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The gut microbiota of bats confers tolerance to influenza virus (H1N1) infection in mice

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Abstract

Pathogens from wild animals cause approximately 60% of emerging infectious diseases (EIDs). Studies on the immune systems of natural hosts are helpful for preventing the spread of EIDs. Bats are natural hosts for many emerging infectious pathogens and have a unique immune system that often coexists with pathogens without infection. Previous studies have shown that some genes and proteins may help bats fight virus infection, but little is known about the function of the bat gut microbiome on immunity. Here, we transplanted gut microbiota from wild bats (Great Himalayan Leaf-nosed bats, *Hipposideros armiger*) into antibiotic-treated mice, and found that the gut microbiota from bats regulated the immune system faster than mice after antibiotic treatment. Moreover, we infected mice with H1N1, and found that the gut microbiota of bats could effectively protect mice, leading to decreased inflammatory response and increased survival rate. Finally, metabolomics analysis showed that the gut microbiota of bats produced more flavonoid and isoflavones. Our results demonstrate that the quick-start innate immune response endowed by bat gut microbiota and the regulatory and antiviral effects of gut microbiota metabolites successfully ensured mouse survival after viral challenge. To our knowledge, our study was the first to use fecal microbiota transplantation (FMT) to transplant the gut microbiota of bats into mice, and the first to provide evidence that the gut microbiota of bats confers tolerance to viral infections.

KEYWORDS
bat, Chiroptera, immunity, isoflavone, microbiome, viral infection

1 | INTRODUCTION

Emerging infectious diseases (EIDs), such as COVID-19, pose a major and growing threat to global health, the global economy and social security (Jones et al., 2008; Morens & Fauci, 2012; Pike et al., 2014). Identifying the causes of the emergence of a disease can both deepen our understanding of a specific disease and help humans more effectively limit its subsequent spread and socio-economic impact (Allen et al., 2017; Morse, 1995). It has been estimated that about 60% of EIDs are caused by pathogens from wild animals, also known as zoonotic diseases (Jones et al., 2008). Among these carriers of zoonotic diseases, bats have been shown to carry more zoonotic pathogens than any other mammals (Brook & Dobson, 2015; Luis et al., 2013; Olival et al., 2017). This view may be biased, as this background survey of viruses in bats was extensively focused on, and is disproportionate to other mammals. Bats are natural hosts for a variety of viruses (Calisher et al., 2006) and are known for their ability to coexist with some of the world's most deadly viruses (which are fatal to humans but have little or no effect

on bat health), including SARS-CoV, MERS-CoV, Hendra virus and Ebola virus (Ge et al., 2012; Halpin et al., 2000; Leroy et al., 2005; Ge et al., 2013; Li et al., 2005; Mandl et al., 2018; Subudhi et al., 2019). Moreover, coronaviruses with high homology to SARS-CoV-2 have also been found in bats (Zhou, Chen, et al., 2020; Zhou, Yang, et al., 2020). Among the many pathogens carried by bats, respiratory viruses transmitted by droplets need to be paid special attention. Respiratory viruses, such as SARS and influenza, are usually highly contagious and have a huge impact on humans and animals. In recent years, two new influenza A viruses, H17N10 and H18N11, have been found in bats (Chan et al., 2013; Tong et al., 2012). Unlike avian and human influenza, which uses sialic acid receptors, bat flu uses MHC II to enter cells (Karakus et al., 2019). Meanwhile, bat H17N10 and H18N11 viruses can also enter cells through the MHC II homologues encoded by pigs, humans and chickens, demonstrating the potential for bat influenza to spread across species (Banerjee, Mossman, et al., 2020; Karakus et al., 2019). Ferrets are a common model for conventional influenza viruses, but bat flu infects ferrets with no disease response (Ciminski et al., 2019). Cell tests have demonstrated that although bat influenza virus cannot replicate in human and mammalian cell lines normally used for conventional influenza viruses, the accumulation of viral mutations is rapid (Zhong et al., 2020). Given that a variety of bats can be infected with conventional influenza (Poole et al., 2014), the possibility of reassorting bat influenza outbreaks remains, and this requires ongoing attention. The exchange of genetic mutations and reassortment is key for the spread of influenza viruses across species into humans. The most striking example was the 2009 swine-derived H1N1 pandemic, which resulted from the recombination of gene fragments from the European 'avian-like' swine (EA-swine) lineage of H1N1, the triple reassortant swine lineage H1N2, and the Eurasian avian H1N1 lineage (Ma et al., 2008; Sun, Xiao, et al., 2020). Bats, like birds and pigs, have become a 'mixing machine' for influenza viruses (Neumann et al., 2009; Poole et al., 2014).

It is critical for human health to understand how bats can carry a lot of pathogens without getting sick because the knowledge is helpful for effectively stopping the spread of pathogens from bats to other animals and even humans. However, due to the high diversity of bat species and the lack of information on their biological backgrounds and commercial reagents (such as antibodies), little is known about bat immunity, especially about their antiviral immunity. It has been hypothesized that bats control viral replication early in the immune response through innate antiviral mechanisms (Baker et al., 2013). Some studies have demonstrated that bats have type I, II and III interferons, that bat interferons also have viral effects, and that bats appear to have high baseline levels of interferons (De La Cruz-Rivera et al., 2018; Kepler et al., 2010; Subudhi et al., 2019; Zhou et al., 2011). It has also been hypothesized that the balanced immune regulation of bats to viral infection may be responsible for their tolerance to the virus (Irving et al., 2021). For examples, the down regulation of NOD-like receptor (NLR) family, pyrin domain-containing protein 3 (NLRP3), partial deletion of the pyrin and HIN domain-containing protein (PYHIN) and stimulator of interferon genes (STING) families help to control inflammation and further contribute to bat immune tolerance to the virus (Ahn et al., 2016; Ahn et al., 2019; Holzer et al., 2019; Shaw et al., 2017; Xie et al., 2018; Zhou et al., 2016). These studies only found that several genes and proteins may help bats to fight virus infection, but the body's immunity is regulated by numerous factors, such as genetics, nutrition and gut microbiota. Thus, understanding antiviral immunity in bats only at the molecular level may be biased and not sufficient for answering this question. Moreover, previous studies in wildlife have focused on the composition of the gut microbiome and its function either via prediction using software or via shotgun metagenomic sequencing, little is known about function of gut microbiota on immunity via verification tests, such as fecal microbiota transplantation (FMT). FMT has been widely used in organisms including humans and mice, but to our knowledge, no research has been conducted on transplanting bat feces into mice to date (Burrello et al., 2018; Paramsothy et al., 2017). Thus, it is necessary to assess the effects of gut microbiota on the immune system using FMT, especially for bats with their unique immune system.

In this study, we transplanted gut microbiota from the great Himalayan leaf-nosed bat (*Hipposideros armiger*) into antibiotic-treated mice via FMT, and investigated the effects of gut microbiota on the immune system. *Hipposideros armiger* was selected as bat dropping donors for the following reasons. First, *H. armiger* is the largest species of the *Hipposideridae* with wide geographical distribution, and its population size is normally large. In this case, sample collection was relatively easy. Second, one previous study showed that *H. armiger* was infected with *Orthomyxoviridae* viruses (Wu et al., 2016). Third, similar to other insectivorous bats, previous studies have indicated that *H. armiger* also have higher levels of *Firmicutes* and *Proteobacteria* in their gut microbiota (Sun , Gao et al., 2020), suggesting that the basic composition of the gut microbiota of insectivorous bats may be similar. Finally, many aspects (i.e., behavior, ecology and genome information) of *H. armiger* have been well documented, which provides a basis for further research (Dong et al., 2017). We tested three hypotheses. First, mice are very common as an animal model in biology and medicine, so we hypothesized that mice could also be used as an animal model to receive transplantation of bat feces. Based on 16S rRNA gene high-throughput sequencing, we predicted that FMT would change the composition of the gut microbiota of mice, making it more similar to that of wild bats. Second, we hypothesized that the gut microbiota of bats has a stronger ability to regulate the immune system. We predicted that transplantation of gut microbiota from bats may recover immune cell levels in mice after antibiotic treatment and tested this using flow cytometry. Finally, gut microbiota mainly regulates host immunity and disease tolerance in many ways, including thallus stimulation and production of bacterial metabolites (Marchesi et al., 2016; Michaudel & Sokol, 2020). Therefore, we hypothesized that the gut microbiota of bats has a similar tolerance role in the host's response to influenza virus. We thus selected the H1N1 virus as a viral stress to demonstrate that the bat gut microbiota imparts viral tolerance to mice. We predicted that differences in the metabolites and pathways in mice between the bat feces transplantation group and mice feces transplantation group would be observed, and may be helpful for the survival of mice undergoing H1N1 infection.

