminants on the human gut microbiota are yet to be investigated. Table 1 summarizes the known effects of heavy metals on host health and gut microbiota. The importance of gut microbiota in the metabolism of heavy metals was demonstrated using GF mice. It was shown that heavy metals significantly accumulated in blood and target organs of GF mice compared to normal mice following exposure (Breton *et al.* 2013*a*).

Arsenic

About 225 million people in over 70 countries in the world are chronically exposed to arsenic (Naujokas *et al.* 2013, Podgorski & Berg 2020), making it the cause of an environmental health crisis with no known treatment. In USA, about 3 million individuals are exposed to arsenic mostly through unregulated domestic well water used for drinking purposes (Ayotte *et al.* 2017). Arsenic is a group I human carcinogen and chronic exposure leads to toxic effects in multiple organs like liver, kidney, bladder, skin, and central nervous system (Banerjee 2011, IARC 2012, Hong *et al.* 2014, Hunt *et al.* 2014, Coryell *et al.* 2019, Garza-Lombo *et al.* 2019). While skin has been reported as the major target organ for arsenic with precancerous and cancerous outcomes (Hunt *et al.* 2014), recent data are shedding light on its adverse effects on lifestyle disorders including diabetes, cardiovascular diseases, and obesity (Navas-Acien *et al.* 2008, Moon *et al.* 2012, 2018, Chen & Karagas 2013, Grau-Perez *et al.* 2017, Bulka *et al.* 2017, Young *et al.* 2018, Farkhondeh *et al.* 2019). Recent studies highlighted that chronic exposure to arsenic has a significant impact on the GI system, leading to irritation, nausea, pain, and vomiting (Jomova *et al.* 2011) as well as perturbation of gut microbiome (Choiniere & Wang 2016*b*). Previous reports suggested that the chronic arsenic exposure abrogates the gut barrier function, with affected

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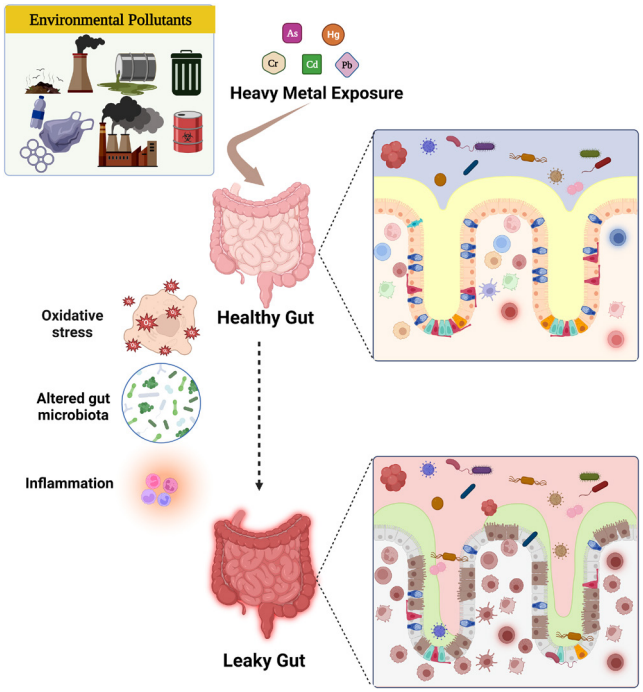

Figure 1

Effects of heavy metals on gut. Heavy metals such as arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), and chromium (Cr) lead to increased oxidative stress, altered gut microbial composition, and inflammation, potentially leading to gut leakiness and gut barrier dysfunction.

