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Evaluation of the Impact of BaP Exposure on the Gut Microbiota and Allergic Responses in an OVA-Sensitized Mouse Model

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BACKGROUND: Exposure to environmental pollutants, including benzo[a]pyrene (BaP), has been implicated in allergic diseases and intestinal microbiota homeostasis, but the environment–microbiota–immunity triangular relationship and to what extent BaP-induced remodeling of the gut microbiota contributes to intestinal allergic inflammation remain to be established.

OBJECTIVES: We investigated the impact of BaP on intestinal allergic inflammation and examined the relationship between this effect and gut microbiota dysbiosis. We explored the potential ability of intestinal bacteria to degrade BaP and alleviate cytotoxicity as a detoxification strategy to counteract the effects of BaP exposure.

METHODS: We combined microbiome shotgun metagenomics with animal histological and intestinal inflammatory responses to assess the effects of BaP (50 μg/mouse per day) in a 23-d toxicity test in antigen-induced allergic female mice. In addition, genome annotation, quantitative analysis of BaP, and in vitro cytotoxicity-tests using CaCo-2 cells were conducted to infer the role of intestinal bacteria in BaP detoxification.

RESULTS: BaP exposure impacted the taxonomic composition and the functional potential of the gut microbiota and aggravated antigen-induced intestinal allergic inflammatory responses. The level of inflammatory cytokines correlated with the abundance of specific bacterial taxa, including Lachnospiraceae bacterium 28-4 and Alistipes inops. We identified 614 bacteria harboring genes implicated in the degradation of BaP, and 4 of these bacterial strains were shown to significantly reduce the cytotoxicity of BaP to CaCo-2 cells in vitro.

DISCUSSION: Using allergic female mice as a model, we investigated the relationship between BaP, microbiota, and host immune reactions, highlighting the role of gut bacteria in BaP-aggravated allergic reactions. Our findings offer novel insight toward establishing the causal relationship between BaP exposure and the occurrence of allergic disorders. Identifying gut bacteria that degrade BaP may provide new strategies for ameliorating BaP cytotoxicity. 

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals with two or more fused aromatic rings.1,2 Benzo[a]pyrene (BaP), a model compound for PAHs, causes great environmental problems and harm to human health. BaP is present in coal tar,3 automobile exhaust,4 tobacco and wood smoke,5 and carbon-baked food,6 but cigarette smoke and diet are the major sources of human exposure to BaP.7 When BaP enters the entero-hepatic circulatory system, it is metabolized by CYP450 monooxygenases8 to form glycol epoxide compounds, which form BaP-DNA adducts to induce mutations and cancer.9 Exposure to PAHs, including BaP, is known to increase the risk for a variety of diseases, including allergic diseases, via immunomodulatory and pro-inflammatory activities. Long-term exposure to high doses of BaP has been reported to increase the risk of allergic disease in a guinea pig model of allergic rhinitis.10 Yanagisawa et al. showed that intratracheal exposure to BaP enhanced allergic airway inflammation in mice by facilitating T-helper 2 cell (Th2) responses and activating mediastinal lymph node (MLN) cells.11 Moreover, a recent study showed that oral administration of BaP enhanced the ear-swelling response and scratching behavior associated with allergic dermatitis in mice upon a challenge with allergens.12 Further, our previous research has shown BaP coexposure with allergens led to airway hyperresponsiveness and increased lung inflammation in mouse models of asthma.13 Collectively, these studies suggested that increased immunological and inflammatory responses are observed irrespective of the route of BaP exposure and that these responses can be detected systemically by elevated serum immunoglobulin E (IgE) antibody levels and locally in the lung or skin. However, the gut is one of the major sites for exposure to BaP, and it is unclear whether coexposure to BaP and allergens aggravates intestinal allergy.

As an important participant in maintaining gut and host health, the gut microbiota plays a crucial role not only in nutrition and energy balance, but it is also important for the maturation and

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modulation of the immune system in mice and humans, in part via the production of metabolites, such as short-chain fatty acids (SCFAs, including butyrinate), and in maintaining intestinal mucosal–bacterial homeostasis. The disruption of host–gut microbiota homeostasis is associated with inflammation and increased intestinal permeability. Furthermore, a number of studies have reported that intestinal bacterial dysbiosis could increase the risk of allergic diseases, and several studies of food allergy and sensitization have shown that gut dysbiosis may precede the development of food allergy. The gut microbiota may influence food allergy susceptibility by modulating type 2 immune responses, influencing immune development and tolerance, regulating basophil populations, and promoting intestinal barrier function. Of note, colonization of germ-free mice with feces from healthy infants protected the mice against anaphylactic responses to a milk allergen, whereas colonization with feces from milk-allergic infants did not. In this regard, exposure to environmental pollutants, including PAHs, has been shown to pose a significant risk to intestinal bacterial homeostasis and host health. It has been suggested that exposure to BaP in mice causes damage of small intestinal and colonic tissues, inflammation of inflammatory cells, and epithelial erosion associated with taxonomic alteration of the composition of gastrointestinal bacteria. However, the exact composition of the gut microbiota and their role in mediating the effect of BaP on host allergic responses remain to be defined.

Although xenobiotics can impact bacterial growth and metabolism, xenobiotics are also metabolized by intestinal bacteria, which may affect their bioavailability or toxicity in the host. Harishankar et al. showed that the intestinal bacteria, including Lactobacillus lactis, L. fermentum, L. plantarum, and Escherichia coli, have a potential for degrading the pesticide chlorpyrifos, and a study using a gastrointestinal simulator investigating the metabolizing capacity of the gut microbiota for different PAHs indicated that the gut microbiota contributes to the bioactivation of PAHs in the human body. Thus, although the intestinal microbiota has been suggested to play a role in the metabolism of drugs and xenobiotics to mitigate their toxicity to the host, the exact composition of the microbiota conferring this ability has not been fully elucidated. Some compounds, such as isoeritinin and 6-gingerol, have been reported to attenuate BaP-induced colonic injury and gut microbiota changes in mice, and Lactiplantibacillus plantarum CCFM8661 has been reported to protect the gut microbiota against BaP-induced toxicity, increase intestinal barrier integrity, and alleviate histopathological changes in mice. But none of these compounds or bacteria effectively counteracted the accumulation of BaP in the gastrointestinal tract. Previous studies have reported that bacteria with the ability to degrade BaP are found in the environment, but little research on the biodegradation of BaP by intestinal bacteria has been performed. Bacteria with BaP detoxification ability isolated from the environment cannot be easily administered to humans or animals, therefore, identifying and isolating BaP-detoxifying strains from the human gut may offer an alternative feasible strategy.

