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Article

Xenobiotics: Interaction with the Intestinal Microflora

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Abstract

The human body is host to 100 trillion gut microbes, approximately 10-times more than all human cells. It is estimated that the approximately 500–1000 species residing in the human gut encode 150-fold more unique genes than the human genome. The gut microbiota has important functions in metabolic processing, such as energy production, immune cell development, food digestion, and epithelial homeostasis. It has been increasingly recognized that a dysregulated gut microbiome contributes in a significant way to a variety of diseases, including diabetes, obesity, cardiovascular diseases, allergies, and inflammatory bowel disease. In particular, accumulating evidence indicates that functional interactions between the gut microbiome and xenobiotics play a role in mediating chemical toxicity and causing or exacerbating human disease. This review summarizes emerging evidence that illustrates how xenobiotics can affect the gut microbiome structure, create functional changes to the gut microbiome, and become biotransformed by the gut microbiome.

Key words: gut microbiome; xenobiotics; interaction

Gut Microbiome and Host Homeostasis

The gut microbiome (GM) has recently been characterized as an "exteriorized organ" (Shetty et al. 2013) composed primarily of bacteria but also of viruses, archea, fungi, and protozoa (Sommer and Backhed 2013). There are approximately 100 trillion microbes in the human gut, with the bacterial phyla *Firmicutes* and *Bacteroidetes* being the most dominant (Ley et al. 2005). Together, the gut microbiome has approximately 150-fold more genetic capacity than the human genome (Tilg and Kaser 2011).

The GM is integral to metabolic processes through the regulation of metabolism-modulating host genes (e.g., *Fiaf* and Gpr41 in murine models) (Tilg and Kaser 2011) and the direct fermentation of nondigestible dietary carbohydrates into metabolites such as the short-chain fatty acids (SCFAs) (Ramakrishna 2013). The GM and its metabolites have also been shown in recent studies to influence host physiology. For example, the GM can directly affect tissue homeostasis, as gnotobiotic mice were found to have reduced epithelial cell turnover and apoptosis (Sommer and Backhed 2013), while gut metabolites like the SCFAs were shown to be involved in colonic epithelial cell repair and differentiation (Ramakrishna 2013).

Gut Microbiome and Obesity

The gut microbiome has been implicated in or associated with human health and disease, especially metabolic disorders such as obesity. The cecal microbiota of obese mice contains more *Firmicutes* and fewer *Bacteroidetes* than nonobese controls (Ley et al. 2005), and similar results have been shown in humans, suggesting that obesity has a microbial aspect to its genesis (Ley et al. 2006). Other studies have furthered this assertion, showing that the GM in both obese rodents and humans have an increased capacity for energy harvest, which promotes obesity development (Tremaroli and Backhed 2012). If this GM phenotype is transplanted into germ-free mice, there is an increase in energy harvest

along with other obesogenic conditions within those mice, suggesting that obesity alters not only the gut microbiome ecology but also its function within the body (Turnbaugh et al. 2006).

Gut Microbiome and Diabetes

In type-2 diabetes in humans, studies have shown a decrease in the microbe *Faecalibacterium prausnitzii*, which is associated with anti-inflammatory effects (Everard and Cani 2013). In contrast, studies show that the treatment of the GM by antibiotics can reduce gut permeability, which reduces bacterial cell wall components such as lipopolysaccharide (LPS) from activating pathways that can lead to decreased insulin sensitivity (Carvalho and Saad 2013). This suggests that the gut microbiota can regulate gut permeability (Tremaroli and Backhed 2012), suggesting that the composition of the GM can play a role in the development of diabetes. These findings are supported by a study that reported transferring the microbiota from lean human donors to those with metabolic abnormalities decreased insulin resistance in the recipients receiving the microbial transplants (Vrieze et al. 2012).