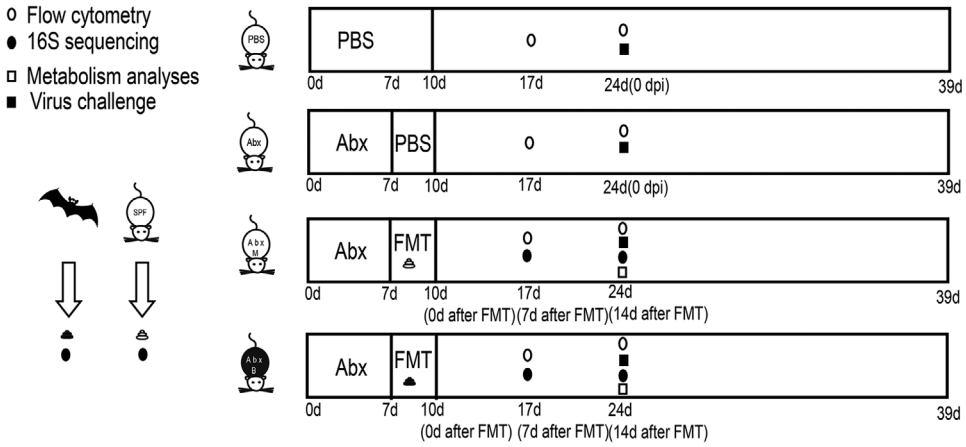

FIGURE 1 Schematic diagram of study design.

2 | MATERIALS AND METHODS

2.1 | Study design

The study design is shown in Figure 1. In brief, changes in the immune cells and gut microbiota and fecal metabolome of mice were detected after bat feces were transferred into mice using FMT, followed by viral challenge of bat gut microbiota mice using H1N1 influenza virus as a viral stress model.

2.2 | Mice

Female 3-week-old C57BL/6N SPF mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and reared in an animal room attached to a biosafety level 2 laboratory. The feed was sterilized using cobalt-60 and a double steam water autoclave. The mice were fed with feed and water freely at 22 ± 2°C and alternated between light and darkness for 12 h.

2.3 | Collection and preservation of feces

Feces from Great Himalayan Leaf-nosed bats was collected in Hanzhong City, Shaanxi Province, China, in May 2019. Bats were captured in mist nets, and placed in autoclaved brown paper bags. The feces were collected every 5 min for a total of 30 min from each bat. The collected feces was weighed and transferred to a sterile cryopreservation tube [containing 20% glycerin with PBS buffer (HyClone, Logan, UT, USA)] and quickly stored in dry ice, and then transferred to the laboratory for storage in a low-temperature refrigerator at −80°C. Fecal samples were collected from 40 adult female bats. Mouse feces were taken from five SPF mice in the same batch and treated in the same way as the feces from bats. We isolated a small amount of feces samples from five bats/mice and labeled them as Bats and Mice for subsequent 16S rRNA sequencing.

2.4 | Fecal microbiota transplantation

We treated mice with antibiotics and FMT according to the methods of Le Roy et al. (2018). In brief, mice were administered a compound antibiotic for 7 days, then the intestinal cavity was cleaned with polyethylene glycol (PEG). After that, each mouse was orally given 200 μl of the transplant bacterial liquid daily for 3 days.

The compound antibiotic solution contained ampicillin, neomycin, metronidazole 200 mg/kg and vancomycin 100 mg/kg, and this was used daily for consecutive 7 days. The administration method was oral irrigation. The PEG solution contained PEG3350 (77.5 g/L), sodium chloride (1.9 g/L), sodium sulfate (7.4 g/L), potassium chloride (0.98 g/L) and sodium bicarbonate (2.2 g/L), and 1.5 ml per mouse was used, being given in five equal portions. Each portion was taken orally once every 30 min after fasting for 2 h on the 8th day of antibiotic treatment.

The bacterial transplant liquid was prepared as follows. To ensure that bacterial transplant liquid was population-representative, we prepared them from a mixture of feces from five bats/mice. First, frozen bat/mouse feces was placed in a water bath at 37°C to thaw. Since viruses in bat feces may affect mice, the supernatant was removed by centrifugation at 7000 × *g* for 5 min. After fully mixing with PBS buffer, the supernatant was centrifuged and the resulting supernatant was discarded. This was repeated three times. Chicken embryo amplification was performed using the supernatant, followed by hemagglutination tests using 1% chicken red blood cell suspensions to confirm that the grafts did not contain H1N1 influenza virus. We adjusted the fecal concentration to 50 mg/ml with PBS buffer containing 20% glycerin, and then packaged this and froze it separately for later use. The above operations were carried out in an anaerobic workshop with CO_2 and N_2 ventilation.

2.5 | 16S rRNA gene sequencing

Fecal microbial composition after fecal bacteria transplantation can demonstrate the changes of gut microbiota in mice. Therefore, 16S

rRNA gene sequencing was performed on the feces of the donor and fecal bacteria on the 7th and 14th days after transplantation. Forty mice were randomly divided into two groups. The AbxM group was treated with antibiotic and transplanted with mice feces, and the AbxB group was treated with antibiotic and transplanted with bat feces. In this study, we did not assess gut microbiota prior to FMT because a previous study has confirmed that initial microbiota depletion constitute a valid alternative to germ-free (GF) mice in FMT studies (Le Roy et al., 2018). On day 7 after transplantation, 10 mice from each group were collected for subsequent testing and labeled AbxM7 and AbxB7. On day 14, the remaining 10 mice from each group were selected for follow-up testing and labeled as AbxM14 and AbxB14. To conduct gut microbiota analysis, the fresh mouse feces were quickly quenched with liquid nitrogen and stored in a low temperature refrigerator at −80°C until they were used.

Microbial community genomic DNA was extracted from fecal samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's instructions. The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F and 806R (Herlemann et al., 2011). PCR reactions were performed following the protocol described previously (Gong et al., 2021). The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform or a NovaSeq PE250 platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Technology Co. Ltd. (Shanghai, China).

The raw sequences were merged by FLASH, version 1.2.7 (Magoc & Salzberg, 2011) and imported and processed with The Quantitative Insights into Microbial Ecology Version 2 (QIIME2) for further bioinformatics analysis (Bolyen et al., 2019). The plugin DADA2 was used to perform quality filtering, denoising and merging of imported paired reads. Classification was performed using the QIIME2 platform's classification plugin q2-feature-classier64, which performs similar calculations using the Scikit-learn Naive Bayes classifier (Bokulich et al., 2018). Finally, the filtered ASVs were classified using a pre-trained, QIIME2-compliant Silva database (Silva SSU release 138 515–806) with a confidence threshold of 0.7 (Pruesse et al., 2007). We performed alpha (ACE, Chao, Sobs, and Shannon indices) diversity analysis using Q2-diversity, a plugin in QIIME2. To analyze the structure of the gut microbial community, we performed principal-coordinate analysis (PCoA) using unweighted UniFrac matrices of beta diversity calculated in QIIME2. Moreover, we visualized changes in the relative abundance of taxa in the gut microbial community between diets at the phylum and genus levels in QIIME2. We also use Linear Discriminant Analysis (LDA) Effect Size (LEfSe), with a threshold logarithmic LDA score of 3.5, to determine the differences in gut microbial characteristics (Segata et al., 2011). We used a receiver operating characteristic curve (ROC) to analyze the mice that received FMT on the 7th and 14th days, and the confidence interval threshold was set to 0.95 to detect whether the composition of these microorganisms could distinguish the two groups of mice (van der Giessen et al., 2020).

2.6 | Flow cytometry

Changes in gut microbiota may alter the immune status of mice, so we next performed a separate experiment to assess the immune response to transplantation. Eighty mice were randomly divided into four groups. The control group received an equivalent dose of PBS buffer. Abx was the control group with antibiotic treatment with no FMT. AbxM was the mice feces transplantation group that received SPF mice feces transplantation after antibiotic treatment. AbxB was the bat feces group, receiving bat feces transplantation after antibiotic treatment. On days 7 and 14 after FMT, 10 mice from each group were randomly selected for flow cytometry. We collected spleen and MLN single cell suspensions using Shi et al.'s (2020) method. T/B/NK cell recognition was performed using the following antibodies and reagents: CD16/CD32 (FC reports block), Fixable Viability Stain 620, CD3e, CD4, CD8α, CD45R and NK1.1. All antibodies and reagents were purchased from BD Biosciences (BD Biosciences, New York, NY, USA) and used according to the manufacturer's instructions.

All the samples were collected using BD FACS Diva software (BD Biosciences) on a BD LSR Fortessa (BD Biosciences) and subsequently analyzed using Flowjo10 (Treestar, Ashland, OR, USA).

2.7 | Virus challenge and viral load of the lung

Bats are generally known for their tolerance of viruses, so we conducted a separate experiment to test the tolerance of mice transplanted with bat gut microbiota to viral infection. First, 60 mice were randomly divided into four groups. PBS group and Abx group were used as controls. AbxM mice were transplanted with the feces of mice, and AbxB mice were transplanted with the feces of bats. Both groups were infected with influenza virus H1N1 on the 14th day after FMT. Influenza A/Puerto Rico/8/34 (H1N1, PR8) virus was amplified using chicken embryos and the hemagglutination titer of the virus was determined using 1% chicken red blood cells. Mice were anesthetized with 1% pentobarbital by intraperitoneal injection and then intranasally inoculated with $1 \times LD_{50}$ ($300TCID_{50}$) in 40 μl PBS buffer per mouse 14 days after FMT. The changes in body weight and mortality in each group were observed and recorded for 14 consecutive days after challenge. For animal ethics and humanitarian reasons, mice that had lost more than 25% of their body mass were euthanized. Five mice were randomly selected from each group to be euthanized on the 5th day after virus infection for the detection of cytokines and pathological sections. The daily weight changes of the remaining mice in each group were recorded until the 14th day after challenge.