Determining the effect of BaP and allergen coexposure on the intestinal health of mice and investigation of the interaction between BaP, bacteria, and the immune system by interrogating shotgun metagenomics data may offer novel insight into a possible causal relationship between BaP exposure and the occurrence of allergic disorders. Furthermore, identifying gut bacteria that harbor genes involved in BaP detoxification may provide new strategies for ameliorating BaP cytotoxicity. Here, we employed a metagenome-wide association analysis to identify microbial species affected by BaP exposure potentially contributing to the induction of an inflammatory state, and importantly, by using a combination of bioinformatics analyses and in vitro experiments, we identified bacterial species with an ability to degrade BaP. We envisage that the combination of comprehensive metagenomics analyses, culture approaches, and in vitro testing not only improves insight into the mechanisms whereby BaP affects the physiology of organisms but also advances the screening for bacteria able to counteract the detrimental effects of BaP exposure.

**Methods**

**Animal Experiment Design and Sampling**

Wild-type female C57BL/6 mice [specific-pathogen-free (SPF), 5–6 wk old] were obtained from Guangdong Medical Laboratory Animal Center. Experiments were conducted following the guidelines of the research ethics committee and approved by animal care and use committee of Shenzhen University. The mice were weighed and held in cages at 22°C ± 1°C and 50 ± 10% relative humidity under a 12-h light/dark cycle and allowed free access to a standard diet (SPF, natural grain feed; Guangdong Medical Laboratory Animal Center) and water ad libitum. After a 7-d acclimatization phase, the mice were randomly split into four groups. The normal control group mice were given BaP-free corn oil (200 μL; control, n = 8). The BaP (Sigma-Aldrich)-exposure group mice were gavaged with BaP dissolved in corn oil orally for 23 d consecutively [50 μg/mouse per day, 200 μL; BaP alone (BAM group), n = 8]. Fifty micrograms of BaP was dissolved in 200 μL of corn oil and stored at 4°C before use. The sensitization group mice were treated with BaP-free corn oil, as described above. They were then sensitized intradermally with ovalbumin (OVA)/aluminum hydroxide [Al(OH)3] adjuvant (catalog no. AC219130250; Thermo) at day 9 and day 13 (Figure S1). The OVA (Sigma-Aldrich) and the Al(OH)3 mixture solution was formulated by adding 100 μg of OVA and 2 mg of Al(OH)3 to 100 μL physiological saline and was prepared before use and stored at 4°C. The challenge was at 100 μg OVA with Al(OH)3 adjuvant 2 mg, 100 μL/mouse. The mice were subjected to sensitization solely with OVA on day 19, day 21, and day 23 by intragastrical administration of 50 μg of OVA [dissolved in 0.3 mL of normal saline; mice sensitized and challenged solely with OVA (OV group, n = 8)]. In the BaP-exposed and OVA-sensitized model, BaP (50 μg/mouse per day, 200 μL) was administered [BaP in combination with OVA (BOM group; n = 8)].

All the mice had their body weight recorded and anal temperature measured every week. Upon euthanization in carbon dioxide (CO2) chambers, blood was taken by eyeball removal, and feces and intestinal tissues were harvested, snap-frozen, and stored in a −80°C freezer before analysis.

**Animal Histological Analyses**

Collected samples were fixed in 4% parafomaldehyde at 4°C overnight, dehydrated, and embedded in paraffin and cut into sections using a Leica RM2235 microtome (4 μm thick; Leica Microsystems) and stained with hematoxylin and eosin for light microscopy examination. The slides were reviewed in a blinded fashion by a pathologist and were assigned a histological score for intestinal inflammation, ranging from 0–4 (Table 1).

**Intestinal Permeability Measurements**

Mice were fasted for 12 h with free access to water, after which a baseline blood sample was collected from the submandibular vein. For the fluorescein isothiocyanate dextran (FITC-dextran) measurements, mice were orally gavaged with 200 μL of FITC-dextran (0.6 mg/g mouse, catalog no. A18557; Adooq Bioscience)
on day 23, and blood was collected 4 h later. After anesthesia, blood taken by eyeball removal was kept at room temperature for 30 min until centrifugation for 15 min at 3,000 rpm, and the serum was collected for analysis. The concentration of FITC-dextran was determined using a fluorimeter (BioTek Synergy H1; Bio Tek Instruments Inc.) with an excitation wavelength at 490 nm and an emission wavelength of 530 nm. The entire assay was performed in a dark environment.

**Enzyme-Linked Immunosorbent Assay Measurements**

Blood taken by eyeball removal was kept at room temperature for 30 min and centrifuged at 4°C at 3,500 rpm for 10 min on day 23. The serum was transferred to centrifuge tubes for enzyme-linked immunosorbent assay (ELISA) analysis. The levels of serum OVA-specific antibodies were detected by ELISA using high-binding plates [Chemical Abstract Services (CAS).514201; Nest] coated overnight with 2 μg/mL OVA. The detection of mouse OVA-specific antibodies was carried out by goat anti-mouse IgE–horseradish peroxidase (HRP; catalog no. 1110-05; Southern Biotech) or goat antimouse IgG1-HRP (catalog no. 1070-05; Southern Biotech) and the Multiskan GO full-wavelength microplate reader (Thermo Fisher Scientific) according to the manufacturer’s recommendations. Serum mouse mast cell protease 1 (MCPT-1) levels were measured by ELISA (catalog no. 88–7503-22; Invitrogen). The levels of cytokines in serum were assayed by ELISA according to the manufacturer’s protocol. Mouse anti-interleukin (IL)-4, anti-IL-5, anti-IL-13 ELISA kit, and mouse anti-IFN-γ were purchased from 4A Biotech Co., Ltd.

**Metagenome Sequencing and Analysis**

DNA extraction from stool, library construction, and sequencing were performed by BGI-Shenzhen, Shenzhen, China. Feces was collected using the MGIEasy Stool Sample Collection Kit (item no. 1000003702; MGI Tech Co., Ltd.), and stool DNA for metagenomics was extracted using MagPure Fast Stool DNA KF Kit B (catalog no. MD5115-02B; Magen Biotechnology Co., Ltd.), following the procedure described previously.39 The quality of the extracted DNA was determined by agarose gel electrophoresis and by using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). Metagenomic sequencing libraries of each sample were then constructed as previously described.40 In short, extracted DNA was fragmented to a length of 150–350 bp using a Covaris S220, polymerase chain reaction amplification was carried out after fragment end-repairing and A-tailing, barcoded adapters were ligated to the fragments, and single-stranded DNA (ssDNA) circularization was performed to yield the libraries. Paired-end sequencing was conducted on the BGI-DIPSEQ platform (read length: 100 bp).