Gut Microbiome and Liver Disease

Another major disease in which the GM is implicated is nonalcoholic fatty liver disease (NAFLD). Patients with NAFLD have been shown to have an altered gut microbiome, small intestinal bacterial overgrowth, and increased intestinal permeability (Li et al. 2013). These factors encourage the gut bacteria and gut-derived bacterial products to cross the intestinal barrier, leading to endotoxemia. These translocated products can subsequently activate various receptors (like TLR4 in a murine model) that can then promote the release of inflammation-promoting cytokines and chemokines by liver cells, which then leads to NAFLD (Li and Jia 2013; Li et al. 2013). It is also known from experimental animal models that a diet deficient in choline causes symptoms of fatty liver (Koch-Weser et al. 1953; Zeisel 1992). Additionally, it has been found that gut bacteria can decrease the amount of ingested choline by forming choline to a metabolite known as trimethylamine (TMA) (Zeisel et al. 1983). A study found that there was a greater increase in the excretion of urinary TMA in male 129S6 mice documented to be susceptible to NAFLD, directly showing the association between the gut-microbial-mediated metabolism of choline and susceptibility to NAFLD (Dumas et al. 2006). In fact, trimethylamine has recently emerged as a promising biomarker for noninvasive diagnosis of NAFLD in children (Alkhouri et al. 2014).

Gut Microbiome and Cardiovascular Disease

Another interesting finding is that increased fasting levels of plasma trimethylamine N-oxide (TMAO), which is converted from TMA by flavin-containing monooxygenase 3 (Russell et al. 2013), was associated with increased risk for an adverse cardiovascular event (Tang et al. 2013), which again illustrates how the GM's interaction with the host can cause disease and which suggests that using probiotics to lessen the GM's production of TMA (thus also TMAO) may reduce the risk of atherosclerosis (Wang et al. 2011).

Gut Microbiome and Cancer

Liver cancer has been one of the few cancers where there is a mechanism behind how the gut microbiome is involved. A study published in *Nature* found that obesity-induced alteration of the gut microbiome increases levels of deoxycholic acid, a metabolite that is formed by the gut microbiome (Yoshimoto et al. 2013). Deoxycholic acid modifies hepatic stellate cells into secreting pro-inflammatory, tumorigenic molecules that facilitate the formation of hepatic carcinoma (Coppé et al. 2010; Yoshimoto et al. 2013).

Dysbiosis of the gut microbiome has also been associated in several studies with colorectal cancer, and a comprehensive review has been published on the topic (Keku et al. 2015). Though the mechanisms by which the gut microbiome affects colorectal cancer are still not very clear, several reports have proposed various mechanisms, including a deoxycholic-acid-mediated mechanism similar to that for liver cancer (Keku et al. 2015; Ohtani 2015).

Gut Microbiome and Mental Disease

Parasympathetic and sympathetic nerves, as well as sensory fibers, allow for two-way signaling between the gastrointestinal tract and the brain, which can regulate various processes including food intake (Konturek et al. 2004). In a review by Foster and McVey Neufeld, several examples of the interplay of the gut microbiome with the "gut-brain axis" are presented (Foster and McVey Neufeld 2013). Among these examples: germ free mice have less activation of the neurons of the enteric nervous system than control mice that are specific pathogen free; perturbations to the gut microbiome by pathogenic bacteria *Citrobacter rodentium* and *Campylobacter jejuni* upregulate the activation of vagal sensory neurons, which can induce stress and anxiety-type symptoms; and both antibiotics and probiotics were found in several animal studies to reduce these kinds of anxiety-like symptoms through the modulation of the gut microbiome (Foster and McVey Neufeld 2013).

The connection between the gut microbiome and mental state has spurred interest into the associations of the gut microbiome with mental diseases. With autism spectrum disorder (ASD) being a mental disease that affects ~1 in 68 children, several research articles and reviews have been published discussing its association with the gut microbiome (Louis 2012; Port et al. 2015). In one study, autistic patients complaining of gastrointestinal problems had a higher abundance of *Clostridium* in their gut microbiome compared with control patients complaining of similar gastrointestinal problems (Mulle et al. 2013; Parracho et al. 2005). Clostridia are known to produce neurotoxins, which could further worsen the behavioral symptoms of autism (Parracho et al. 2005). In another study, autism-associated dysbiosis of the gut microbiome was associated with a deficiency in the expression of enzymes necessary for carbohydrate digestion and transport, suggesting the role of the gut microbiome as a potential agent in gastrointestinal disorders as a result of an autistic disease state (Benach et al. 2012; Williams et al. 2011). Yet another study found an increase in the gut-microbial metabolite *p-cresol* in autistic children compared with controls, further implicating the involvement of the gut microbiome in autism (Altieri et al. 2011). Still, as the bulk of the studies are correlational and the underlying mechanisms are not largely understood, several researchers agree that more studies are needed to better elucidate the role of the gut microbiome in autism (Mayer et al. 2014; Toh and Allen-Vercoe 2015).