individuals exhibiting symptoms like dyspepsia, gastroenteritis, and chronic diarrhea (Guha Mazumder & Dasgupta 2011, Choiniere & Wang 2016*a*, Chiocchetti *et al.* 2019*a*,*b*, Fernández Fernández *et al.* 2019). In rodents, arsenic metabolites (e.g. monomethylarsonic acid) target the GI tract, leading to enlargement of the intestinal wall, edemas, hemorrhages, necrosis, and ulcerations as well as increase in the incidence of squamous metaplasia of absorptive epithelial cells of the colon and rectum (Arnold *et al.* 2003). Sub-chronic exposure to arsenic led to disruption of the colonic epithelial structure and barrier function (Chiocchetti *et al.* 2018). The transmission electron microscope (TEM) image of Caco-2 cells after arsenic exposure (As (III) ≥ 0.075 mg/L; As (V) ≥ 0.75 mg/L) for 14 days showed the disruption in microvillus structures, resulting in increased paracellular transport and barrier dysfunction (Chiocchetti *et al.* 2018). Trivalent forms of arsenic significantly induce inflammatory cytokines such as IL-6, IL-8, and TNF-α as well as oxidative stress in colon epithelial cells (Calatayud *et al.* 2013, 2014). Recently, it was shown that gut microbiota is required for full protection against acute arsenic toxicity using mouse models (Coryell *et al.* 2018). In this study, the authors showed that when mice were given the antibiotic cefoperazone before being exposed to inorganic sodium arsenate (iAsV), the inorganic arsenic (iAs) accumulated significantly in their organs. These mice also exhibited lower levels of iAs in their stools compared to the mice that did not receive antibiotics. Furthermore, in line with these findings, mice without gut microbiota (GF mice) exposed to iAs also showed higher levels of iAs in their organs compared to mice with normal microbiota. In essence, these studies suggest that gut microbiota is necessary for effectively processing/metabolizing iAs for excretion from the host's body. This study also demonstrated that transplantation of human microbiota into GF mice protected them from arsenic-induced adverse effects. It was shown that functional arsenic detoxification enzyme (As3mt) and a specific bacterium called *Faecalibacterium* are essential for protection against acute arsenic toxicity in mouse models (Coryell *et al.* 2018). iAs speciation is significantly impacted by microbial-dependent iAs metabolism (reviewed in (Tsai *et al.* 2009, Kruger *et al.* 2013)). The iAs is transported through glycerol or phosphate transporters in bacteria. Arsenate can be reduced to arsenite, which may be expelled by ArsAB. Alternatively, arsenite can be methylated by ArsM to MMAs (III), DMAs (III) and TMAs. Briefly, biotransformation of iAs by microbes can be divided into four functional groups: (i) As(III) oxidation, (ii) iAs(V) reduction, (iii) iAs methylation and demethylation, and (iv) iAs transport (Zhao *et al.* 2019). The toxic forms of As(III)/MMAs(III) can be actively expelled from bacterial cells using arsenic transporters encoded by *arsB*, *arsP*, *or acr3* (genes associated with resistance). Arsenic methylation, governed by *arsM* (related to resistance), results in the release of arsenic through volatilization or the formation of products like mono-, di-, or trimethylarsines. It is possible that lack of microbes in the host (either in antibiotic-treated or germ-free mice) failed to metabolize iAs and expel from host body, leading to the accumulation of iAs in target organs, including the GI tract.

Gut microbiome analysis of individuals exposed to arsenic revealed that abundance of pathogenic bacteria is positively correlated with and commensal gut bacteria are negatively correlated with increased arsenic concentration (Brabec *et al.* 2020, Chen *et al.* 2021). It was shown by Chi *et al.* that exposure to environmentally relevant levels of As (100 ppb) in mice led to significant changes in the functional metagenome. These include increase in the genes involved in energy metabolism, LPS synthesis, oxidative stress responses, and DNA repair (Chi *et al.* 2017). Additionally, arsenic exposure also enriched genes that encode conjugative transposon proteins, components of the multidrug efflux system, and the synthesis of multiple vitamins (Chi *et al.* 2017). Human gut microbiota can biochemically transform arsenic-containing compounds (arsenicals), leading to arsenic speciation and bioavailability (Lu *et al.* 2013, 2014, Yin *et al.* 2022). It is possible that either microbiota metabolizes arsenic to produce less-toxic arsenicals or microbial metabolites can counteract

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Table 1 Effects of heavy metals on host health and gut microbiota.

		Changes in Gut microbiota	
Heavy metal	**Effect on health**	Increase	Decrease
Arsenic	Pathological changes in skin; gut barrier dysfunction; inflammation; carcinogenesis; microbial dysbiosis; cardiovascular diseases	Bacteroidetes, *Bifidobacterium*, *Faecalibaculum*, Enterobacteriaceae, Gammaproteobacteria	Firmicutes, Enterobacteriaceae
Lead	Increased bodyweight; reduced MUC2, ZO-1, occludin, and claudin 1; increased gut permeability; elevated oxidative stress and inflammation; dysregulated hepatic metabolism; microbial dysbiosis; hepatotoxicity; nephrotoxicity	Bacteroidetes, Clostridiaceae, Ruminococcus, *O*scillibacter, Parabacteroides, Desulfovibrionaceae, *Clostridium* XIVb, *Barnesiella*	Firmicutes, Proteobacteria, *Turicibacter*, Akkermansia, Dehalobacterium, *Lactococcus*, *Enterorhabdus*, Caulobacterales
Mercury	Neurotoxicity, oxidative stress and inflammation, leaky gut, mitochondrial dysfunction, increased lipid peroxidation, altered calcium homeostasis	Firmicutes/Bacteroidetes ratio, *Akkermansia*	*Lactobacillus*, Proteobacteria
Cadmium	Inflammation; microbial dysbiosis; intestinal damage; junctional protein, mucus and glycan distribution alteration; hepatotoxicity; energy metabolism dysregulation; endocrine disruption; genome instability	Bacteroidetes, *Akkermansia muciniphila*, *Prevotella* spp., *Escherichia coli*, *Shigella*	Firmicutes, γ-Proteobacteria, *A. muciniphila*, *Clostridium cocleatum*, *Lachnoclostridium*
Chromium	Oxidative stress, cancer, GI distress, microbial dysbiosis, DNA damage, lipid peroxidation, liver toxicity	Bacteroidetes, Tenericutes, *Prevotella*, *Clostridiales*, S24-7, Actinobacteria	Firmicutes, Lachnospiraceae