After quality control of sequence reads (read length: >80 bp), the reads mapping to the mice genome (GRChm38)42 were removed using SOAP243 (default parameters, except -m 100 -x 1000 -r 1 -l 30 -v 5 -c 0.95 -u). High-quality reads were imported for the following analyses: Taxonomic composition and function profiling were performed using MetaPhAn 3 and HUMAnN 3 pipelines using default parameters.44,45 respectively, with ChocoPhAn V 30 (201901) and the full UniRef 90 databases (https://www.uniprot.org) (retrieved 1 October 2020). For functional analyses, microbial genes were binned to Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologies (KO) based on the annotations in HUMAnN 3, and then the KOs were binned to gut metabolic modules (GMMs).46 GMMs reflect bacterial and archaeal metabolism, with a focus on anaerobic fermentation processes. Briefly, the reference pathway database in GMM is a flat file where pathway/module reactions are listed according to their order in the pathway. Modules were defined with a detection threshold of >66% coverage of enzymes in a given pathway, and the module abundance was calculated as the median of KO abundance in the pathway with maximum coverage.47 Bioinformatic analyses were performed using R (version 3.6.1; R Development Core Team). Analyses of differential abundances of species were performed comparing two groups using Wilcoxon rank-sum testing, and p-values were adjusted using the Benjamini–Hochberg method. Non-metric multidimensional scaling (NMDS) was performed using the Bray–Curtis dissimilarity matrix calculated using the vegan R package (version 2.5.4). Cross-correlation Spearman’s correlation matrices for network analysis were generated with the lessR R package (version 2.5.4) and visualized with Cytoscape (version 3.8.2). Spearman’s correlations and p-values between levels of allergy and abundances of bacteria were calculated by the lessR package (version 2.5.4) in R. Linear discriminant analysis Effect Size (LEfSe) analyses47 were performed to estimate metabolic potentials that differed significantly among the groups. LEfSe was calculated using p < 0.05 for the factorial Kruskal–Wallis test and p < 0.05 for the pairwise Wilcoxon test. A LEfSe score of >2 was used as the threshold cutoff (http://huttenhower.sph.harvard.edu/galaxy).

**BaP Degradation Pathway Annotation**

In a previous study, we established a high-quality catalog of non-redundant reference genomes of cultivated human gut bacteria based on whole-genome sequencing.48 To identify whether gut bacteria harbor genes involved in BaP metabolism, the high-quality genomes (data obtained from our laboratory)49 were functionally annotated. Gene functions were assigned according to the best match of alignments using Blastx to the gut bacteria genomes and the KEGG (version 93).49 with query and subject match length >100 bp (amino acid) and coverage >80%, with identity >60%.

**Bacterial Culture**

The Coprococcus comes 1 strain, Coprococcus comes 2 strain, Streptococcus salivarius subsp. strain, Streptococcus salivarius subsp. salivarius NCTC8618 strain, Klebsiella pneumoniae subsp. rhinoscleromatis ATCC13884 strain, Escherichia coli strain, Bacillus mycoides DSM2048 strain, and Megasphaera indica strain, isolated from feces of healthy human donors in our laboratory,48 were stored in a glycerol suspension (20%, vol/vol) containing 0.1% cysteine at –80°C. Cultures of each of the eight bacterial species were suspended in peptone yeast glucose broth (PYG) medium,48 spread on PYG plates, and incubated at 37°C for 12 h under anaerobic conditions. Each bacterial strain was then grown in PYG medium at 37°C and harvested at the exponential growth phase. Finally, the
bacterial suspensions were washed twice and resuspended in phosphate-buffered saline for subsequent use. The concentration of bacteria was detected by measuring the optical density at 600 nm.

Twenty microliters of purified and resuspended bacterial suspension [1.0 × 10⁸ colony-forming units (CFUs)] were incubated in PYG medium with BaP (50, 25, 12.5, or 5 mg/L) as the sole carbon source and grown at 37°C under anaerobic condition for 6 d. Growth was monitored by measuring turbidity at 80°C for 2 min, then the oven was heated to 180°C at a rate of 20°C/min, held for 5 min, followed by an increase to 290°C at 5°C/min, and held for 5 min. The solvent cut time was set to 3 min. The GC-MS interface temperature was maintained at 290°C. Mass spectra were recorded at 1 scan/s under electronic impact with an electron energy of 70 eV, mass range of 45–450 AMUs. The temperature of the ion source was set at 230°C.

**Screening of BaP-Degrading Bacterial Strains**

A colorimetric assay was used to quantify the degradation of BaP for preliminary screening of Coprococcus comes 1 strain, Coprococcus comes 2 strain, Streptococcus salivarius subsp. strain, Streptococcus salivarius subsp. salivarius NCTC8618 strain, Klebsiella pneumoniae subsp. rhinoscleromatis ATCC13884 strain, Escherichia coli strain, and Megasphaera indica strain. Methylene blue (2%, vol/vol, as a redox indicator; CAS.61-73-4; Sigma-Aldrich) was added to the PYG medium with BaP (5 mg/L) as the sole carbon source, and the resuspended bacterial suspension (1.0 × 10⁸ CFU) was incubated in an anaerobic incubator at 37°C for 144 h. All experiments were performed with three biological replicates.

**Standard Solutions and Sample Preparation**

For the quantitative analysis, a BaP-d₁₂ (CAS. 63466-71-7; Alta-Scientific) deuterated internal standard (IS) was prepared in acetone at a concentration of 100 µg/mL. This stock solution stored in the dark at −20°C was stable for 1 month. A stock solution of BaP was prepared by dissolving BaP in acetone to a final concentration of 250 µg/mL, and further diluted in acetone to a final concentration of 25 µg/mL (DS1) and 5 µg/mL (DS2). Isotope ratio standard (std) solutions were prepared by mixing IS and BaP-d₁₂ as detailed below:

- **Std 1 solution:** 5 µL IS, 95 µL acetone (final concentrations: BaP, 0 µg/mL; BaP-d₁₂, 5 µg/mL)
- **Std 2 solution:** 100 µL DS2, 25 µL IS, 375 µL acetone (final concentrations: BaP, 1 µg/mL; BaP-d₁₂, 5 µg/mL)
- **Std 3 solution:** 500 µL DS2, 50 µL IS, 450 µL acetone (final concentrations: BaP, 2.5 µg/mL; BaP-d₁₂, 5 µg/mL)
- **Std 4 solution:** 200 µL DS1, 50 µL IS, 750 µL acetone (final concentrations: BaP, 5 µg/mL; BaP-d₁₂, 5 µg/mL)
- **Std 5 solution:** 500 µL DS1, 50 µL IS, 450 µL acetone (final concentrations: BaP, 12.5 µg/mL; BaP-d₁₂, 5 µg/mL)
- **Std 6 solution:** 75 µL DS1, 6.25 µL IS, 43.75 µL acetone (final concentrations: BaP, 15 µg/mL; BaP-d₁₂, 5 µg/mL).