Taken together, the diseases mentioned above constitute only a short list of many diseases in which the gut microbiome may play a role. These disease case studies show the interplay between the gut microbiome, gut metabolites, the baseline genetics/epigenetics of the organism, and environmental factors in causing disease. A related, relevant interest is the functional interaction between xenobiotics, substances foreign to the body such as environmental chemicals or drugs, and the gut

microbiome. With the development of culture-independent, next-generation sequencing methods to determine GM composition (D'Argenio et al. 2014), and the use of -omics approaches, researchers have focused on elucidating the mechanisms centered on how the GM promotes or mediates xenobiotics toxicity to cause or exacerbate human disease.

We will thus begin this review with a general survey on how xenobiotics alter the GM community structure. Next we will discuss how xenobiotics can change the function of the GM, referring to studies that have examined the mechanisms whereby the GM can affect xenobiotics biotransformation and the resulting toxicological consequences.

Effects of Xenobiotics on the Gut Microbiome

Xenobiotics Alter the Gut Microbiome Community Structure

In general, xenobiotics have been known to alter the GM for some time (see, for example, George et al. 1989). However, recent developments in culture-free methods, such as 16S rRNA sequencing, have allowed us to actually profile the specific changes that occur in the GM community structure as a result of exposure to xenobiotics (Robinson and Young 2010). In this section, we will focus on studies that show the effects that several typical substances, such as antibiotics, pesticides, air pollutants, polychlorinated biphenyls (PCBs), and heavy metals, have on the GM structure. This is by no means a complete list of xenobiotics that could perturb the gut microbiome structures in different models.

Antibiotics

An early study using molecular methods (in this case, 16S rRNA gene sequencing) to monitor changes in the diversity within the GM as a result of antibiotic exposure involved a human patient who began to develop complications as a result of treatment with amoxicillin-clavulinic acid (Young and Schmidt 2004). Though the main component of the gut community of this patient consisted of members of the *Bacteroides* genus, the detection of bacteria in the family *Enterobacteriaceae* increased by 32% just 4 days after exposure to antibiotics. The *Enterobacteriaceae* were not detected two weeks after the cessation of antibiotic consumption, suggesting that the change occurred as a result of antibiotic exposure (Young and Schmidt 2004). In a mouse model treated with vancomycin, a similar trend of an increased presence of *Enterobacteriaceae* was observed (*Firmicutes* remained the dominant phyla detected in these samples), followed by a lack of *Enterobacteriaceae* after the mice had a recovery period without vancomycin treatment (Robinson and Young 2010). Since vancomycin is effective mostly against gram-positive bacteria, and *Enterobacteriaceae* are gram-negative, Robinson and Young (2010) suggested that the observed increase in *Enterobacteriaceae* may be a result of reduced competitive inhibition from perturbed gram-positive bacterial species, illustrating the selective nature by which antibiotics affect the gut community structure (Robinson and Young 2010). Another antibiotic that consistently has been shown to alter the GM is ampicillin. In a *Tenebrio molitor* larval model of the GM, ampicillin decreased the diversity and size of the gut bacterial community, although there was not a clear trend in the decrease of one phyla of bacteria over another (*Tenericutes* was the dominant phyla) (Jung et al. 2014). However, a study with three human volunteers found that ampicillin increased members of the *Bacteroidetes* phyla (Maurice et al. 2013).

Due to their selectivity of effect on the gut microbiome, antibiotics are being used for their efficacy to treat gut-microbiome-mediated diseases (Kerman and Deshpande 2014). Several recent publications using animal models suggest that antibiotic alteration of the gut microbiome shows promise in treating metabolic and gastrointestinal disorders such as insulin resistance, body weight gain, and irritable bowel syndrome (Davey et al. 2013; Hwang et al. 2015; Laterza et al. 2015). However, there is still a need for more clinical trials in order to formulate appropriate antibiotic therapies for humans (Kerman and Deshpande 2014).