against arsenic-induced toxicity or organ damage. Our group showed that gut microbial metabolite urolithin A (UroA) protected colon epithelial cells against inorganic arsenic exposure by reducing inorganic trivalent arsenic (iAs^{3+})-induced oxidative stress and enhancing TJ proteins (Ghosh *et al.* 2022*a*,*b*). We have demonstrated that UroA protected from arsenic-induced cell death in colon epithelial cells and alleviated iAs^{3+}-induced barrier dysfunction in both colon epithelial cell monolayers and a human 3D small intestinal tissue model. Importantly, UroA treatment significantly protected from arsenic-induced gut barrier permeability by enhancing TJ proteins such as zonula occludens 1, occludin, and claudin 4. Mechanistically, we showed that UroA treatment reduced iAs^{3+}-induced reactive oxygen species (ROS) through regulating genes involved oxidative stress pathways. Further *in vivo* studies are required to determine the impact of metabolites in ameliorating arsenic-induced toxicities. Moreover, use of probiotics like *Bifidobacterium* and *Lactobacillus* modulated arsenic transformation abilities by enhancing excretion and detoxification (Liu *et al.* 2022*b*). It is pertinent to recall the epidemiological observation that people from Northport, WA, USA, who had chronic exposure to iAs had 15 times more IBD incidence than the national average (Pynn 2013). Therefore, targeting gut barrier dysfunction and inflammation simultaneously by beneficial microbiota and microbial metabolites would offer better protection against arsenic-induced adverse events.

Lead

A non-essential heavy metal, lead (Pb) exerts its toxic effects on several organs including liver, kidney, nervous system, cardiovascular, GI, and reproductive systems (Flora *et al.* 2012, Liu *et al.* 2021, Yu *et al.* 2021*b*). Soil contamination with gasoline and paint exposes humans to Pb-related toxicity. An epidemiological study by Lanphear *et al.* determined the concentrations of Pb in blood of adults aged 20 years or older and confirmed that low-level exposure to Pb was associated with risk factors for cardiovascular disease mortality in the USA (Lanphear *et al.* 2018). Pb-induced oxidative stress by ROS is one of the causes for Pb poisoning and related adverse health effects. Hence, the modulation of cellular thiols by antioxidants against ROS production has emerged as a key therapeutic approach (Flora *et al.* 2012). Several studies elaborated the adverse effects of Pb on GI tract and gut microbiota in several species. Mice exposed to low-dose (chronic model) or high-dose (acute model) Pb exhibited reduced colonic MUC2 levels, TJ proteins such as ZO-1, claudin 1, and occludin and increased intestinal permeability, suggesting its direct impact on GI functions (Zhai *et al.* 2019*b*). IEC damage was observed in honeybees when they were exposed to lead oxide (PbO) and/or cadmium oxide (CdO) nanoparticles for 9 days (Dabour *et al.* 2019). In these studies, they also showed using TEM methodologies that CdO and PbO induced cytological alterations in IECs (Dabour *et al.* 2019).