For the determination of pulmonary virus titers, we referred to the method from Kumaki et al. (2011). In short, the supernatant of homogenate was filtered using a 40-μm filter and different concentrations of viral supernatant in MDCK cells (infected by TPCK-trypsin)

were detected by CPE assay, while the virus $TCID_{50}$ was determined with MDCK cells using the Reed-Muench method.

2.8 | Paraffin sectioning and immunofluorescence after virus challenge

Histopathological examination can directly reflect the severity of a lesion, so we conducted histopathological examination on the 5th day of virus challenge in mice. Samples of lungs were collected and fixed in tissue boxes with 4% paraformaldehyde solution. Paraffin blocks were then made and serial step sections were processed and stained with hemotoxin and eosin. Immunofluorescence was performed using section based on a previous study (Jin et al., 2018). In brief, the antigen was retrieved with sodium citrate antigen repair solution and the sections were stained with rabbit anti-mouse H1N1 HA antibody, FITC-Donkey anti-rabbit IgG antibody and DAPI.

2.9 | Bronchoalveolar lavage fluid and serum cytokine detection after virus challenge

Cytokine testing at the initial stage of viral challenge can assess a host's response to an immune challenge, so we performed cytokine testing on mice 5 days after challenge. Bronchoalveolar lavage fluid (BALF) was obtained as previously described (Xu et al., 2006). Cytokine expression was assessed using a CBA Mouse Th1/Th2/Th17 Cytokine Kit (BD Biosciences) and Anti-Virus Response Panel (13-plex) (BioLegend, San Diego, CA, USA) in accordance with the manufacturer's instructions. The detection limit of the Th1/Th2/Th17 Cytokine Kit: IL-2 was 0.1 pg/ml, IL-4 was 0.03 pg/ml, IL-6 was 1.4 pg/ml, IFN-γ was 0.5 pg/ml, TNF was 0.9 pg/ml, IL-17A was 0.8 pg/ml and IL-10 was 16.8 pg/ml. Test values beyond these ranges can be calculated by referring to the manufacturer's manual. The detection limit of the Anti-Virus Response Panel: CCL2 was 6.8 pg/ml, CCL5 was 1.85 pg/ml, CXCL1 was 0.66 pg/ml, CXCL10 was 1.63 pg/ml, GM-CSF was 1.31 pg/ml, IFN-α was 1.86 pg/ml, IFN-β was 1.7 pg/ml, IFN-γ was 0.73 pg/ml, IL-1β was 1.55 pg/ml, IL-6 was 1.67 pg/ml, IL-10 was 2.81 pg/ml, IL-12p70 was 2.47 pg/ml and TNF-α was 0.92 pg/ml.

2.10 | Metabolism analyses on feces from AbxM and AbxB mice

Since gut microbiota may influence in the host's immune system through its metabolites, we performed a metabolomics analysis of the feces from mice on day 14 after fecal bacteria transplantation, that is, before the viral challenge. Fresh feces from AbxM and AbxB were collected on day 14 after FMT for metabolomic detection. Thermo Field's ultra-performance liquid chromatography tandem Fourier transform mass spectrometry system was used for the LC-MS analysis. The samples were ionized by electrospray ionization, and the mass spectrum signals were collected in both the positive and negative ion scanning modes.

We used the metabolic data analysis method from Liu et al. (2019). Ropls1.6.2 was used for orthogonal partial least-squares discrimination analysis (OPLS-DA). In order to ensure the reliability of these results, the OPLS-DA model was tested 200 times by permutation tests. Scipy 1.0 was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, pathway topology analysis, and correlation analysis. Expression profiles and variable importance in projection (VIP) values were analyzed using Ropls 1.62 and Scipy 1.0, and the VIP threshold was set as the TOP 50 metabolites. Ropls 1.62 and Scipy 1.0 were also used for correlation analysis between cytokines and metabolomics.

2.11 | Statistical analyses

Data analysis of flow cytometry and virus challenge is displayed as follows. Statistical analyses were performed using Graphpad Prism 8.0 software (GraphPad Software, San Diego, CA, USA). We tested whether the data conformed to a normal distribution using the Shapiro–Wilk test. When the two groups of data were normally distributed, an unpaired Student's *t*-test was used for comparison. The non-parametric Mann–Whitney U test was used to measure the differences between two groups whose data were not normally distributed. An one-way ANOVA followed by Tukey's test was used for comparisons between three or more groups when the data were normally distributed or Kruskal–Wallis test followed by Dunn's test when the data were not normally distributed. All tests were two-sided. The survival curve of mice was analyzed using a Gehan–Breslow–Wilcoxon test.

Data analysis of the microbiome and metabolome was performed as follows. Alpha diversity analysis was performed using a one-way ANOVA. Pearson's correlation coefficients were used to analyze the correlation between gut microbiota and the metabolome.

3 | RESULTS

3.1 | Changes in the gut microbiome of mice after FMT

First, antibiotic-treated mice received a colony of transplanted bat or mouse feces and were then observed closely for 14 consecutive days, with no signs of abnormal behavior or acute disease being observed. Our results showed that the ACE, Chao, Sobs, and Shannon indices of the gut microbiota from bats were significantly lower than those of mouse, which indicated that the richness and diversity of the gut microbiota from bat feces were lower than those in mouse feces (ACE index, $p < 0.001$, Chao index, $p < 0.001$, Sobs index, $p < 0.001$, Shannon index, $p < 0.001$, Figure 2a–d). The correlation index of gut microbiota from mice after bat fecal transplantation at days 7 and 14 was similar to that of bats, and showed a higher abundance of *Proteobacteria* (Figure 2e, Supplementary Figures S1a and S2a).

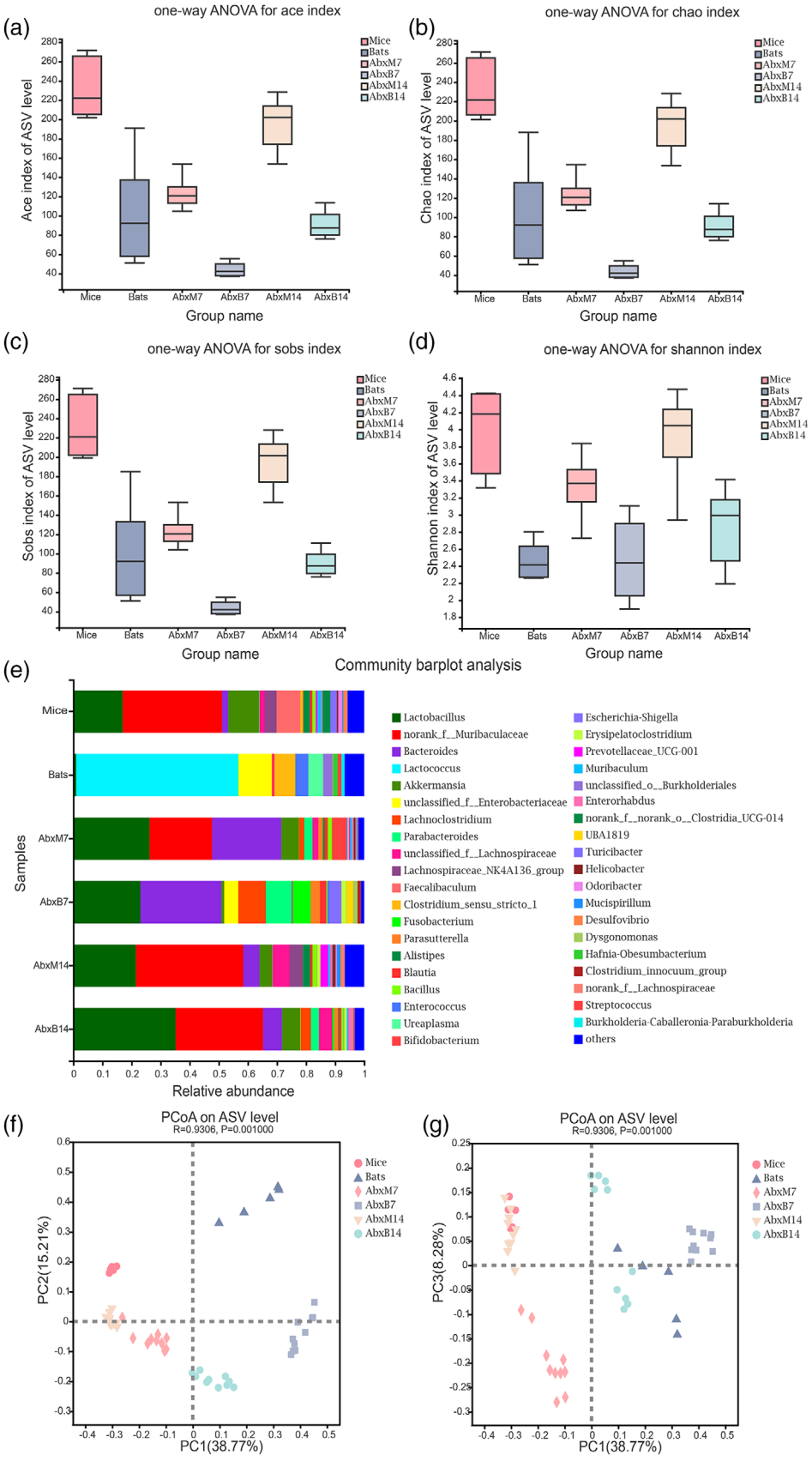

FIGURE 2 Changes in the gut microbiota profiles of mice after FMT. Differences in ACE index (a), CHAO index (b), Sobs index (c) and Shannon index (d) among the different groups. (e) Relative contribution of the dominant phyla in all samples (phylum level). (f) Differences in the ASV level between the different groups from PCoA based on an unweighted UniFrac distance matrix by PC1 and PC2. (g) Differences in the ASV level between the different groups from PCoA based on an unweighted UniFrac distance matrix by PC1 and PC3. The number of mice in each group was 5 or 10. Box plots indicate median (middle line), 25th and 75th percentile (box) and 5th and 95th percentile (whiskers) (a–d).