Pesticides

Pesticides are a significant environmental hazard, especially considering the fact that there are 10,000–20,000 pesticide-related poisonings diagnosed every year among the 2 million farmworkers in the United States, and little is known about the impact of pesticides on the digestive system and especially the gut microbiome (Joly et al. 2013; National Institute for Occupational Safety and Health Division of Surveillance 2013). One study in a rat model observed that chronic, low-dose exposure to chloripyrifos (an organophosphate insecticide) was associated with a decrease in *Lactobacillus spp*. and *Bifidobacterium spp*. This is interesting because these two species are considered to be probiotic; thus their decrease is a sign of microbial dysbiosis that could further lead to disease (Joly et al. 2013). An in vitro study employing the poultry microbiome found that glyphosate, a herbicide known to be genotoxic and teratogenic, was associated with a decrease in beneficial bacteria such as *Enterococcus* spp., previously noted to have protective effects against disease-causing bacteria (Shehata et al. 2013). However, both studies depended on culture-based methods in order to profile the GM, which does not allow for a proper analysis of the effects of pesticides on the entire gut microbial community.

Air Pollutants

Several public health studies have associated air pollution exposure with adverse health effects such as lung cancer, sickle cell disease, asthma, high blood pressure, and gastrointestinal diseases (Barbosa et al. 2015; Brandt et al. 2015; Chan et al. 2015; Salim et al. 2014; Yang et al. 2015). The association of air pollution with gastrointestinal diseases is important because the gut microbiome plays a significant role in the development of these diseases. The literature on the association of air pollutants and its effect on the gut microbiome has been recently reviewed (Salim et al. 2014). In one study, PM-10 (particulate matter on the order of 10 micrometers or less) exposure in a mouse model of colonic inflammation was associated with alterations to the composition of the gut microbiome as well as greater decrease in butyrate, a metabolite formed by the gut microbiome previously found to suppress colonic inflammation by inhibition of interferon-gamma STAT1 (Kish et al. 2013; Zimmerman et al. 2012). It could be that PM-10-driven perturbation to the gut microbiome encourages a pro-inflammatory colonic environment that increases susceptibility to colonic diseases such as inflammatory bowel disease (IBD), especially since previous studies showed that germ-free mice do not develop colitis, and the same study showed increases in pro-inflammatory cytokines associated with PM-10 exposure (Kish et al. 2013). However, mechanistic studies are needed to prove this assertion, and there is also a need for an analysis of the effects of other major components of air pollution on the gut microbiome, such as carbon monoxide, sulfur dioxide, and nitrogen oxides.

Polychlorinated Biphenyls (PCBs)

Although there is evidence to suggest that polychlorinated biphenyls (PCBs) cause cancer, have negative effects on male

and female reproduction, and induce metabolic disorders such as adipose inflammation and impairment of glucose and insulin tolerance (Baker et al. 2015; Diamanti-Kandarakis et al. 2009), there has been only one published study to date centered on the effect of PCBs on the gut microbiome. In a mouse model, PCBs decreased the overall abundance of gut bacteria (by 2.2% from baseline) and primarily decreased the levels of proteobacteria (Choi et al. 2013). Interestingly, exercise by the mice altered the PCB-associated perturbation to the gut microbiome and elevated bacterial abundance (about 2.9% from baseline) (Choi et al. 2013). Although the authors did not do any further analysis of their results, they theorized that physical activity may have stimulated excretion of antimicrobial bile acids to the gastrointestinal tract, which could have selectively inhibited growth of some bacterial species while promoting growth of others (Choi et al. 2013). However, the relationship between exercise and the gut microbiome is complex (Clarke et al. 2014), and it is unclear if exercise would attenuate the effects of other kinds of chemical exposure on the gut microbiome similar to what was observed for PCBs.