Following culture growth, any remaining BaP was extracted by liquid–liquid extraction. Briefly, samples were taken after culturing for 6 d in a medium with BaP as the sole carbon source, and bacteria were separated from the medium by centrifugation at 8,000 rpm for 5 min, and the supernatants were collected. The supernatants were mixed with an appropriate amount of the IS solution (BaP-d₁₂ final concentration: 5 µg/mL), and subsequently supplemented with an equal volume of acetonitrile and vortexed for 10 min for mixing and equilibration. After the phases had separated, the organic phase was retained. The aqueous fraction after extraction was acidified with concentrated hydrochloric acid to pH 2 and extracted again with three equal volumes of ethyl acetate. The residual extracts were dried over anhydrous sodium sulfate and the combined extract was dried in a flow of nitrogen gas at 40°C. The extract was finally dissolved in hexane/acetonitrile (1:1, vol/vol) and subjected to gas chromatography–mass spectrometry (GC-MS) analysis.

**Quantitative Analysis of the Degradation of BaP**

The GC-MS analysis was performed using an HP 6890 gas chromatograph with an HP 5973 mass spectrometer system (Agilent 7890B-5977A). The column was a DB-5MS (30 m × 0.25 mm, 0.25 µm). Helium was the carrier gas, with a constant flow of 1.0 mL/min. The column temperature was held at 80°C for 2 min, then the oven was heated to 180°C at a rate of 20°C/min, held for 5 min, followed by an increase to 290°C at 5°C/min, and held for 5 min. The solvent cut time was set to 3 min. The GC-MS interface temperature was maintained at 290°C. Mass spectra were recorded at 1 scan/s under electronic impact with an electron energy of 70 eV, mass range of 45–450 AMUs. The temperature of the ion source was set at 230°C.

**Cell Culture and Subcultivation Model**

CaCo-2 cells were obtained from Shenzhen University and were grown in Dulbecco’s Modified Eagle’s medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), and 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco). Cells were grown in an atmosphere of 5% CO₂ and 90% relative humidity at 37°C. The medium was changed every 48 h and the cells were subcultured every week at a 1:3 split ratio by treatment with trypsin–ethylenediaminetetraacetic acid (EDTA) solution (0.25% trypsin, 10 mM EDTA). Bacteria used for BaP degradation were separated from the medium by centrifugation at 8,000 rpm for 5 min, and the supernatants were collected. A total of 2 × 10⁵ CaCo-2 cells were resuspended in 100 µL DMEM in each well of a 96-well plate. After incubation for 24 h, 10 µL of the bacterial supernatants were added to the CaCo-2 cell cultures, and incubation was continued for 48 h. Cytotoxicity was assessed by the manufacturer’s instruction and using a MultiSkans microplate spectrophotometer (Thermo). BaP-incubated medium without bacterial inoculation was used as a control. All experiments were performed with three biological replicates.

**Statistical Analysis**

Analyses were performed using R (version 3.6.1; R Development Core Team) or Prism (version 7; GraphPad Software, Inc.). For comparisons between multiple groups, two-way analysis of variance (ANOVA) with Tukey’s post hoc test was performed and adjusted p-value levels of <0.05 were considered significant. In addition, we used Kruskal–Wallis tests as indicated in the figure captions. The statistical significance of differential relative abundance (shotgun metagenomics) was computed using nonparametric two-sided Wilcoxon rank-sum tests with the Benjamini–Hochberg false discovery rate (FDR) correction for multiple hypotheses. The significance levels were *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001. A p < 0.05 was accepted as the level of statistical significance. All analyses were performed on individual animals or in independent experiments, as indicated in the text.
Results

Evaluation of Allergic Inflammation in Mice following BaP Exposure

To investigate the potential impact of BaP exposure on intestinal allergic inflammation, we used an established mouse model of intestinal allergic inflammation, using the BAM group or the BOM group, or the OVM group (Figure S1). The histological examination of the jejunum mucosa of normal control mice revealed an intact mucosa with neatly arranged intestinal villi without injury or inflammatory cell infiltration. The morphology of the jejunum mucosa was also intact in the OVM group, but the intestinal villi were narrowed, with slight serrated damage at the villi edge and a low degree of inflammatory cell infiltration. Compared with the control and OVM groups, the BAM group showed edema of the jejunum mucosa and slight erosion of intestinal villi, and the BOM group revealed intestinal villi of the jejunum displaying serious erosion, with especially severe damage at the top of the intestinal villi, and inflammatory cell infiltration (Figure 1A). The pathological score of the jejunum in the BOM group (average score: 2.4) was higher than that of the BAM group (average score: 1.4) and the OVM group (average score: 1.2) (Figure 1B and Table 1). Moreover, the numbers of eosinophils and neutrophils in the BOM group were significantly higher than in the control, the OVM, and the BAM groups (Figures 1C,D). The FITC-dextran permeability assay was used to examine the intestinal permeability in each group. Compared with the control group, the intestinal permeability was significantly higher in the BOM group (Figure 1E; p < 0.05).

Compared with the controls, we observed a modest up-regulation in the levels of circulating IL-4, IL-5, and IL-13 in mice exposed to BaP alone, and a further increase was noted in mice of the OVM group. The levels of IL-4 (p < 0.005), IL-5 (p < 0.005), and IL-13 (p < 0.005) were markedly higher in the allergic mice exposed to BaP (BAM group) compared with the OVM and the BAM groups (Figure 1F). In contrast, although the level of the Th1 cytokine IFN-γ (p < 0.005) was lower in mice challenged with OVA (OVM group) and in mice coexposed to BaP and OVA (BOM group) compared with the control and BaP groups (Figure 1F), there was no significant difference in IFN-γ levels in mice of the BOM group compared with the OVM group. The rectal temperatures of the BOM or OVM group mice were significantly lower than that of the control and BAM groups (Figure 1G, p < 0.005). The levels of OVA-specific IgE (p < 0.005), OVA-specific IgG1 (p < 0.005), and MCPT-1 (p < 0.05), which are indicators of allergic responses, were also significantly higher in the BOM group as compared with the OVM group (Figure 1H).