Compared with mouse fecal transplantation, the richness of *Lactococcus*, *Enterobacteriaceae* and *Enterococcus* in mice with bat fecal transplantation was higher and on 7th and 14th days after FMT, the feces of the AbxB group also displayed higher levels *Enterobacteriaceae*, *Lachnoclostridium*, and *Enterococcus* (Supplementary Figure S2b–d). However, it should be noted that the richness of *Enterobacteriaceae* and *Enterococcus* gradually decreased over time (Figure 2e and Supplementary Figure S2b–d). At the phylum level, mice successfully inherited *Proteobacteria* from bat feces rather than *Lactococcus* (Figure 2e and Supplementary Figure S2a). Nevertheless, as shown in the genus bar diagram, there were some differences in the composition of the mouse gut microbiota from the donor bat (Figure 2b).

PCoA results based on the Unweighted Unifrac distance algorithm showed that FMT changed the composition of the gut microbiome of mice, making it more similar to that of bats (Figure 2f and g). Based on microbial data from the 7th and 14th days post-transplantation, a random forest model and ROC analysis showed that the microbial composition could be used to distinguish AbxM from AbxB mice (AUC = 1 and 0.93, Supplementary Figure S1b and c).

3.2 | Immune regulation of mice after bat fecal transplantation

It has been found that immune cell levels in antibiotic-treated mice are reduced relative to untreated mice, and bacterial replenishment can restore normal immune cell levels. Therefore, we used flow cytometry to test for the recovery of $CD3^+$ cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, $CD45R^+$ B cells, $CD3^-NK1.1^+$ NK cells and $CD3^-NK1.1^+CD45R^+$ activated NK cells in the murine spleen and MLN cells at 7th and 14th days after transplantation. The flow cytometry gating is shown in Supplementary Figure S3.

In the spleen, on day 7 after FMT, we found that bat gut microbiota could maintain $CD3^+$ cells and NK cells, while mouse gut microbiota could not, and the proportion of $CD3^+$ cells was further reduced in the AbxM group (Figure 3a and k). Interestingly, the change in the levels of $CD3^+$ cells disappeared on the 14th day post transplantation, while the NK cell levels persisted on the 14th day (Figure 3b and l). $CD3^+CD4^+$ T cells and $CD3^+CD8^+$ T cells did not change significantly on the 7th day after FMT (Figure 3c and e), but the proportion of $CD3^+CD4^+$ T cells in the AbxM group increased by the 14th day, while the opposite result was found for $CD3^+CD8^+$ T cells (Figure 3d and f). $CD45R^+$ B cells (Figure 3g and h) showed no significant change except that the Abx group was higher than the AbxM group on day 7. NK cells in the AbxM group increased on day 7 after FMT, but there was no difference on the 14th day (Figure 3i and j). On days 7 and 14 after FMT, the relative proportion of activated NK cells in the AbxB group was higher than that in the AbxM group (Figure 3k and l). In addition, we also detected the number of immune cells in each group, and the results showed that the trend of the number change was similar to the relative counts in general (Figure 4).

In MLN, on the 7th day after FMT, transplanted bat gut microbiota restored the reduced $CD3^+$ cell levels of mice induced by antibiotic treatment, while the gut microbiota from SPF mice did not recover them until the 14th day after FMT. Moreover, the number of $CD3^+$ cells that could not be recovered by the gut microbiota of mice was still lower than that of the control group (Figure 5a and b). The number of $CD3^+CD4^+$ T cells (Figure 5c) in the AbxB group decreased on the 7th day, while the number of $CD3^+CD8^+$ T (Figure 5e) cells in the AbxM group was higher than that in the control group on the 14th day (Figure 5d and f). On day 7, the levels of $CD45R^+$ B cells increased in the AbxM group, but there was no difference between the AbxB and control groups, or between the four groups on day 14 (Figure 5g and h). The increase of NK cell levels appeared in the AbxM group on the 7th day after transplantation, though the number of activated NK cells was relatively small (Figure 5i and k). We also noted that on day 14, the relative proportion of NK cells in the AbxB group was higher than that in the control group, but the activated NK cell levels showed no differences (Figure 5j and l). In addition, the absolute cell count showed that only the $CD3^-NK1.1^+CD45R^+$ cells in the MLN on day 7 were different (Figure 6).

By balancing Th1, Th2 and Th17 cytokines, the gut microbiome plays a very important role in the formation of responses to different antigens. We therefore examined Th1, Th2 and Th17 cytokines (IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ and TNF-α), and interestingly, although the AbxB mice showed significant changes in their microbiome, only TNF-α was elevated (Supplementary Figure S4).

3.3 | The roles of the gut microbiome from bats in resisting H1N1 infection

We next tested the anti-viral effect of transplanted bat fecal microbiota in mice. We found that body mass of mice in the AbxM group began to decrease from the 5th day, while that in the AbxB group began to decrease from the 7th day and the body mass of the two groups of mice was statistically different (Figure 7a). For animal ethics and humanitarian reasons, mice that had lost more than 25% of their body mass were euthanized. We found that while no death occurred in the AbxB group over this time period and five mice died in the AbxM group ($p = 0.0126$, Figure 7b).

Pathological sections showed less inflammatory cell infiltration in the lungs of mice in the AbxB group than in the AbxM group, showing reduced bronchitis and alveolitis (Figure 7c). $TCID_{50}$ results showed decreased viral load in the lungs of mice in the AbxB group relative to other groups (Figure 7d).

Furthermore, the cytokine levels in the blood serum and BALF were measured, showing that the CXCL10 ($p = 0.0079$) and IFN-α ($p = 0.0024$) levels in the serum of mice in the AbxB group were higher than the levels in the AbxM group (Figure 7e). In addition, the content of GM-CSF in serum of Abx group was significantly lower than that of AbxB group. IL-10 ($p = 0.0341$), IL-12p70 ($p = 0.0248$), TNF-α ($p = 0.0431$) and IFN-β ($p = 0.0012$) were higher in the AbxB group than AbxM group (Figure 7f). No differences (AbxM vs AbxB) were detected in the remaining tested cytokines (Supplementary Figure S5). These results showed that our mouse model with bats gut microbiota

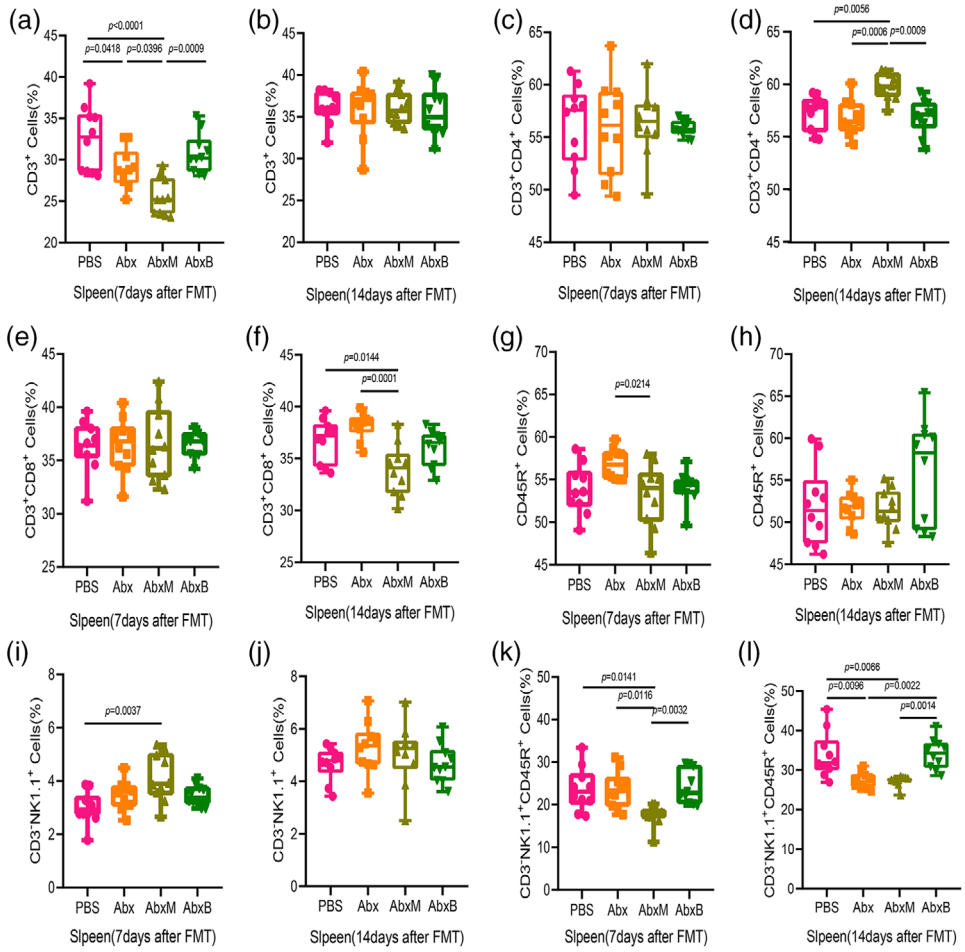

FIGURE 3 Changes in T, B and NK cells in the spleen at 7th and 14th days after fecal microbiota transplantation (FMT). Control: PBS group; Abx: only antibiotic treatment without fecal microbiota transplantation; AbxM: mouse feces transplantation group with antibiotic treatment; AbxB: bat fecal transplantation group treated with antibiotics. Each group included 10 mice. One representative of three independent experiments is shown. Box plots indicate median (middle line), 25th and 75th percentile (box) and 5th and 95th percentile (whiskers) as well as individual scores (single points). Statistical significance was tested by one-way ANOVA followed by Tukey's tests (a–j) or Kruskal–Wallis test by Dunn's test (k, l). $p < 0.05$ was considered significantly different from each group.

transplantation helped mice resist H1N1 infection, resulting in lower weight loss and mortality rates.