Heavy Metals

Mercury. Mercury exposure from contaminated fish is a current environmental health hazard, with several epidemiological studies reporting poorer intellectual function associated with prenatal exposure in countries with high fish consumption (Debes et al. 2006; Jacobson et al. 2015). Mercury exposure has also been implicated in various metabolic disorders (Tinkov et al. 2015), and a study published in 1993 was the first to show the effects of mercury exposure on the gut microbiome. This study reported that mercury exposure altered the gut community structure by increasing both the abundance of mercury-resistant bacteria—several of which were also antibiotic resistant—as well as antibiotic-resistant plasmids in the GM of monkeys (Summers et al. 1993). A later study employing 16S rRNA profiling of the gut microbiome of *Porcellio scaber* (an isopod) not only confirmed that mercury exposure increased the abundance of mercury-resistant bacteria, but also found that mercury exposure completely eliminated *Bacteroidetes* and elevated levels of *Actinobacteria*, *Betaproteobacteria*, and *Alphaproteobacteria* (Lapanje et al. 2010). Though the former study did not detail the implications of these changes to promoting disease, a recent epidemiological study found that probiotics have a protective effect against increases to blood levels of mercury in pregnant women (Bisanz et al. 2014), suggesting that modulation of a mercury-exposed gut microbiome could possibly counter the effects of mercury-exposure-related disease. However, there remains a need for more experimental studies that use animal models greater of similarity to humans as well as cutting-edge microbiome profiling techniques in order to better establish the relationship between mercury exposure and the gut microbiome.

Cadmium and Lead. Oral exposure to cadmium and lead has been linked to various diseases and harmful effects, such as a decrease in hemoglobin (Breton, Daniel et al. 2013; Chen et al. 2015). Researchers in one study exposed mice to either cadmium or lead and found that neither was associated with any significant changes to the gut community structure at the phylum level, yet both were associated with gut bacterial changes at the family level, notably lower numbers of Lachnospiraceae and higher numbers of Lactobacillaceae and Erysipelotrichaceacae compared with control animals (Breton, Massart et al. 2013). However, another study found that cadmium exposure had significantly diminished Bacteroidetes growth and decreased levels of short-chain fatty acids such as the anti-inflammatory metabolite butyrate (Liu et al. 2014), which signifies that cadmium exposure could perturb the gut microbiome and promote gut inflammatory diseases. Previous literature has also connected lower numbers of Lachnospiraceae with colonic inflammation , and higher amounts of *Turcibacter* (part of the Erysipelotrichaceacae family) have been associated with appendicitis and colitis (Breton, Massart et al. 2013). However, these trends have not been clearly established and so more studies will be needed to understand the impact of gut microbiome exposure to cadmium and/or lead (Breton, Massart et al. 2013).

Xenobiotics Change the Functions of the Gut Microbiome

Although the aforementioned studies establish that exposure to xenobiotics can perturb the gut microbiome by changing the community structure, the authors tend to not follow up their analysis with other kinds of data, such as metatranscriptomics or metabolomics profiling, that can link perturbations to the gut microbiome as a result of xenobiotics exposure to specific changes to the metabolic or physiological functions that the gut microbiome serves and which could adversely affect the host. In this section, we will look at two examples of xenobiotics, antibiotics and arsenic, where studies have been conducted in this regard.

Antibiotics

Exposure in a mouse model to several different kinds of antibiotics, such as ampicillin, kanamycin, and chloramphenicol, was found to differentially express 1728 gene clusters in the gut microbiome (Maurice et al. 2013). Out of these gene clusters, there was an upregulation in genes related to stress response and antibiotic resistance, suggesting that antibiotic exposure creates metabolic stress as well as selective pressure, which changes how the GM responds to environmental exposure (Maurice et al. 2013).

Along with metagenomics analysis, a metabolomics study examined the effects of penicillin exposure in a rat model (Sun et al. 2013). Penicillin exposure resulted in the decrease of some bile acids, vitamins, and indole-containing metabolites, as well as several urinary metabolites conjugated with sulfate or glucuronide, which suggested that perturbation of the GM can affect gut-mediated pathways important to host metabolism as well as detoxification pathways of xenobiotics (Sun et al. 2013). This trend is similar to what we have found with arsenic, as noted in the next section.

Arsenic

Arsenic exposure is a major problem in the United States, with almost 25 million people consuming water containing arsenic levels greater than the 10 μg/L guideline of the World Health Organization and U.S. Environmental Protection Agency (EPA) (Lu, Abo et al. 2014). We have recently studied the effects of arsenic on the GM community structure and found that arsenic exposure via drinking water significantly perturbed the gut microbiome composition in C57BL/6 mice, with a significant decrease in several species of the *Firmicutes* phylum (Lu, Abo et al. 2014). It is also evident that arsenic perturbation to the gut microbiome disturbed its metabolic profile at the functional level, with changes to several metabolites that are either formed, processed, or mediated by the gut microbiome (Lu, Abo et al. 2014). For example, fatty-acid carnitines, involved in fatty acid oxidation, were reduced in the urine of arsenic-treated mice, suggesting that an arsenic-altered GM could decrease energy metabolism by the host (Lu, Abo et al. 2014). We also found the

reduction of several glucuronide metabolites in the urine, which suggests that gut-microbiome perturbation could also negatively affect phase-II detoxification within the body (Lu, Abo et al. 2014). Such changes to the metabolic profile could promote or exacerbate disease.