The Effects of BaP Exposure on Gut Microbiota Communities

We next investigated the changes of the gut microbiota upon oral intake of BaP and its functional relationship with BaP-aggravated allergic inflammation. The structural and functional characteristics of the microbiota were determined by shotgun metagenomics sequencing. In total, 59 fecal samples from mice were sequenced and a mean of 118 million clean reads for each sample were generated after quality control. Species annotations were performed by Metagenomic Phylogenetic Analysis 3 (MetaPhAn 3).44 We determined the top 10 species present in all four groups of mice, and termed these bacteria the “core” microbiota (Figure S2). Similarly, we identified the top 10 most abundant genera present in all four groups (Figure S2). We next analyzed the impact of exposure to BaP, comparing the microbiota of control mice and the BAM group at day 23. Based on Bray–Curtis dissimilarities, we did not observe a significant separation at the species level between the two groups (Figure S3). By contrast, comparing the OVM and the BOM groups based on Bray–Curtis dissimilarities at day 23, we observed a clear separation at the species level (Figure S3). To determine in more detail the alterations in the microbiota, we identified species that differed in abundance in pairwise comparisons between different groups after treatment for 23 d. We identified seven bacterial species that differed in relative abundance between mice not exposed to and exposed to BaP. Of these, Butyricimonas virosa and Neisseria subflava were present in higher relative abundance in the BAM group compared with the control group, whereas Bacteroides uniformis and Lachnospiraceae bacterium COE1 were present in lower relative abundance in the BAM group compared with the control group (Figure 2; Mann–Whitney–Wilcoxon test). Comparing the OVM group with the BOM group, we observed that the relative abundances of Lachnospiraceae bacterium 3-2, L. bacterium COE1, and Prevotella sp. MGM1 were significantly higher in the BOM group than in the OVM group, whereas the abundance of Faecalibacterium prausnitzii was significantly lower in the BOM group compared with the OVM group (Figure 2).

We assessed the gut microbial metabolic potential after BaP exposure using HUMAnN344 and GMMs for the analyses.46 At the level of gene families, KOs, and module profiles, significant differences were found among the groups at day 23. Importantly, we observed similarities in abundances between the BAM and the BOM groups, indicating that BaP exposure affected community functions of the gut microbiota (Figure S4; Adonis test p = 0.01). Consistent with previous analyses of microbial functional potential,55 the abundance of pathways involved in the metabolism of carbohydrates, amino acids, and nucleotides were significantly different than those in control and the OVM groups (Figure 3). Some pathways were enriched only in the BAM group or the BOM group. Thus, genes associated with queuosine biosynthesis were enriched specifically in the BAM group (marked with a black font in the BAM group in Figure 3), whereas those associated with seleno amino acid biosynthesis were enriched specifically in the BOM group (marked with a black font in the BOM group in Figure 3). In spite of the distinct differences, we also observed a substantial overlap in pathways affected by BaP in the BAM and the BOM groups (marked with red bold font in Figure 3). Our analyses indicated that Bacteroides vulgatus and Bacteroides vulgatus species were major contributors to the enrichment of metabolic pathways in both the BAM and BOM groups. In addition, we found that Prevotella sp. MGM2, Parabacteroides goldsteinii, and B. virosa were the main contributor to the differences in the abundance of community functions in the BAM group, whereas Prevotella sp. MGM1, L. bacterium COE1, and Bacteroides sartorii were the main contributors to differences in community functions in the BOM group (Figure 3). Different bacteria may contribute to differences in the abundance of a single metabolic pathway; for example, both L. bacterium COE1 and B. vulgatus contributed to the enrichment members of the adenine and adenosine salvage III pathway in the BOM group (Figure 3).

Identification of Bacteria Associated with Intestinal Dysbiosis and Inflammatory Responses

To investigate how bacteria in the gut ecosystem responded to BaP exposure, we adopted a co-abundance approach to analyze the community structure in the microbial ecosystem. Co-occurrence networks of bacteria at the species level were constructed based on Spearman’s rank correlations. These analyses revealed that BaP exposure affected the interaction relationships between gut bacteria (Figure 4A; Figure S5), where the number of bacteria whose abundance was significantly negatively correlated in the BOM group was higher than that in the
OVM group (Figure 4A), and the same phenomenon was observed comparing the BAM and the control groups (Figure S5). Some bacteria whose abundances were not significantly correlated in the control group or the OVM group were negatively correlated after BaP exposure, including *Bifidobacterium pseudolongum*, *Lactobacillus intestinal*, and *Lactobacillus johnsonii*. Furthermore, *B. virosa*, detected only in the mice exposed to BaP, was negatively correlated with *L. johnsonii* and *Lachnospiraceae bacterium* (Figure 4A).
Interestingly, BaP exposure shifted the relationship among certain bacteria. Thus, Lactobacillus murinus was positively correlated with Aclistibacter muris in the OVM group, whereas these two bacteria were negatively correlated in the BOM group (Figure 4A).

We identified specific gut bacteria associated with the levels of allergic responses, and we systematically analyzed the data from the four experimental groups by using a metagenome-wide association approach. Our analyses revealed that several bacteria correlated with allergic cytokines [Spearman’s rank correlation (r), FDR < 0.1]. L. bacterium 28-4 was positively correlated with the levels of OVA-specific antibodies, allergic cytokines, and intestinal permeability [Spearman’s rank correlation (r), FDR < 0.1] (Figure 4B; Figure S6A). The abundances of Muribaculaceae bacterium DSM103720, Alistipes inops, and Bacteroides stercorisroisoris were positively correlated with the levels of IFN-γ and rectal temperature (Figure 4B; Figure S6B). These bacteria were found to be negatively correlated with the abundance of other bacteria in the BOM group (Figure 4A), suggesting selective competition for niches among intestinal bacteria upon BaP exposure, concomitant with increased allergic inflammation. Prevotella and Alistipes were positively correlated with allergic cytokines at the genus level, whereas L. bacterium COE1, exhibiting higher abundance in the controls compared with the treatment groups, were negatively correlated with allergic cytokines (Figure 4B; Figure S6C).