3.4 | The metabolome of AbxM and AbxB mice

Through the analysis of our OPLS-DA model, we found that the metabolites were significantly different between AbxM and AbxB group mice, which was consistent with the results of 16S rRNA gene amplicon high-throughput sequencing (Figure 8a and b). The intercept of the Q2 regression line was less than 0, and this model was not overfitted (Supplementary Figure S6a and b).

According to KEGG topology analysis and KEGG enrichment analysis, we found that Isoflavonoid biosynthesis ($p = 0.0009$), D-glutamine and D-glutamate metabolism ($p = 0.0173$) and the tryptophan metabolism ($p = 0.018$) pathways were significantly different in these two groups (Figure 8c and d).

Our results also showed that Daidzein, Formononetin and Genistein from the Isoflavonoid biosynthesis pathway were significantly positively correlated with *Proteobacteria* and *Fusobacteriota*. Moreover, although *Proteobacteria* was not only an important phylum in the gut microbiota of bats, it was successfully transplanted to mice by bats through fecal transplantation (Figure 8e and Supplementary Figure S6c). Our results also showed that 3-methylquercetin 7- [Galactosyl -(1- > 4)-glucoside] and 3-Hydroxychavicol 1-[rhamnosyl-(1 - > 6)-glucoside] had a high VIP value (Supplementary Figure S6d). Our results also showed that flavonoids/isoflavones such as marmesin rhamnoside and daidzein were significantly positively correlated with IFNα and CXCL10 (Supplementary Figure S7).

4 | DISCUSSION

In this study, changes in the composition of the gut microbiome of mice after FMT were observed, supporting our first hypothesis. Moreover,

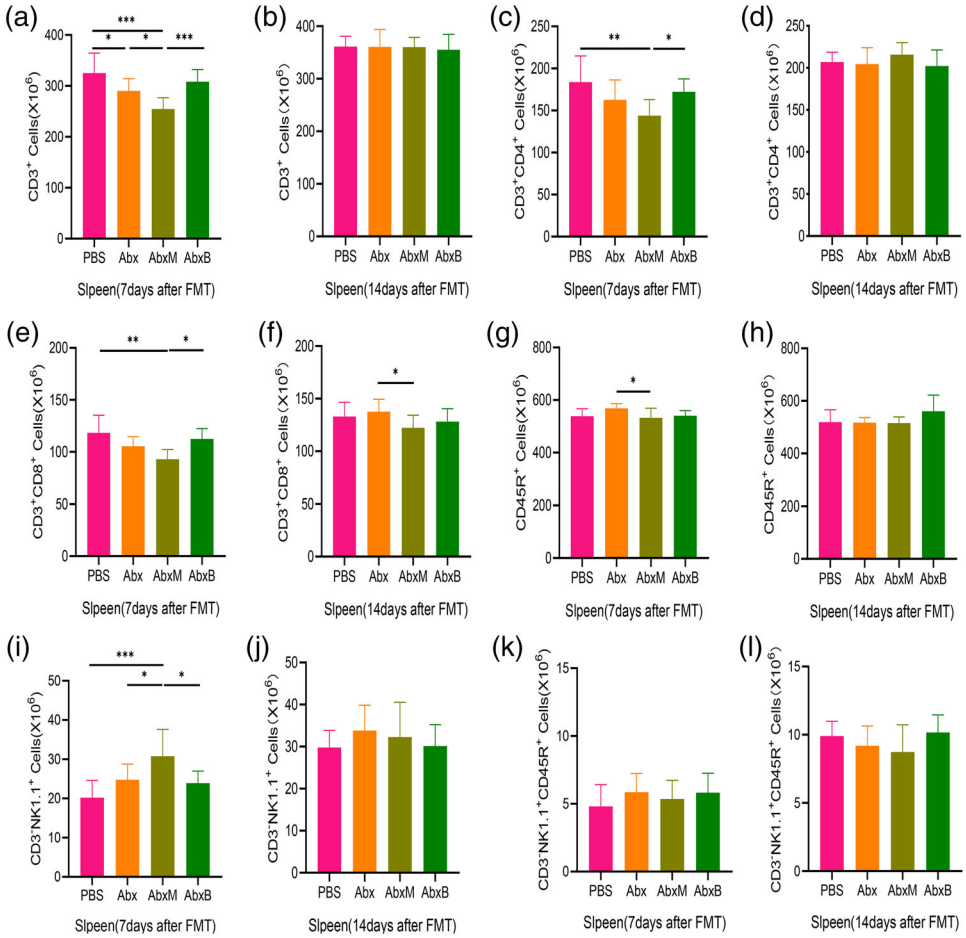

FIGURE 4 Changes in counts of T, B and NK cells in spleen on days 7 and 14 after fecal microbiota transplantation (FMT). Differences in Antiviral-related cytokine on the 5th day after H1N1 challenge. Each group had 5 mice. One representative of three independent experiments was shown. Data are expressed as the mean ± SD. Statistical significance was tested by one-way ANOVA followed by Tukey's tests. $p < 0.05$ was considered significantly different from each group.

flow cytometry demonstrated that the immune cell levels of mice after antibiotic treatment were restored by the gut microbiota of bats, which confirmed that the gut microbiota of bats might regulate the immune system, supporting our second hypothesis. Finally, we found that some metabolites and pathways were significantly different in mice between the AbxM and AbxB groups, and the key compounds isoflavones may influence the survival of mice during H1N1 influenza viral infection, supporting our third hypothesis.

Mice successfully received bat gut microbiota transplantation, which can be used as a model for studying bat gut microbiota. Mammals have a widely diverse and highly active gut microbial community that coevolves with a host through sustained mutual partnerships, and plays a role in behavior, nutrition, and immunity (Cryan et al., 2019; Rooks & Garrett, 2016; Valdes et al., 2018). Unfortunately, it is difficult to determine the function of the gut microbiota from wild animals due to the poor environmental conditions, complex biological backgrounds, lack of targeted reagents and protection of many wild species (Banerjee, Baker, et al., 2020). Mice are a commonly used animal model that has been widely applied to the study of human beings and captive animals, providing a good alternative for many wild species. Previous studies have successfully transplanted the gut microbiota of wild mice, brown bears and other species into mice by FMT (Ma et al., 2018; Rosshart et al., 2017; Sommer et al., 2016). In this study, the colonization of the gut microbiome after FMT was confirmed in mice, and the composition of the gut microbiota was found to be similar to that of wild bats to some extent. In this case, our study provides the first evidence that mice also can be used to transplant the gut microbiota of bats as an animal model. Previous studies have shown that the gut microbiota of bats is different from that of other mammals, having such characteristics as low α diversity and high levels of *Proteobacteria* (Ley et al., 2008; Song et al., 2020). These results were also confirmed by our study, which showed high levels of *Proteobacteria* in the feces of mice at 7th and 14th days after FMT, implying the success of microflora transplantation in this study. We also noticed that the abundance of *Lactococcus* was high in bats, while *Lactococcus* was not identified in the treated mice (AbxB group). This may have been due to the differences in the genetic backgrounds, feeding habits and ecological niches between bats and mice, which have shaped different characteristics of the gut microbiome during the long evolutionary process (Ley et al., 2008; Sun, Gao, et al., 2020; Zhang et al., 2010). It may, therefore, be presupposed that mice