Other investigators approached this issue by analyzing the metabolic genes of the gut microbiome through profiling of an arsenic-exposed mouse fecal metagenome (Guo et al. 2014). They found a decrease in the abundance of genes for secondary metabolites biosynthesis, transport, and catabolism, as well as inorganic ion transport and metabolism (Guo et al. 2014). These findings continue to emphasize the negative effects of arsenic exposure to metabolism documented by our metabolomics analysis.

Impact of the Gut Microbiome on Xenobiotic Biotransformation

Xenobiotics are usually metabolized to reach target tissues or are excreted from the host. Thus, factors that mediate the biotransformation of xenobiotics could affect the functions and toxicity of xenobiotics. Xenobiotics have been shown to induce the GM to express genes having to do with the metabolism of xenobiotics, even during short-term exposure (Maurice et al. 2013). Thus, the biotransformation of several xenobiotics by the GM have been well characterized, especially by using in vitro methods, which include adding xenobiotics to human or animal fecal suspensions or fecal enzyme suspensions mixed with specific purified bacteria and then analyzing the metabolites (Kang et al. 2013). In vivo methods such as using mice with an altered gut microbiome have also been employed (Lu et al. 2013). The potential difference between an in vivo and in vitro model of the gut microbiome should be taken into consideration when analyzing studies of xenobiotics biotransformation, as some xenobiotics (like methamphetamine) can be metabolized by the GM in vitro but have little risk of being metabolized in humans due to efficient absorption before reaching the lower portions of the gut where gut bacteria are more prevalent (Sousa et al. 2008). On another note, recent studies have shown how the GM can indirectly regulate xenobiotic metabolism in the liver (Bjorkholm et al. 2009; Meinl et al. 2009), which means that the gut microbiome may not have to "see" a particular metabolite in order to be able to affect its metabolism. Thus, the examples discussed here are only an attempt to review the role of the gut microbiome in mediating the biotransformation of specific classes of xenobiotics with a focus on those that could cause or exacerbate disease. Our analysis is by no means an exhaustive list of the metabolic potential mediated by the GM.

Drug Metabolism

One paper identified 30 drugs whose biotransformation is mediated by the GM (Sousa et al. 2008), while other publications have discussed other drugs (Davey et al. 2012; Kang et al. 2013). Sousa and colleagues (2008) provide a detailed review of the specific metabolic pathways of several of these drugs (Sousa et al. 2008).

The metabolism of sorivudine is probably the most serious example of how the GM's metabolism of xenobiotics can adversely affect human health. Sorivudine, an antiviral drug that treats herpes zoster, is converted by the GM into (E)-5-(2-bromovinyl) uracil (BVU) (Nakayama et al. 1997). In 1993, there were 18 deaths in Japan of people who had taken sorivudine but had also taken an anticancer drug 5-flurouracil (5-FU) (Li-Wan-Po 2013). Later studies in a rat model provided a possible mechanism for this unfortunate outcome: the gut-microbial metabolite bromvalerylurea (BVU) is reduced in the liver by an enzyme known as dihydropyrimidine dehydrogenase (DPD). DPD also mediates the hydrogenation of 5-FU into other metabolites. In the rat model, reduced-BVU inactivated DPD, which promotes the buildup of 5-FU and leads to toxic conditions (Okuda et al. 1998). As there is substantial variation in the enzymatic activity of DPD amongst different people (Watabe et al. 2002), the co-administration of these drugs presents a great risk because some people may be more adversely affected by the drugs than others.