Identification of Enzymes Involved in Degradation of BaP in Intestinal Bacteria

We examined the potential for BaP degradation in intestinal bacteria based on the whole-genome sequence data set in our previously reported gut bacterial strain collection.48 A total of 2,924 bacterial genomes were used to annotate BaP degradation-related enzymes. By alignment to the KEGG, we identified 614 bacteria harboring 1,129 bacterial genes encoding BaP degradation-related enzymes (Figure S7A). The 1,129 annotated genes could be classified into BaP-4,5-dioxygenases (66 genes), BaP-11,12-epoxidas (117 genes), 4,5-dihydroxy-BaP dioxygenases (181 genes), cis-4-(8-hydroxyxypren-7-yl)-2-oxobut-3-enoates (596 genes), and 4,5-chrysenedicarboxylate decarboxylases (169 genes) (Figure S7A). Of the annotated genes, 36.8% encoded one of three enzymes (BaP-4,5-dioxygenases, 4,5-dihydroxy-BaP dioxygenases, and 4,5-chrysenedicarboxylate decarboxylases) (Figure S7A), which are known to be involved in BaP detoxification, catalyzing ring cleavage and generation of intermediate metabolites that eventually enter the tricarboxylic acid (TCA) cycle (pathway iv) (Figure S7B).

In addition, 52.8% and 10.4% of the annotated genes encode cis-4-(8-hydroxyxypren-7-yl)-2-oxobut-3-enoatlyase (pathway ii) and BaP-11,12-epoxidase (pathway v), respectively, which belong to two additional BaP degradation pathways. These enzymes participate in the main steps of BaP degradation in gut bacteria, comprising detoxification pathways involving ring cleavage (Figure S7B), suggesting that gut bacteria have the potential to degrade BaP by different pathways. According to the type of annotated enzymes and pathway integrity, 87 species, including “beneficial” bacteria and opportunistic pathogens belonging to 29 genera, have a potential for degradation of BaP (Figure 5). Interestingly, BaP-detoxifying genes were found in the Parabacteroides distasonis genome (Figure S8B), whose abundance was shown to be negatively correlated with intestinal permeability and positively correlated with the level of IFN-γ (Figure 4B; Figure S8A). Lactobacillus johnsonii FI9785 and Parabacteroides goldsteinii, in which BaP-detoxification genes were also identified, were negatively correlated with allergic cytokines (Figure 4B; Figure S8B).

Figure 2. Impact of BaP exposure on the mouse gut microbiota. Bacterial species exhibiting different relative abundance in the BaP-exposed groups. Midline, median; box limits, upper and lower quartiles; whiskers, 1.5 × interquartile range; points, outliers. Mouse fecal samples were collected on day 23. n = 5–8 mice in each group. p-Values were estimated using Wilcoxon rank-sum tests, p < 0.05. The data are provided in Excel Table S2. Note: BAM, BaP alone; BaP, benzo[a]pyrene; BOM, BaP in combination with ovalbumin; OVM, sensitized and challenged solely with ovalbumin.
Figure 3. Comparison of the relative abundance of predicted functional modules (pathways) in the gut microbiota of mice. Linear discriminant analysis Effect Size (LEfSe) analysis was performed. (A) The comparison between the control and the BAM groups. (B) The comparison between the OVM and the BOM groups. Mouse fecal samples were collected on day 23. n = 5–8 mice in each group. p-Values were estimated using Wilcoxon rank-sum tests, p < 0.05. A LDA score > 2 was used as the threshold cutoff. Marked with bold font: Overlapping pathway affected by BaP in the BAM and the BOM groups. The roman fonts indicated by superscript were estimated using Wilcoxon rank-sum tests, p < 0.05. A LDA score > 2 was used as the threshold cutoff. Marked with bold font: Overlapping pathway affected by BaP in the BAM and the BOM groups. The roman fonts indicated by superscript were estimated using Wilcoxon rank-sum tests, p < 0.05. A LDA score > 2 was used as the threshold cutoff.
Figure 4. Bacterial networks in the OVM and the BOM groups and the correlation between inflammatory responses and intestinal bacteria. (A) Graphic representations of networks representing significant microbial interactions in OVA-sensitized mice with or without administration of BaP at day 23. Nodes represent bacteria at the species level. Edges represent significant positive (purple) and negative (orange) correlations between bacterial species. The thickness of the edges indicates the strength of the correlation. (B) Heatmap of the correlations between bacterial abundances and phenotypic data. Spearman’s correlation was used to calculate the correlation. The color of the rectangles in the panel represents the correlation coefficient. Bacteria enriched in the BaP-exposure group are shown in bold. Spearman’s correlation was used to calculate correlation and adjusted p-values were determined using the Benjamini–Hochberg procedure. (A) FDR <0.05 and (B) FDR <0.1. The data are provided in Excel Table S4. Note: BaP, benzo[a]pyrene; BOM, BaP in combination with ovalbumin; FDR, false discovery rate; IFN-γ, interferon gamma; IL, interleukin; Ipe, intestinal permeability; MCPT-1, mouse mast cell protease 1; OVA, ovalbumin; OVM, sensitized and challenged solely with ovalbumin; RTh, rectal temperature.
The Ability of Intestinal Bacteria to Degrade BaP and Relieve Cytotoxicity in Vitro

Based on pathway integrity and type of annotated enzymes, strains with BaP-detoxification genes were selected for further analysis. We identified strains that could degrade BaP by using colorimetric assays and bacterial growth (Figures 6A,B). Seven strains were able to utilize BaP efficiently as the sole carbon source in the PYG medium. The growth of the strains differed in the presence of four concentrations of BaP, and compared with the other strains, K. pneumoniae subsp. exhibited better growth at the various concentrations (Figure 6A). Colorimetric assays were used to determine the degradation ability of the strains at a concentration of 5-mg/L BaP (Figure 6B). The results showed that these strains effectively degraded BaP. To confirm the capacity for BaP degradation, we used GC-MS to quantify the amount of BaP remaining in the media after culturing for 6 d with the individual bacterial species.

The GC-MS results showed that 59% and 56% of the added BaP were metabolized by K. pneumoniae subsp. and B. mycoides respectively (Figure 6C; Figure S9). Although the gene encoding the enzyme BaP-cis-4,5-dihydroyl dehydrogenase was not annotated in the genome sequence of these bacteria (Figure 6D). For C. come and E. coli, only a part of the BaP degradation pathways was annotated, and 37% and 46%, respectively, of the added BaP were degraded. B. mycoides DSM2048, capable of degrading BaP in the environment, was used to evaluate the degradation ability of the intestinal bacteria. We found that 54% of the added BaP was degraded by this bacterium, showing that the BaP degradation capacities of the screened strains were equivalent to or better than that of B. mycoides DSM2048 (Figure 6C).