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Xia *et al.* showed that chronic exposure to Pb (15 weeks) induced gut microbial dysbiosis and metabolic disorder in mice (Xia *et al.* 2018). Especially, Pb exposure caused significant increase in the levels of hepatic triglycerides, total cholesterol as well as genes involved in lipid metabolism. Further, Pb exposure led to change in the structure and richness of the gut microbiota, especially relative abundance of Firmicutes and Bacteroidetes was altered compared to normal control mice (Xia *et al.* 2018). Even early exposure to Pb during developmental period enhanced the risk for obesity in adulthood by altering the gut microbiota independent of gender (Wu *et al.* 2016). Gao *et al.* demonstrated by using multi-omics approaches that Pb exposure affected gut microbiome trajectories including metabolic pathways in C57BL/6 mice (Gao *et al.* 2017). Fecal microbiota transplantation from donors supplemented with galactooligosaccharide (GOS) altered the gut microbiota composition and improved the recovery of the gut barrier function in mice after Pb exposure. Zhai *et al.* showed that the mice treated with antibiotics exhibited accumulation of higher levels of Pb in their blood and primary organs, along with reduced Pb levels in their feces (Zhai *et al.* 2019*a*) indicating importance of microbiota in clearance of Pb from host. Typically, certain gut microorganisms can boost the healing of intestinal mucosal injuries, support the balance of gut immunity, and contribute to the reduction of gut inflammation. It was shown that the Pb exposure leads to gut dysbiosis, resulting in a decrease in beneficial bacteria such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Oscillibacter ruminantium* (Zhai *et al.* 2017). Interestingly, oral administration of these Pb-intolerant gut microbes, *A. muciniphila*, *F. prausnitzii*, and *O. ruminantium* reversed the Pb-induced toxicity in mouse models (Zhai *et al.* 2019*a*,*b*). Oral administration of these bacteria led to reduction in Pb burden in target organs and bloodstream and increased fecal Pb excretion in chronically Pb-exposed mice (Zhai *et al.* 2017). Moreover, oral supplementation of these Pb-intolerant gut microbes (especially *F. prausnitzii* and *O. ruminantium*) enhanced gut barrier function by upregulating TJPs such as ZO-1, occludin, and claudin 1 proteins in the colon and small intestine. Further, these treatments increased the levels of short-chain fatty acids (SCFAs), which have been shown to have a significant impact on gut barrier function. Mechanistically, treatment with these microbes significantly reduced Pb-induced hepatic and renal oxidative stress in mice. These studies further showed that the three Pb-intolerant gut microbe strains showed a high adsorption capacity for Pb^{2+} compared with *E. coli* K12 (Zhai *et al.* 2017). In summary, these findings indicate that gut microbiota can limit the absorption and accumulation of Pb in host tissues. When Pb-intolerant intestinal microbes like *F. prausnitzii* and *O. ruminantium* were orally administered to mice, there was a significant decrease in Pb accumulation and alleviate Pb toxicity. This underscores the significance of adjusting gut microbiota as a potential strategy for minimizing the Pb toxicity in the host. Pb exposure to GF mice resulted in increased accumulation of Pb in blood and organs compared to conventional SPF mice, suggesting that gut microbiota plays a major role in excretion of heavy toxic metals (Breton *et al.* 2013*a*). Recently, a human cohort study revealed that increased urinary Pb is associated with the presence of *Proteobacteria*, suggesting that environmental toxins potentially induce microbial dysbiosis and lead to adverse health in humans (Eggers *et al.* 2019). Further in-depth studies are required to define cause or consequence and mechanisms of actions of Pb in various disease conditions.

Mercury

Mercury (Hg) is a well-known biohazard that exists in the environment in its elemental form, as inorganic mercury, or as organic mercury (Rice *et al.* 2014). The most common and major source of Hg in the ecosystems is methylmercury (MeHg), i.e. Hg in its organic methylated form. MeHg is highly toxic, as it covalently binds to the glutathione and cysteine residues of any protein in the host system and causes alterations in the protein structure and function (Pinto *et al.* 2020). Mercury exposure causes DNA damage, induction of oxidative stress, mitochondrial dysfunction, increased lipid peroxidation, and altered calcium homeostasis at the cellular level. Mercury poisoning affects all organs, as it can readily pass into the circulation system, ultimately leading to death. Likewise, gut barrier permeability and microbial dysbiosis have been closely associated to exposure to mercury. Vázquez *et al.* showed that inorganic divalent mercury (Hg (II)) and MeHg can exert toxicity on IECs and mucosa by generating redox imbalance (Vazquez *et al.* 2014). Generation of ROS or reactive nitrogen species (RNS) and decrease in glutathione by mercury redistributed the F-actin and ZO-1 protein in the intestinal cells, resulting in elevated barrier permeability (Vazquez *et al.* 2014). It is shown that Hg exposure significantly downregulated expression of intercellular junction proteins like claudin 1, occludin, ZO-1, and junctional adhesion molecule 1 (JAM1) in colon epithelial cells. Hg also increased the intestinal cell volume and membrane permeability without any loss in cell viability; thus, it promotes the uptake of other toxic metals in same conditions (Aduayom *et al.* 2005). Bolan *et al.* reported that gut microbes like *Escherichia coli* and *Lactobacillus acidophilus*, or chelating agents (EDTA and 2,3-dimercapto-1-propanesulfonic acid (DMPS)) significantly reduced the Hg-induced permeability in *in vitro* GI/Caco-2 cell intestinal epithelium model (Bolan *et al.* 2021). It was suggested that microbiota-dependent protection against heavy metal-induced intestinal permeability may be associated with indirect intestinal sequestration of metals by gut bacteria through adsorption on bacterial surface (Bolan *et al.*