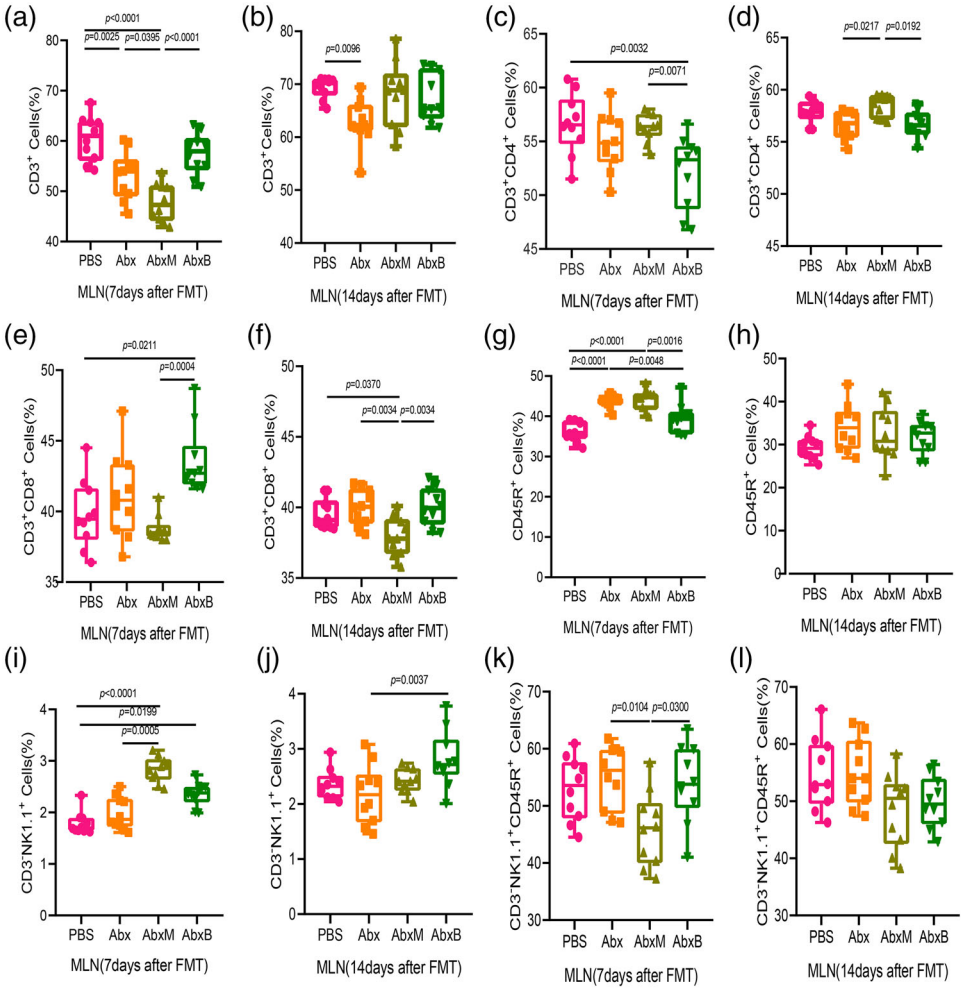

FIGURE 5 Changes in T, B and NK cells in the MLN at 7th and 14th days after fecal microbiota transplantation (FMT). The experimental groups were same to that in the spleen (see Figure 2). Each group included 10 mice. One representative of three independent experiments is shown. Box plots indicate median (middle line), 25th and 75th percentile (box) and 5th and 95th percentile (whiskers) as well as individual scores (single points). $p < 0.05$ was considered significantly different from each group.

transplanted with bats' feces would not retain the entirety of the bat gut microbiome.

The microbiota of bats altered the balance of host immune cells, indicating the ability to regulate the host immune system. It is well known that the coordinated interaction between gut microbiota and the host immune system plays a crucial role in maintaining host immune balance and resisting infection (Pickard et al., 2017). Therefore, we monitored the changes in the levels of immune cells after FMT, and our results showed that the transplanted gut microbiota could restore immune cells that were maladjusted under the action of antibiotics, which was consistent with previous studies on the regulation of the immune system by gut microbiota (Ekmekciu et al., 2017). Additionally, the gut microbiota of bats in mice made the proportions of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells and activated NK ($CD3^-NK1.1^+CD45R^+$) cells recover more quickly. The gut microbiota of bats altered the balance of host immune cells, indicating the ability to regulate the host immune system. Compared with the mouse gut microbiome, bat gut microbiota can regulate the immune system faster, which may be due to the fact that wildlife faces great survival pressure in a natural environment than laboratory bred and housed animals. Another possibility is that the intestinal tract of bats is relatively short, significantly reducing the retention time of food in the gut, which prompts the bat's gut microbiota to evolve more efficiently (Caviedes-Vidal et al., 2007; Klite, 1965; Price et al., 2015). On the whole, our data have highlighted the roles of complex symbiotic microbes in the gut of innate and adaptive immune cells. These results also showed that the gut microbiota of bats might recover disorders of the relative abundance of immune cells faster due to broad-spectrum antibiotics treatment, which may have a far-reaching influence on the immune function of the body. It should be noted that TNFα was elevated in the AbxB group. Normally, bats maintain low levels of inflammatory factors, and the increased TNFα in the AbxB group may have been due to the different physiological structures and immune cell types between bats and mice. Thus, we caution should be exercised in making conclusions based on animal model. For example, the results of some studies in mice are different from those in humans,

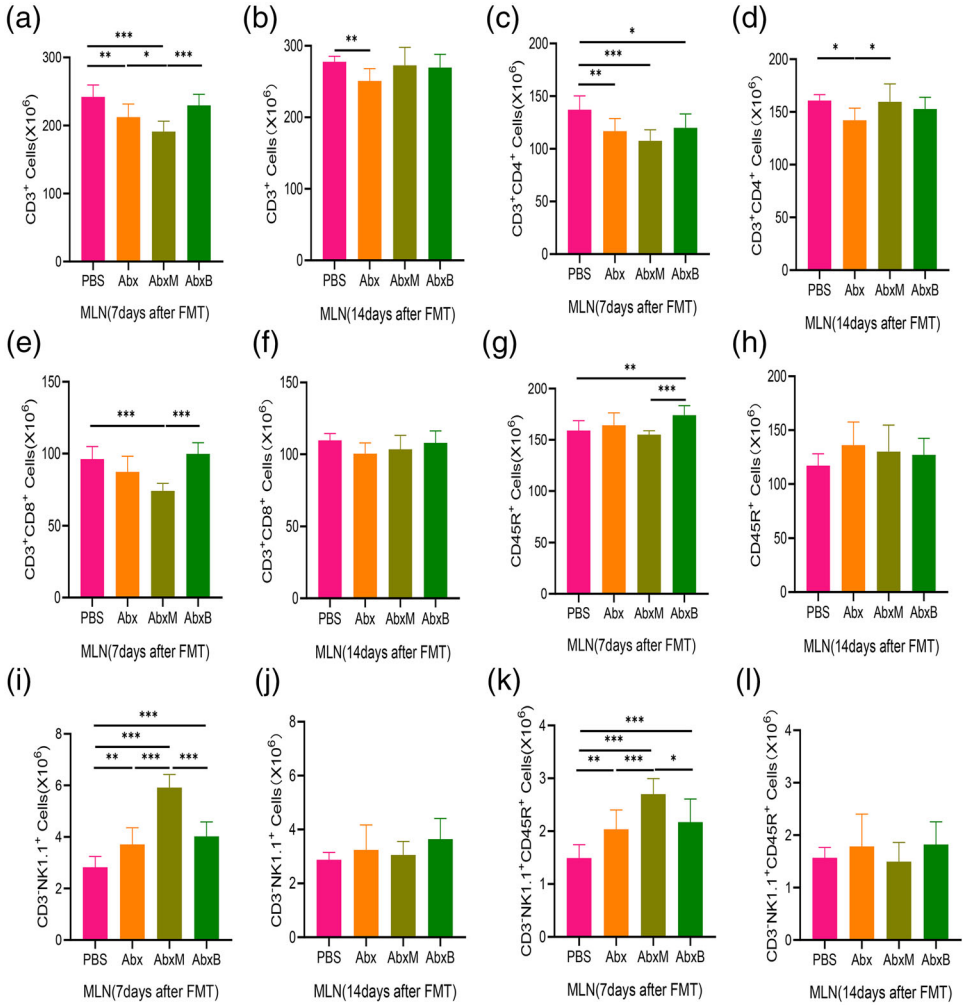

FIGURE 6 Changes in counts of T, B and NK cells in MLN on days 7 and 14 after fecal microbiota transplantation (FMT). Differences in antiviral-related cytokine on the 5th day after H1N1 challenge. Each group had 5 mice. One representative of three independent experiments was shown. Data are expressed as the mean ± SD. Statistical significance was tested by one-way ANOVA followed by Tukey's tests. $p < 0.05$ was considered significantly different from each group.

which was also one of the limitations of this study (Pulendran & Davis, 2020).

The bat gut microbiota imparts viral tolerance to mice, reducing mortality and disease severity from influenza virus challenges. The immune regulation effect of the gut microbiota on the body has been acknowledged by many and has been shown to play a role in various diseases, such as allergic airway disease and influenza (Steed et al., 2017; Trompette et al., 2014). Previous studies have shown that in influenza infection, germ-free mice and antibiotic treated mice have a higher mortality and show pathological damage, and the recovery of the gut microbiome can restore a host's antiviral ability. Moreover, the gut microbiota of wild mice has stronger antiviral and anticancer abilities than laboratory mice (Abt et al., 2012; Rosshart et al., 2017). It is usually the immunopathology of a host to an infection, rather than a pathogen itself, that is most responsible for the death of a host during a viral infection (Flerlage et al., 2021; Rouse & Sehrawat, 2010; Taubenberger & Morens, 2008). Our results showed that in the face of influenza virus infection, bat microflora led to an increase in IL-10, IL-12p70, IFN-α and IFN-β in BALF, which might have reduced viral titers and reduced pathological damage in the lungs, improving mouse survival. This was consistent with the previous findings that bats infected with this virus showed little pathological damage (Malmlov et al., 2019; Swanepoel et al., 1996; Watanabe et al., 2010). These results strongly confirmed the multiple protective effects of gut microbiota from bats on hosts during influenza infection, and implying that bat tolerance to infection with this virus may also be related to specific metabolites produced by their gut microbiome.