Another drug that interacts with the gut microbiome is digoxin, a drug that is used to treat heart failure and atrial fibrillation. Digoxin was found to be inactivated by the GM into reduced metabolites that were unable to have any medical effect (Lindenbaum et al. 1981). A later study determined that the particular gut bacterium *Eubacterium lentum* was most likely responsible for this (the modern name is *Eggerthella lenta*) (Saha et al. 1983). A recent study has elucidated a mechanism involved in the metabolism of digoxin by showing the upregulation of a two-gene cytochrome-encoding operon in *E. lenta* that was co-administered with digoxin (Haiser et al. 2013). This cardiac glycoside reductase (cgr) operon is thought to produce a protein (known as Cgr1-Cgr2 complex) that can bind to digoxin and cause the formation of reduced metabolites like dihydrodigoxin (Haiser et al. 2014). The authors of this study proposed the use of a high-protein diet to help prevent the GM deactivation of digoxin, since arginine was found to inhibit the conversion of digoxin by *E. lenta* (Haiser et al. 2014).

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are substances that are formed during incomplete burning of fossil fuels and waste. They are also present in cigarette smoke and grilled meats. Among the environmental chemicals that can be biotransformed by the GM, the polycyclic aromatic hydrocarbons are known to be transformed into potentially toxic metabolites. One study reported that the human GM can modify several polycyclic aromatic hydrocarbons (PAHs; naphthalene, phenanthrene, pyrene, and benzo(a)pyrene) to produce esterogenic metabolites, which resulted in a significant, positive signal in a gene-reporter assay for the human estrogen receptor. This study indicates that the risk of PAH toxicity to humans may be underestimated if bacterial metabolism is not considered (van de Wiele et al. 2005). Two other PAHs, 1-nitropyrene and 6-nitrobenzo[a]pyrene, lead to potentially toxic metabolites. 1-nitropyrene (1-NP) is metabolized to 1-nitropyrene oxides (1-NPO). Though some 1-NPOs by themselves have been found to be mutagenic to human cell lines (Kim et al. 2008), the 1-NPOs can be transformed and excreted from the liver as a cysteine conjugate, which can then be metabolized by a specific GM enzyme known as β-lyase (Kinouchi et al. 1992). The addition of β-lyase in vitro has been found to promote increased mutagenicity of the 1-NPO cysteine conjugate by a *Salmonella* sp. mutagenicity assay, as well as by an assay that showed increased binding of a more active 1-NPO cysteine conjugate product metabolite to thymus calf DNA (Kataoka et al. 1995). 6-nitrobenzo[a]pyrene can be transformed to 6-nitrosobenzo[a]pyrene by human intestinal microbiota, and 6-nitrosobenzo[a]pyrene was found to strongly induce mutagenicity in a *Salmonella* sp. mutagenicity test (Fu et al. 1988).

Gut Microbiome and Mycotoxin

Gut microbial biotransformation may reduce the toxicity of some environmental chemicals. For example, deoxynivalenol (DON) is

a mycotoxin present in grain that has been shown to undergo de-epoxidation by the GM in both rats and pigs (Kollarczik et al. 1994; Worrell et al. 1989). This de-epoxidated metabolite (DOM-1) was correlated with less cytotoxicity in a MTT-cell-culture assay using swine kidney cells (Kollarczik et al. 1994). Although one group did not find these de-epoxidated metabolites in an in vitro assay of human fecal microbiota (Sundstol Eriksen and Pettersson 2003), another group reported that one volunteer had fecal microbiota capable of biotransformation of DON into DOM-1 (Gratz et al. 2013). Variations in the GM, possibly due to environmental factors such as diet (Dall'Erta et al. 2013), could provide a rationale for these phenomena and provide a new avenue for manipulating GM-mediated toxicity.

Gut Microbiome and Heavy Metals

The literature has some information on how heavy metals or heavy-metal-containing products could be biotransformed by the GM. Mercury-resistant bacteria in the fecal flora of primates can biotransform Hg(II) to volatile Hg(0) in a detoxification pathway; this biotransformation is mediated by the *mer* operon present in these bacteria (Liebert et al. 1997). On the other hand, bismuth, even at low administered concentrations, can be transformed into the toxic, volatile trimethylbismuth by the GM of both humans and mice (Michalke et al. 2008). Diaz-Bone and van de Wiele have a detailed review on GM-mediated biotransformation of several metalloids (Diaz-Bone and van de Wiele 2009).