2021). Moreover, probiotics strain *Lactobacillus brevis* 23017 exhibits strong mercury-binding capacity, which may potentially reduce Hg exposure to the host cells and protects the gut barrier integrity against Hg-induced toxicity (Jiang *et al.* 2018). It was shown that the *L. brevis* 23017 reduced the Hg-induced inflammation and oxidative stress through regulating MAPK and NF-κB pathways (Jiang *et al.* 2018). Additionally, *L. brevis* 23017 supplementation maintained a normal mucosal barrier via modulation of TJ proteins. Depletion of gut microbiota by antibiotics significantly increased the levels of Hg accumulation in several organs like cerebellum, liver, and lungs in mice that are exposed to Hg in comparison with the mice that harbored normal microbiota (Seki *et al.* 2021). It was shown that MeHg is captured and inactivated by the hydrogen sulfide and hydrogen persulfide (by forming sulfur adducts to excrete out) produced by gut microbes, which resulted in reduced MeHg toxicity (Seki *et al.* 2021). Furthermore, gut microbes also regulate Hg biotransformation and bioaccumulation in freshwater fish (*Micropterus salmoides*), marine fish (*Acanthopagrus latus*), and polar bear (*Ursus maritimus*) (Watson *et al.* 2021, Yang *et al.* 2021*b*, Tan *et al.* 2022). Zhao *et al.* showed that subchronic oral mercury caused loss in body weight, intestinal injury and led to changes in gut microbiota and aggravated apoptosis in mice (Zhao *et al.* 2020). In case reports, it was shown that occupational exposure to mercury vapors led to increased episodes of disease reactivation in patients with chronic ulcerative colitis (Cummings & Rosenman 2006). It is interesting recall that mercury released from dental 'silver' fillings provoked an increase in mercury- and antibiotic-resistant bacteria in oral cavity and intestines (Summers *et al.* 1993). These mercury- and antibiotic-resistant isolates include Staphylococci, Enterococci, and members of the family Enterobacteriaceae. Mercury exposure led to generation of mercury and antibiotic resistance plasmids in the normal microbiota of primates (Summers *et al.* 1993). Further studies are required to define the effects of mercury on human GI system and its role in promoting GI-related disorders.

Cadmium

Cadmium (Cd) is a highly toxic environmental pollutant that majorly gets accumulated from cigarette smoking and diet. Cd exposure is related to several diseases like diabetes, chronic kidney disease, osteoporosis, obesity, liver disease, cardiovascular diseases, and cancer (Tellez-Plaza *et al.* 2012, Tinkov *et al.* 2017, 2018). Cd induces cytotoxicity by enhancing inflammation, gut barrier dysfunction, oxidative stress, endoplasmic reticulum stress, endocrine disruption, and genomic instability (Rafati Rahimzadeh *et al.* 2017). Several studies reported that exposure to Cd induced leaky gut, with an irregular distribution and reduced expression of TJ proteins like ZO-1, ZO-2, JAM-A, occludin, and claudin 1 in IECs and mice (Duizer *et al.* 1999, Rusanov *et al.* 2015, Zhai *et al.* 2016, Liu *et al.* 2020*a*). Relative changes in transepithelial/transendothelial electrical resistance (aka TEER) after high doses (100 and 300 μM) of Cd exposure were the result of damaged tight intercellular junctions and loss of the monolayer integrity (Rusanov *et al.* 2015). Even low-dose Cd exposure significantly caused gut microbial dysbiosis in mice and aggravated liver injury by increased intestinal permeability (Liu *et al.* 2020*a*). In this study, they showed that Cd exposure significantly downregulated the expression of TJ proteins such as ZO-1, JAM-A, and occludin, leading to increased intestinal permeability and liver injury. Importantly, it was shown that low Cd exposure led to decrease in the *Akkermansia muciniphila*, a commensal known to protect the gut barrier integrity, suggesting adverse impacts of Cd exposure in regulating gut microbiota composition (Liu *et al.* 2020*a*). Cd interfered with gut mucosa and goblet cells in a dose- and site-dependent manner in zebrafish (*Danio rerio*) (Motta *et al.* 2022). Cd altered the mucosal efficiency by changing localization and distribution of glycan residues and metallothionein expression in intestinal cells (Motta *et al.* 2022). Moreover, oral intake of Cd changed the adaptive immune activities in mice, leading to increased gut permeability, intestinal tissue damage, and inflammation. Importantly, Cd exposure reduced the population of the beneficial commensal *Lactobacillus* strain (Ninkov *et al.* 2015). It was shown that Cd exposure modified the gut–liver axis along with gut dysbiosis in ApoE4-KI males, which are the most susceptible mice strain to neurological damages (Zhang *et al.* 2021). Exposure to Cd in mice also modulated the levels of gut microbiota like *Eisenbergiella*, *Blautia*, *Clostridium* XlVa, *Lactobacillus*, *Bifidobacterium* and decreased levels of microbial metabolites such as short chain fatty acids (e.g. butyrate) (Liu *et al.* 2014, Li *et al.* 2019). It was shown that Cd exposure in mice led to significant alteration in the gut microbiota population including several butyrate producers along with changes in the bile acid fraction of the gut metabolome (Li *et al.* 2019). Moreover, Cd exposure caused a decrease in the thickness of inner mucus layer and reduced the growth of *Bacteroidetes*, *Lactobacillus*, and *Bifidobacterium* followed by altered SCFA metabolism (Liu *et al.* 2014). Yang *et al.* showed that Cd exposure had adverse effects on liver, kidney, and ovary functions in adolescent rats. Moreover, their study unveiled alterations in the microbiota, characterized by decreased levels of *Prevotella* and *Lachnoclostridium*, and increased levels of *Escherichia coli* and *Shigella* (Yang *et al.* 2021*a*), whereas use of gut microbes like *E. coli* and *Lactobacillus acidophilus* reduced the Cd-mediated IEC permeability (Bolan *et al.* 2021). Probiotic *Lactobacillus plantarum* CCFM8610 also reduced that Cd-induced toxicity and intestinal motility dysfunction in mice with decreased Cd content in the tissues and blood of animals along with enhanced fecal cadmium excretion (Liu *et al.* 2020*b*). Moreover, *Lactobacillus plantarum* CCFM8610 exhibited excellent