Metabolites of bat gut microbiota may be responsible for improving virus tolerance in mice. In this study, our results showed that the metabolites in the feces of the two groups of mice (AbxB and AbxM) were significantly different. The mice in the AbxB group were resistant to the H1N1 virus, probably because the transplantation significantly altered the host's gut microbiome to produce different metabolites, which may regulate the immune system of the host to protect itself from H1N1 infection because of the following three metabolic pathways. First, the differential compounds were concentrated in the

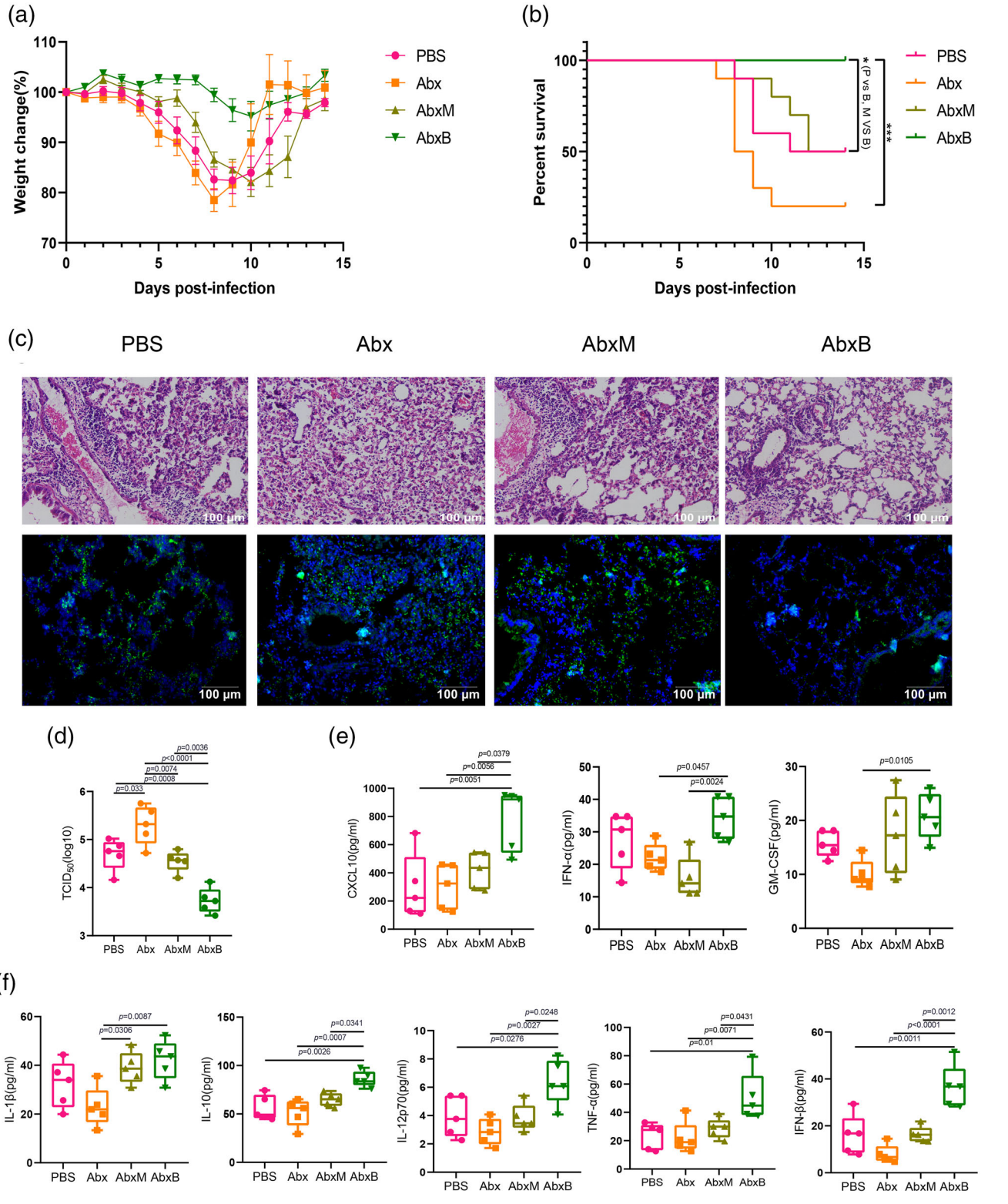

FIGURE 7 Gut microbiota from bats abrogates lung injury induced by H1N1 infection. (a) Changes in body mass during a specified period of time after infection with a half lethal dose of H1N1 virus in mice from the PBS, Abx, AbxM and AbxB groups. (b) Survival of mice in the PBS, Abx, AbxM and AbxB groups. (c) Hematoxylin and eosin staining and immunofluorescence of the lung. (d) $TCID_{50}$ of lung tissue from the PBS, Abx, AbxM and AbxB groups. (e) Antiviral-related cytokines in the serum. (f) Antiviral-related cytokines in BALF. Each group included five mice (10 mice in a and b, respectively). One representative of three independent experiments is shown. Box plots indicate median (middle line), 25th and 75th percentile (box) and 5th and 95th percentile (whiskers) as well as individual scores (single points) in d–f and mean ± SEM in a. $p < 0.05$ was significantly different from each group.

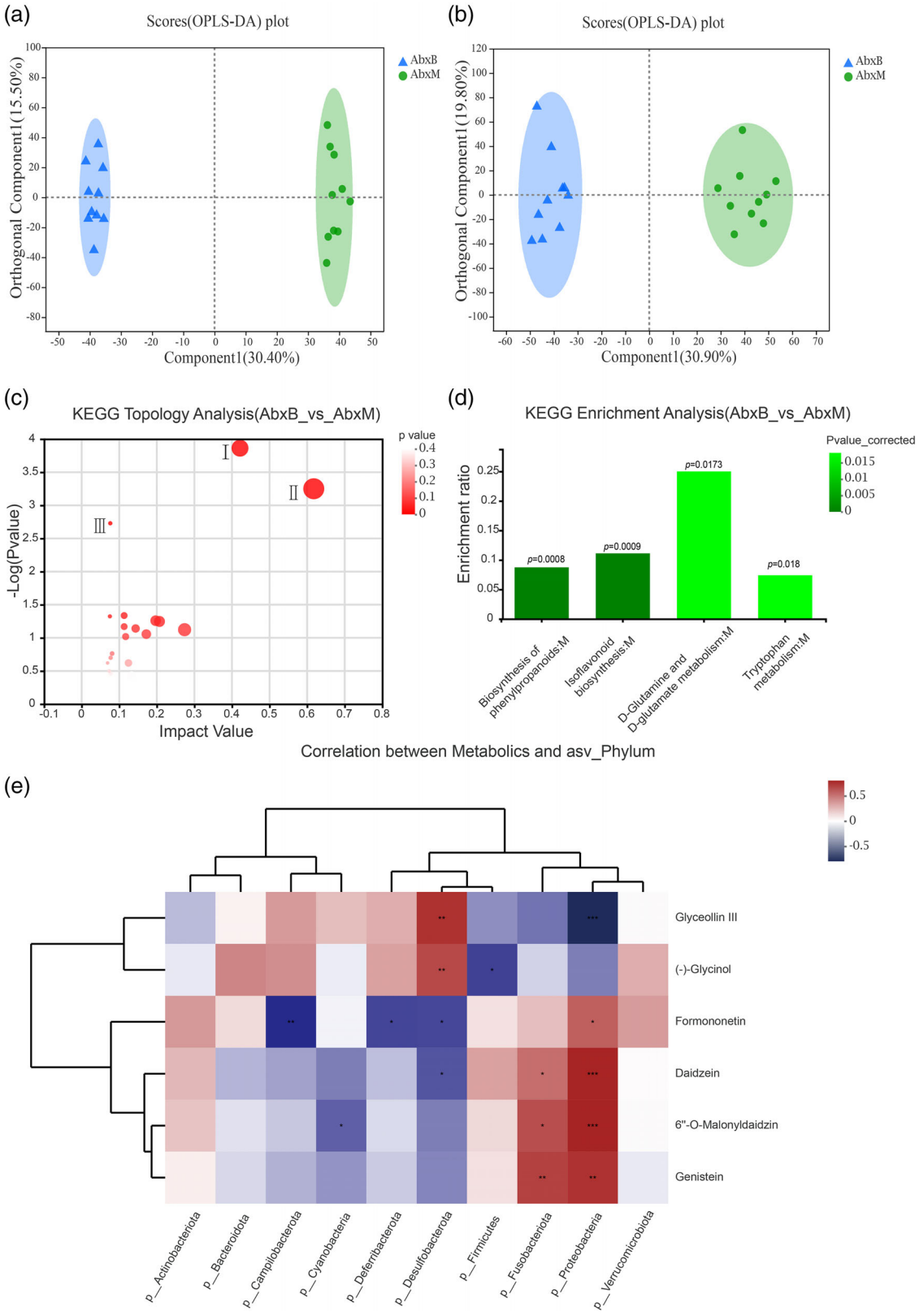

FIGURE 8 Fecal metabolome profiles of AbxM and AbxB mice. (a) OPLS-DA score of fecal samples from the AbxB and AbxM groups from electrospray ionization (ESI)+ mode MS data on day 14 after FMT (cations). (b) OPLS-DA score of fecal samples from the AbxB and AbxM groups from ESI- mode MS data on day 14 after FMT (anions). (c) The metabolic pathways of the AbxM and AbxB groups were topologically analyzed. The *X* axis is the path influence and the *Y* axis is the path enrichment. Larger sizes and darker colors represent richer paths and higher path-impact values, respectively. I: isoflavonoid biosynthesis, II: D-glutamine and D-glutamate metabolism, III: tryptophan metabolism. (d) Metabolic pathway enrichment study of differentially presented metabolites between the AbxB and AbxM groups; $p < 0.05$ was significantly different from each group. (e) Correlation between intestinal bacteria and isoflavone metabolic pathways. Each group included 10 mice.