Gut Microbiome and Arsenic Metabolism

Arsenical biotransformation mediated by the GM has been studied in several labs, including our own. The biotransformation of arsenic is complicated, with multiple arsenic species and enzymes being involved in the process. Inorganic arsenic (iAs^V and iAs^{III}) is primarily metabolized to dimethylarsinic acid (DMA^V) in what is considered to be a detoxification pathway (Conklin et al. 2006). However, studies using in vitro culture of human colonic microbiota found that arsenic can be biotransformed to monomethylarsonic acid (MMA^V) and monomethylarsonous acid (MMA^{III}). MMA^{III} is known to be more toxic than inorganic arsenic (van de Wiele et al. 2010). The same authors also found another metabolite, monomethylmonothioarsonic acid ($MMMTA^V$), which they stated could also be toxic given that some methylated thioarsenicals are more efficiently absorbed by mammalian cells (van de Wiele et al. 2010). A follow-up study, also using human colonic microbiota, found that the presence of a rice matrix could lower the bioaccessiblity of arsenic and could potentially lower its toxic metabolism by the GM (Alava et al. 2013).

The GM has also been found to modify arsenic in other ways, sometimes even derivatives that may be less toxic. One study documented the in vitro conversion of dimethylmonothioarsinic acid ($DMMTA^V$) to trimethylarsinine sulfide ($TMAS^V$) by cecal anaerobic microbiota of mice. $DMMTA^V$ is known to be very genotoxic (Kuroda et al. 2004) and cytotoxic (Naranmandura et al. 2007; Yoshida et al. 2003), while $TMAS^V$ was found to be much less toxic (Rick Irvin and Irgolic 1995; Suzuki et al. 2007). Another study found that an arsenosugar could be converted to its sulfur analog by mice cecal microbiota (Conklin et al. 2006). Although this study did not find that this sulfur analog was toxic, more recent studies examining other arsenosugar metabolites indicated they could be potentially toxic to cells (Leffers et al. 2013). These findings argue that the metabolism of arsenosugars by the GM either promoting or lessening toxicity requires further investigation.

We have examined the effect of environmental and genetic-driven perturbations to the gut microbiome on the biotransformation of arsenic. In one study, C57BL/6 mice were challenged with *Helicobacter trogontum* and also administered arsenic in drinking water. Levels of $DMTA^V$, DMA^V, and MMA^{III} decreased, while iAs^V increased, suggesting that perturbation of the GM by environmental factors inhibits the detoxification of arsenic (Lu et al. 2013). In another study, we found that arsenic-exposed, immunocompromised IL-$10^{-/-}$ mice (which were associated with a decrease in GM diversity in previous studies [Maharshak et al. 2013]) showed an increase in MMA^V, iAs^V, and the ratio of MMA^V/DMA^V, suggesting that an abnormal genetic background is another factor that can contribute to an altered gut microbiome that interferes with the detoxification of arsenic (Lu, Mahbub et al. 2014).

Conclusion

It is increasingly apparent that xenobiotics can affect the gut microbiome profile, create functional changes to the gut microbiome, and become biotransformed by the gut microbiome into metabolites that could be more or less toxic. Additionally, other factors such as genetics, diet, and preexisting disorders may perturb the xenobiotics-gut microbiome relationship in either a positive or negative manner. The majority of published studies aim to characterize the changes of gut microbiome community structures following exposure to xenobiotics, and it is expected that future studies will concentrate more on deciphering the functional impact of these exposure-induced microbiome alterations in the host. Thus, the gut microbiome and associated functional changes may soon serve as potential biomarkers for the development of various kinds of diseases and disorders (Ross et al. 2013; Xiao and Zhao 2014).

Because human subjects are not directly used to study the relationship between xenobiotics and the GM, the use of in vitro simulators of the human gut microbiome, as well as the use of suitable animal models, would be highly valuable to address the xenobiotics-microbiome interaction. In particular, although previous studies have consistently demonstrated significant changes in the relative abundance of several phyla, families, or genera of gut bacteria as a result of xenobiotic exposure, the effects of these changes on the host and in disease development are largely unexplored and remain to be further elucidated. Combining well-designed animal models and exposure scenarios would allow us to discover the mechanisms behind how xenobiotics-induced microbiome alterations, GM-mediated production of toxic metabolites, or the perturbations to metabolic/detoxification pathways can actually disrupt human health, which currently represents a significant knowledge gap on delineating functional interactions between xenobiotics and the microbiome.

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