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To study whether the cytotoxicity of BaP could be diminished by these bacterial strains, an in vitro cell model system was applied. We cultured the strains in medium in the presence of BaP as the sole carbon source for 6 d and subsequently applied the supernatants to CaCo-2 cell cultures. The cytotoxicity of BaP was then assessed using the cell counting (CCK-8) assay. The results showed that cells exposed to BaP preincubated with each of five bacterial strains—C. comes 1, S. salivarius subsp. salivarius NCTC8618, B. mycoides DSM2048, K. pneumoniae subsp., and E. coli—exhibited significantly greater cell viability than those exposed to BaP preincubated with other strains and the control (Figure 6E; \( p < 0.05 \)). However, it should be noted that cells exposed to S. salivarius subsp., which showed greater degradation of BaP than E. coli, did not exhibit greater cell viability, suggesting that other metabolites produced by the individual bacteria may play a role.

**Discussion**

In the present study, we examined how BaP exposure aggravated histologically detected injury and inflammatory infiltration of the gut mucosal lining in a mouse model of OVA-induced allergy. Intestinal mucosal damage is known to increase intestinal permeability, and large molecules of antigenic substances are more likely to cross the intestinal epithelium, causing severe stress responses.\(^{57}\)

Intestinal allergic inflammatory responses, as observed in humans with food allergen-mediated intestinal allergic responses, are typically associated with an imbalance of the Th1/Th2 cell ratio and function,\(^{38,59}\) which is associated with a drop in rectal temperature,\(^{60}\) local accumulation of inflammatory cells, and activation of mucosal mast cells as evidenced by the increase of MCPT-1 in the intestinal mucosa, and the secretion of many Th2-associated cytokines, including IL-4, IL-5, and IL-13.\(^{61,62}\)

As expected, we observed a higher intestinal permeability and a lower rectal temperature in OVA-allergic mice exposed to BaP compared with the OVA-allergic mice not exposed to BaP. The OVA-allergic mice exposed to BaP had a higher levels of the Th2-associated cytokines IL-4, IL-5, IL-13, and OVA-specific IgE, IgG1, and MCPT-1 than the OVA-allergic mice. These results suggested that BaP exposure aggravated allergic inflammation, as evidenced by the enhancement of common allergic inflammatory markers and impaired intestinal barrier function.

**Figure 6.** Identification of gut bacteria capable of degrading BaP. (A) Growth measurement of bacteria in medium with BaP as the sole carbon source. Colors with fill patterns mark the concentration of BaP. Percentage of BaP degradation after inoculation with bacteria by (B) color assay analysis and (C) GC-MS. The control refers to cells exposed to a bacterial medium containing BaP as the sole carbon without bacteria. Data are shown as mean ± SD of three independent experiments (n = 3). The data are provided in Excel Table S6. Note: *B. mycoides DSM2048, Bacillus mycoides DSM2048; BaP, benzo[a]pyrene; C. comes 1, Coprococcus comes 1; C. comes 2, Coprococcus comes 2; CaCo-2 cells, human colon carcinoma cell line; CCK-8 assay, Cell Counting Kit-8; E. coli, Escherichia coli; ELISA, enzyme-linked immunosorbent assay; GC-MS, gas chromatography and mass spectrometry; K. pneumoniae subsp., Klebsiella pneumonie subsp. rhinoscleromatis ATCC13884; KEGG, Kyoto Encyclopedia of Genes and Genomes; M. indica, Megasphaera indica; S. salivarius subsp., Streptococcus salivarius subsp.; S. salivarius subsp. salivarius NCTC8618, Streptococcus salivarius subsp. salivarius NCTC8618; SD, standard deviation; TCA, tricarboxylic acid cycle.
In the present study, we demonstrated marked differences in the composition and functional potential of the fecal microbiota in response to intake of BaP accompanied by an increase in serum level of Th2 cytokines. Previous studies have shown that oral administration of BaP led to moderate intestinal inflammation in the ileal and colonic mucosa of C57BL/6 mice, and 16S rRNA gene amplicon sequencing revealed that this was accompanied by a shift in intestinal mucosa-associated bacteria. In our study we observed that, compared with control mice, mice exposed to BaP exhibited a higher relative abundance of B. virosea and N. subflava, whereas the abundances of B. uniformis and L. bacterium COE1 were lower. Compared with OVA-sensitized mice, we observed that OVA-sensitized mice exposed to BaP exhibited higher relative abundances of L. bacterium 3-2, L. bacterium COE1, and Prevotella sp. MGM1, paralleled by an aggravation of intestinal inflammation.

For a few species, we observed a different direction of changes in the response to BaP exposure in the BAM group compared with controls, as well as in the BOM group compared with the OVM group. Thus, compared with the control group, mice exposed to BaP exhibited a lower relative abundance of B. pseudolongum and L. johnsonii, whereas compared with the OVM group, mice in the BOM group exhibited a higher relative abundance of the same bacterium. This result indicates that the response to BaP may differ depending on whether the mice had been subjected to a prior sensitization.

Such difference in response to BaP was also reflected in our analyses of microbial community functions. Although we observed that the abundance of several pathways in mice of both the BAM and the BOM groups were higher than that of the control and the OVM groups, respectively, numerous pathways also exhibited distinct different patterns of enrichment in the two groups of mice. In addition, the networks of interactions between bacteria in control mice and OVA-sensitized mice also differed. Thus, B. pseudolongum, L. intestinal, and L. johnsonii, which were not significantly correlated in the control group or the OVM group, were negatively correlated in the BAM group. These findings suggest a possible link between intestinal bacteria and intestinal permeability. Co-occurrence networks revealed that L. bacterium 28-4 was negatively correlated with B. pseudolongum in the BAM group, indicating that changes in the gut microbiota in response to exposure to BaP may be linked to allergic responses. Our study further revealed that M. bacterium DSM103720, positively correlated with rectal temperature, was negatively correlated with B. vulgarus, a key player in the change in a community function BOM group compared with the OVM group. Thus, competition for ecological niches between intestinal bacteria in allergic mice may lead to a deteriorated gut environment after BaP exposure. These findings suggest a possible link between BaP-induced changes in the gut microbiota and the aggravated allergic responses in allergic mice and, thus, the interplay between BaP, the gut microbiota, and the immune system may profoundly affect host allergic inflammation, at least in part, via changes in the gut microbiota.

We found that the abundance of species harboring BaP metabolizing genes, including L. johnsonii FI9785 and P. goldsteini, were inversely correlated with inflammatory markers, indicating that intestinal bacteria may serve a role in reducing BaP toxicity. In support of this notion, L. plantarum CCFM8661 with BaP-binding ability has been reported to protect against BaP-induced toxicity, to increase intestinal barrier integrity, and to alleviate histopathological changes in mice. However, it should be noted that some opportunistic pathogens, such as K. pneumoniae subsp. rhinoscleromatis ATCC13884, and E. coli, also harbor enzymes related to BaP metabolism and can effectively degrade BaP, suggesting a multifaceted function of intestinal bacteria. Of note, we present a comprehensive KO annotation of a BaP-detoxifying enzyme, which we envision will facilitate the comparison of metabolic networks of multiple organisms. This approach combined with genomics analysis may help in identifying BaP-degrading enzymes in bacteria, facilitating the discovery of detoxification genes and degradation pathways.