Isoflavonoid biosynthesis, D-glutamine and D-glutamate metabolism and tryptophan metabolism pathways. D-glutamine and D-glutamate have a wide range of effects on the immune system (van Sadelhoff et al., 2020). As an energy supply available to immune cells, low concentrations of glutamine can promote T-cell proliferation, while high concentrations have immunosuppressive properties, but it has not been found to have direct antiviral effects (Kong et al., 2018; van Sadelhoff et al., 2020). Second, tryptophan metabolism can produce immunomodulatory substances such as indole-3-acid-acetic and indole-3-propionic acid, which are both ligands of the Aryl Hydrocarbon Receptor (AhR), and play a role in the tolerance of a variety of diseases (Agus et al., 2018). Finally, isoflavones (Daidzein, Formononetin and Genistein) have a wide range of antiviral effects, the mechanisms of which include influencing viral binding, inducing transcription factors, and regulating the secretion of host cytokines (Andres et al., 2009; Bernatoniene et al., 2021; Gomez-Zorita et al., 2020). Moreover, the antiviral effects of Genistein have been studied extensively and it has been shown to inhibit infection with a variety of viruses, such as adenovirus, herpes simplex virus and influenza virus (Li et al., 2000; Lyu et al., 2005; Nagai et al., 2019). Previous studies have shown that genistein can confer tolerance to viruses through a variety of mechanisms. Genistein has been shown to act as an antiviral in the early stages of infection by inhibiting tyrosine kinase activity and preventing adenoviruses from entering cells (Guo et al., 2013; Stantchev et al., 2007). In cytomegalovirus and arenavirus infection, the suppression of protein phosphorylation mediated by genistein reduces the activator of Fe transcription 2 (ATF-2), cyclic AMP response element-binding protein (CREB) and nuclear factor kappa-B (NF-KB) transcription factor levels to resist viral infection (Andres et al., 2009). It has also been reported that genistein can inhibit the DNA synthesis and life cycle of African swine fever virus, indicating that genistein can also play an antiviral role in the protein synthesis and release stage of this virus (Arabyan et al., 2018). However, because isoflavones are natural estrogen receptors, excessive amounts of genistein can cause immunosuppression, leading to decreased thymus weight and decreased cell-mediated immunity (Ganai & Farooqi, 2015). Previous studies on mouse fecal metabolome did not find daidzein, formononetin, or genistein, indicating the uniqueness of fecal isoflavone metabolism in mice in the AbxB group (Yuan et al., 2018). These results indicated that the KEGG enrichment of transplanted bat gut microbiota in mouse feces may have been unique. Additionally, to our knowledge, Ball et al. (2018) has provided the only bat fecal metabolomics data available. However, this metabolome was collected from captive bats of different ages, and our previous studies have shown that the intestinal microbiota of captive bats are quite different from that of true wild bats (Xiao et al., 2019). Different gut microbiota has different metabolome results, so we did not refer to the data provided by Ball et al. In this study, our results found a high correlation between Isoflavones and *Proteobacteria* levels, which was consistent with higher *Proteobacteria* abundance in bat gut microbiota and AbxB mice. These results suggested that isoflavones produced by the gut microbiota of bats may enhance host tolerance to influenza viruses and may also help bats to resist viral infections.

Metabolomics has also revealed the role of certain monomer compounds in modulating immunity and antiviral activity. Quercetin is a flavonoid with a wide range of antiviral properties, and regulatory action on inflammation and immunity (Colunga Biancatelli et al., 2020; Li et al., 2016). Quercetin usually exists in nature in the form of glycosides, which may be metabolized by the body's own enzymes and those of intestinal flora after entering the body (Miles et al., 2014). Quercetin has been found to have various mechanisms of action, such as blocking virus entry into cells (H1N1), inhibiting reverse transcriptase (HIV), increasing interferon and activating NK cells (Alvarez et al., 2006; Li et al., 2014; Nair et al., 2002; Wu et al., 2015). In addition, hydroxychavicol has anti-inflammatory and immunosuppressive effects (Pandey & Bani, 2010). In this study, our results also showed that the VIP values of 3-methylquercetin 7- [Galactosyl -(1 - > 4)-glucoside] and 3-hydroxychavicol 1-[rhamnosyl-(1 - > 6)-glucoside] in the feces of mice in the AbxB group were higher. These results suggest that quercetin may be involved in host immune regulation and increase host tolerance to viruses. In our results, we also found higher levels of interferon α in the AbxB group and a positive correlation with many flavonoids and isoflavones such as hydroxychavicol and daidzein. Therefore, we believe that bat gut microbiota and its flavonoids and isoflavones may be the key to regulating the immune system of mice and improving their tolerance to viruses. Bat gut microbiota can quickly adjust the immune cells of a host in response to viral pressure, and rapid innate immune responses can be effective against viruses. Moreover, flavonoids and isoflavone bioactive substances play roles at the same time, exerting an anti-inflammatory effect to reduce disease pathological damage, functioning as antivirals in order to reduce the virus proliferation and so on. The combination of gut microbiota and metabolites allowed mice to survive challenge with influenza virus.

Over the past few years, a series of important advances have been made in the genomic, transcriptomics and cell biology of bat immunity (Pavlovich et al., 2018; Periasamy et al., 2019; Wynne et al., 2016; Zhang et al., 2013). The exact mechanism by which bats tolerate viruses is unclear. One hypothesis is that bats have particularly strong innate antiviral defenses that control viral replication in the early stages of infection (Baker et al., 2013). Our results showed that mice with bat gut microbiota had the ability to rapidly modulate immune cells, producing more interferons under challenge with influenza virus H1N1. Moreover, our metabolome results revealed high levels of flavonoids and isotopes flavonoids. These results supported the hypothesis that bats have a rapid innate immune response to pathogens and suppress viral reproduction early in infection. It has also been hypothesized that balanced immune regulation of bats to viral infection may be responsible for their viral tolerance (Irving et al., 2021). Our results showed the ability of bat gut microbiota to rapidly modulate immune cells, supporting this hypothesis. Bats appear to have an 'always ON' immune state, characterized by elevated baseline levels of interferon and a rapid response to pathogens (De La Cruz-Rivera et al., 2018; Subudhi et al., 2019). However, our results did not show a baseline increase in IFNs, which may have been due to the fact that previous studies focused on the bat genome/transcriptome, whereas our study focused on the

bat gut microbiota's response to viruses. Our study complements these and focused on the relationship between bat gut microbiota and immunity, which contributes significantly to the development of bat immunity.

5 | CONCLUSION

In summary, our study demonstrated that mice as an animal model can be used to transplant the gut microbiota of bats, and compared with the gut microbiota of mice, the gut microbiota of bats can promote faster recovery of immune cell disturbances caused by antibiotic treatment. Moreover, reduced pathological damage and viral titers in the lungs improved mouse survival. Further metabolism analysis indicated that the flavonoids and isoflavones produced by the gut microbiota from bats might enhance a host's tolerance to influenza viruses. Our results confirmed that the gut microbiota of bats might help bats to tolerate viral infections via the regulation of the immune system. Additionally, this also showed that bat gut microbiota endowed the host with the ability to rapidly initiate innate immune responses, which was in line with the hypothesis that bats have a rapid and powerful innate antiviral response. Therefore, our results demonstrated that bat intestinal microbiota and its flavonoid and isoflavone metabolites enhanced the host's tolerance to viruses through regulating the immune system, endowing the host with a rapid innate immune response and anti-inflammatory effects. To our knowledge, our study was the first to employ FMT to transplant the gut microbiota of bats into mice and provided the first experimental evidence that the gut microbiota of bats plays an important role in tolerance to viral infections and increases host fitness. A limitation of this study was that there are limitations to extrapolating from mouse model results to an original animal subject. Our study highlighted the facts that the gut microbiota of bats is a huge probiotic storehouse. Further studies are needed to determine the function of the gut microbiota of bats on tolerance to viral infections at the organic, cellular and molecular levels, and to determine the relative importance of taxonomy, the metabolome and regulation to the immune system of bat gut microbiota with respect to the tolerance of viral infection.

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ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. Animal experiments were conducted in an animal biosafety level 2+ facility and laboratory animals are managed in accordance with the Animal Welfare Guidelines of the World Animal Health Organization (WOAH, OIE). All experiments were approved by the Animal Welfare and Ethics Committee of Jilin Agricultural University, approval number: 2019 07 25 001.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be accessed from NCBI Sequence Read Archive under BioProject PRJNA750299. Flow cytometry data is available upon reasonable request.

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SUPPORTING INFORMATION

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