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Cd binding and antioxidative capacity, which helped in protection of the Cd-mediated disruption of TJs in IECs. Overall, Cd exposure leads to gut microbial dysbiosis and exerts its impact on gut barrier integrity, leading to increased gut permeability and promoting GI-related disorders; supplementation of beneficial certain probiotics could protect from Cd-induced adverse effects.

Chromium

Chromium, a known mutagenic, metallic contaminant gets bioaccumulated in animals from solid waste disposal, pesticides, fertilizers, mining activities, and residues from industrial productions. Cr is available in multiple oxidation states, but trivalent and hexavalent forms are the most stable (Balali-Mood *et al.* 2021). Cr in trivalent state is required for lipid metabolism, protein metabolism and as a cofactor for insulin action, whereas hexavalent Cr is responsible for disease pathogenesis like organ failure, rhinitis, asthma, gut barrier dysfunction, and cancer. A recent meta-analysis from human study showed that Cr exposure increased mortality and occurrence of larynx, lung, thyroid, bone, kidney, bladder, and testicular cancer (Deng *et al.* 2019). Another human study reported that groundwater contamination of Cr caused severe GI distress and digestive problems in both male and females along with changes in hematological parameters like RBC and platelet counts (Sharma *et al.* 2012). Studies revealed that long-term exposure to Cr aggravated GI symptoms and body weight loss in colorectal cancer model with change in microbial abundance (Zhang *et al.* 2020). Moreover, Cr affected the GI tract of earthworm (*Eisenia fetida*) via nuclear damage in gut epithelia, with hemorrhage and ulceration (Tang *et al.* 2019). The TEM images revealed that the Cr-exposed earthworm contained subcellular injury with short, messy, and rough gut villus and damaged organelles. A recent study showed that exposure to hexavalent chromium in ducks led to gut barrier damage through downregulation of ZO-1, occludin, claudin 1, and MUC2 expression (Xing *et al.* 2022). Activation of NLRP3 inflammasome and generation of oxidative stress contributed to the shortening of the intestinal villi and gut barrier dysfunction. In mice with Cr-induced damage, the use of *Lactobacillus plantarum* TW1-1 reduced Cr accumulation and restored gut bacterial homeostasis (Wu *et al.* 2017). Cr exposure effectively dysregulated Bacteroidetes–Firmicutes homeostasis and increased the abundance of S24-7, *Prevotella*, and Clostridiales but lowered Lachnospiraceae. The lower butyrate producer Lachnospiraceae hampered the development of IECs, leading to inflammation and oxidative stress after Cr exposure in mice (Wu *et al.* 2017). Long-term Cr exposure also increased the presence of Firmicutes and Actinobacteria and removed the presence of *Deferribacteres*, *Intestinimonas*, *Butyricimonas*, *Butyricicoccus*, Lachnospiraceae FCS020 group, and Ruminococcaceae V9D2013 group in chickens (Li *et al.* 2021). Several studies in Wistar rats and chickens proved that gut microbiota is essential for first line of defense against Cr toxicity and microbes may act as a prebiotic or probiotic to attenuate Cr toxicity (Shrivastava *et al.* 2005, Li *et al.* 2022, Wang *et al.* 2022*b*). Upreti *et al.* developed resistance to Cr up to 64 ppm in probiotic Lactobacilli strains with chronological chronic exposures. These probiotic strains showed no antibiotic resistance but ameliorated Cr-induced GI toxicity (Upreti *et al.* 2011). Thus, this study showed that alteration of microbial composition can be therapeutic for Cr-induced GI damage and toxicity.