In previous studies, we established a high-quality catalog of non-redundant reference genomes of cultivated human gut bacteria based on whole-genome sequencing. Here, we exploited this resource for identifying bacterial strains capable of degrading BaP based on the available genome information. We identified 87 species belonging to 29 genera that have a potential for degradation of BaP. Interestingly, most of these strains, 67 in total, are facultative anaerobes, such as Escherichia coli and Enterococcus faecalis, whereas only 14 were strictly anaerobic bacteria and 6 were unknown. A large fraction of the annotated enzymes were oxidoreductases that require molecular oxygen, such as BaP-4,5-dioxygenases, 4,5-dihydroxy-BaP-dioxygenases, and BaP-11,12-epoxidases. Even though the intestine is often considered anaerobic, the intestine provides a range of oxygen microenvironments that favor the formation of local niches for specific microorganisms. Moving inward from the intestinal submucosa along the radial axis, oxygen concentrations decrease to near anoxia at the midpoint of the lumen, but the intestine and microbes can utilize the minute amounts of oxygen close to the mucosa supporting oxygen-dependent redox processes. However, even though it has been demonstrated that the cleavage of the BaP ring structure can proceed via the function of a series of enzymes, such as mono-oxygenases, dioxygenases, and hydrolases, the metabolic pathway and terminal metabolic products of BaP metabolism in prokaryotes are not completely elucidated. Of note, studies have demonstrated that the TCA cycle can work under anaerobic conditions, producing metabolites in bacteria. Although this finding provides support for intestinal bacteria to metabolize BaP by the TCA cycle even in an anaerobic environment, the exact metabolic mechanism by which BaP is degraded still needs to be clarified. In our study, we provide evidence that intestinal bacteria can metabolize BaP, and this suggests that such bacteria may function as part of a detoxification strategy. Still, the exact mechanism needs to be further explored.

Our finding that the gut harbors bacteria with a potential for degrading BaP suggested that such bacteria might counteract the detrimental effects of exposure to BaP. To determine whether the cytotoxicity of BaP could be diminished by such bacterial strains, we first determined the ability of seven candidate strains to grow on BaP as the sole carbon source. These experiments showed that these bacteria grew efficiently and degraded BaP. When we subsequently applied the medium conditioned by the bacteria to CaCo-2 cell cultures, we could demonstrate that medium conditioned by C. comes 1, S. salivarius subsp. salivarius NCTC8618, B. mycoides DSM2048, K. pneumoniae subsp., and E. coli, significantly reduced the cytotoxicity compared with medium not conditioned by these bacteria. Although these experiments pointed to a relation between the degradation of BaP and the reduced cytotoxicity, it should be noted that S. salivarius subsp., which showed greater degradation of BaP than E. coli, did not...
improve cytotoxicity. We did not evaluate the bacterial supernatant-alone control. Therefore, we cannot exclude the possibility that decreased cytotoxicity was due to other metabolites produced by the BaP-degrading bacteria. Establishment of a direct causal relationship between decreased cytotoxicity and metabolism of BaP would require targeted deletion of the genes predicted to be involved in the degradation of BaP. It should be noted that BaP is a high-molecular-weight PAH, and microbial degradation requires significant energy. Although our study found that some gut bacteria can degrade BaP, BaP may not be preferentially utilized in the presence of multiple carbon sources in the human gut. Further verification of the ability of gut bacteria to degrade BaP in the gut environment is needed in animal studies.

There are limitations of the present study. Because female mice are more prone to develop allergic inflammation than male mice, female mice were used as models in our study. Sex-specific differences in immune system9,9 and gut microbiota composition68 are known to exist. Microbiota-independent sex differences in the immune system may select a sex-specific gut microbiota composition, which in turn further contributes to sex-specific differences in the immune system.99 We recognized that although the relationship between the gut microbiota and allergy in female mice exposed to BaP was assessed in our study, the interaction between environmental pollutants, gut microbiota, and immunity in male mice most likely differ from that of female mice. In addition, the species or abundance of gut bacteria and the levels of allergy cytokines may differ between female and male mice. Further dedicated and more comprehensive studies are needed to properly address the issue of sex difference.

The mice developed severe intestinal tissue damage after repeated administration of OVA. The jejunum was used for assessment of intestinal tissue damage in OVA-allergic mice. Several studies have reported that food allergies also cause colonic tissue damage, and histological analyses demonstrated that dramatic infiltration of eosinophils and mast cells occurred in the large intestine.70,71 The expression of Th1 cytokines (e.g., IFN-γ) and Th2 cytokines (e.g., IL-4, IL-5, and IL-13) have been reported to be markedly increased in the proximal colons of OVA mice.72 Therefore, although detailed analyses of bacteria in the jejunum would have been of interest, the fecal microbiota may still serve as a proxy for the gut microbiota, in keeping with the results of a large number of metagenomics studies.

Limitations of the present study in relation to the impact of BaP on humans relates to the well-established significant differences between the gut microbiota in mice and humans.73 In addition, there are significant differences between the immune system in mice and humans, which means that a translation of our findings to a human setting clearly requires further studies. In a real-life setting, humans are exposed to complex mixtures of chemicals and nutrients that also may impinge on the development of different types of allergy. However, given that the bacterial strains we tested for their ability to degrade BaP all were derived from human fecal samples, this also implies that humans indeed harbor bacteria with a capability to degrade BaP.

In summary, evidence from a series of metagenomic association analyses suggested that BaP exposure in allergic mice was able to aggravate intestinal allergic inflammation associated with changes in the composition and functional potential of the gut microbiota but also that the aggravating effect of BaP potentially may be reversed by bacteria with BaP-degrading capability, as indicated by our in vitro analyses showing how bacteria capable of degrading BaP reduced BaP toxicity in a CaCo-2 cell model.

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All data needed to evaluate the conclusions in the paper are presented in the text and the Supplemental Material. Microbiome sequencing data have been deposited in the China National GeneBank (CNGB) Nucleotide Sequence Archive under the accession code CNP0002413 (CNSA: https://db.cngb.org/cnsa/). The databases referred to in microbiome analyses are as follows: Metacyc (https://metacyc.org/), KEGG (https://www.genome.jp/kegg/), and UniRef (https://www.uniprot.org/help/uniref).

References