Moreover, some metals like aluminum (Al) despite not having a high density like 'heavy metals' are considered toxic due to their level of toxicity. Aluminum is the most abundant metal element in the Earth's crust, and continuous exposure to Al leads to bioaccumulation and severe toxicity in several tissues including the gut (Vignal *et al.* 2016). Studies have reported that excessive Al induced apoptosis of IECs and disrupted TJ proteins, leading to increased intestinal permeability and gut barrier dysfunction (Vignal *et al.* 2016, Hao *et al.* 2022). Damage of the gut barrier due to Al exposure leads to impaired immune system in affected individuals. Yu *et al.* reported that dietary Al exposure altered the human gut microbiota composition and that supplementation with probiotics like *L. plantarum* CCFM639 abrogated the toxicity of Al (Yu *et al.* 2021*a*). In Wistar rats, dietary intake of Al diminished the natural gut microbiota diversity and affected the physiological homeostasis (Wang *et al.* 2022*a*).

Outstanding questions and major challenges

- What are the molecular and cellular mechanisms of heavy metal-mediated gut microbial dysbiosis and gut barrier dysfunction? Especially, how do heavy metals disrupt epithelial junctional proteins? Is the metal-induced oxidative stress solely responsible for long-term adverse effects that are observed in the GI tract?
- How does microbiota regulate metal toxicity and is there any specific bacterium or bacterial consortium responsible for detoxification of specific metal toxicity? For example, is there any bacterial specificity for heavy metals that either increase or decrease toxicity? If so, what are the mechanisms for metal specificity?
- What are interactions of microbial metabolites with heavy metals and their implications on gut barrier functions in humans? Especially, it will be interesting to determine whether direct supplementation of microbial metabolites renders benefits against heavy metal toxicity and helps overcome the microbial dysbiosis?

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- The effects of heavy metal exposure on skin and lung microbiome are less explored. It is important to evaluate these effects, as metals directly influence the pathogenesis of skin and respiratory disorders.
- Developing appropriate models to investigate metal toxicity is a major challenge in this area of research. For instance, recapitulation of human exposure (low vs high) of heavy metals in animal models is a major constraint due to limitations in duration of exposure, and controlling the route of administration may not provide the complete array of effects observed in humans. Secondly, the metabolic rates of metals are significantly different between humans and animal models (mice or rats), which adds another layer of complexity. Thirdly, the effects of heavy metals on gut environment may not be detectable in early stages, but metal-induced gut barrier dysfunction may promote and cause other organ damage due to leakage of constant bacterial endotoxins and inflammatory cytokines. Therefore, developing methods and biomarkers to detect gut barrier functions would significantly aid in the design and development of therapeutics.

Summary

The gut epithelial barrier is the most important system for nutrient absorption and required for protection from exogenous pathobionts and environmental pollutants (Gillois *et al.* 2018, Ghosh *et al.* 2021). In homeostatic conditions, the gut microbiota and gut

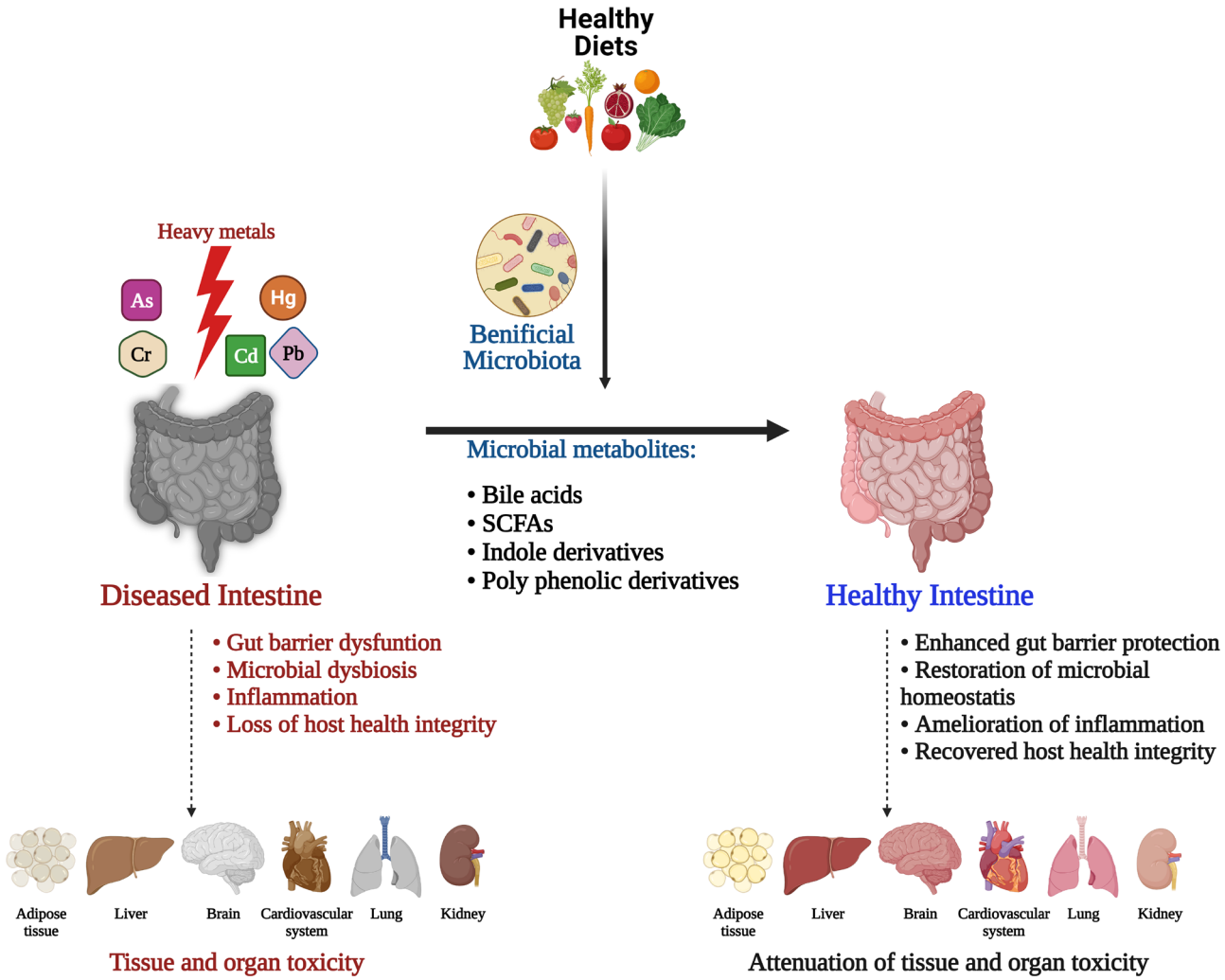

Figure 2

Effect of heavy metals on human health. Environmental pollutants such as arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), and chromium (Cr) lead to several adverse health effects. Heavy metal-induced loss of gut barrier integrity and microbial balance initiates inflammation and toxicity in host tissue and organs. Consumption of healthy diets and treatment with beneficial gut microbiota or microbial metabolites potentially mitigate the adverse effects of heavy metal toxicity and restore gut homeostasis.

barrier play a protective role against toxic effects of environmental pollutants and reduce the risk factors for GI-related disorders. Nevertheless, several exogenous and endogenous factors such as diet, xenobiotics, heavy metals, and microbial dysbiosis as well as increased inflammation contribute to the enhanced barrier dysfunction and systemic toxicity. The extent and duration of exposure are crucial factors in determining the hazards that may jeopardize the host's overall health. Failure to promptly address these issues could result in the continuous deterioration of epithelial barriers, posing an increased risk for a range of GI and non-GI disorders. Alterations in gut physiology, encompassing but not limited to the degradation of the mucus layer, disruption of junctional proteins, heightened intestinal permeability, inflammation, and microbial imbalances, are the underlying causes of gut barrier dysfunction and immune irregularities. Heavy metal exposure impairs the metabolic activity of the gut microbiome, giving rise to inflammatory responses and cellular damage. In contrast, the restoration of microbial equilibrium and the sequestration of heavy metals by specific microbiota hold potential benefits for the host. The concept of 'Intestinal Bioremediation' is emerging as a valuable tool for immobilizing toxic metals by selectively employing relevant microbes to shield against their harmful effects (George *et al.* 2021), although the precise mechanisms underpinning this microbiota-mediated defense against environmental pollutants continue to be under investigation. Recent research has unveiled that gut bacteria communicate with the host signaling system through metabolites, which in turn regulate intestinal immunity and barrier defense (Fig. 2). The vital role played by the gut microbiota in maintaining gut homeostasis can thwart the nonessential toxicity of heavy metals. Therefore, leveraging beneficial microbiota and their metabolites as a therapeutic approach holds great promise in treating pollutant-induced gut barrier dysfunction.

Declaration of interest

VRJ is one of the scientific cofounders of Artus Therapeutics. SG and SPN have no conflicts of interest to declare.

Funding

VRJ is supported by NIH/NCI (CA191683), NIH/NIGMS CoBRE grant (P20GM125504-01), NIH/NIEHS (P30ES030283), The Jewish Heritage Fund for Excellence Research Enhancement Grant, and UofL Health-BCC.

Author contribution statement

SG and VRJ collected the literature, conceptualized the review, and wrote the manuscript. SP contributed by conceptualizing, proofreading, and editing the manuscript.

Acknowledgements

VRJ is supported by NIH/NCI (CA191683), NIH/NIGMS CoBRE grant (P20GM125504-01), NIH/NIEHS, (P30ES030283), The Jewish Heritage Fund for Excellence Research Enhancement Grant, and UofL-Health Brown Cancer Center. The authors thank Dr Bodduluri Haribabu for proofreading the manuscript and insightful discussions. Images were prepared using Biorender.com.

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