Parasitology Research (2019) 118:693–699
https://doi.org/10.1007/s00436-018-6179-x

HELMINTHOLOGY - SHORT COMMUNICATION

Chinese liver fluke *Clonorchis sinensis* infection changes the gut microbiome and increases probiotic *Lactobacillus* in mice

Ju Yeong Kim[1,2] · Eun-Min Kim[1] · Myung-hee Yi[1] · Jinyoung Lee[1] · Seogwon Lee[1] · Younjee Hwang[2,3] · Dongeun Yong[3] · Woon-Mok Sohn[4] · Tai-Soon Yong[1]

Received: 5 September 2018 / Accepted: 11 December 2018 / Published online: 8 January 2019

Abstract

Chinese liver fluke *Clonorchis sinensis* changes the host's immune system. Recently, it has been reported that helminths including *C. sinensis* can ameliorate immune-related diseases such as allergy. In addition, recent studies showed that helminth infection can alleviate immune-mediated disorders by altering the gut microbiome. However, changes in the gut microbiome due to *C. sinensis* have not been reported yet. In this study, changes in the gut microbiome of C57BL/6 mice infected with *C. sinensis* metacercariae were evaluated over time. Stool was analyzed by 16S rRNA amplicon analysis using high-throughput sequencing technology. There was no apparent difference in species richness and diversity between the infected and control groups. However, the composition of the microbiome was different between the infected and control groups at 20 days and 30 days post-infection, and the difference disappeared at 50 days post-infection. In particular, this microbiome alteration was associated with a change in the relative abundance of genus *Lactobacillus* and the probiotic *Lactobacillus* species that are known to have an immune-modulation role in immune-mediated diseases.

Keywords *Clonorchis sinensis* · Gut microbiome · *Lactobacillus* · Metagenomics · Liver fluke

Introduction

Recent epidemiological and clinical evidence has supported the hygiene hypothesis, which proposes that the elimination of intestinal helminths in developed countries contributes to the increased incidence of immune-mediated diseases (Cooper et al. 2003; Feary et al. 2011; Maizels and Yazdanbakhsh 2003; Wammes et al. 2014). Chinese liver fluke (*Clonorchis sinensis*) infection is a major public health problem in some Asian countries, including the Republic of Korea. This endemic disease provokes the loss of liver function, chronic liver diseases, and even cholangiocarcinoma (Hong 2003; Rim 2005). On the other hand, it has been known that *C. sinensis* considerably changes the host's immune system (Choi et al. 2003; Kim et al. 2017). In a recent study, *C. sinensis*–derived total protein attenuated airway inflammation in a murine asthma model, induced regulatory T cells, and modulated dendritic cell functions (Jeong et al. 2011). An epidemiological study also showed that the skin prick test and IgE reactivity for aeroallergens vary by *C. sinensis* infection status, suggesting its potential effect on immune-mediated diseases (Choi et al. 2011).

Numerous studies have focused on changes in the microbiome caused by helminths (Broadhurst et al. 2012; Li et al. 2012; Osborne et al. 2014; Plieskatt et al. 2013; Rausch et al. 2013; Walk et al. 2010). Intestinal helminths have been reported to alter intestinal physiology, permeability, mucous secretion, and production of antimicrobial peptides, all of which

Section Editor: Ramaswamy Kalyanasundaram

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-018-6179-x) contains supplementary material, which is available to authorized users.

✉ Tai-Soon Yong
tsyong212@gmail.com

[1] Department of Environmental Medical Biology, Institute of Tropical Medicine, and Arthropods of Medical Importance Resource Bank, Yonsei University College of Medicine, Seoul 03722, Korea

[2] Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

[3] Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul 03722, Korea

[4] Department of Parasitology and Tropical Medicine, and Institute of Health Sciences, Gyeongsang National University College of Medicine, Jinju 52727, Korea

may impact the gut microbiome (Zaiss and Harris 2016). Furthermore, changes in the gut microbiome due to helminth infection have been reported as one of the mechanisms of helminth-induced modulation of immune-mediated disorders (Zaiss et al. 2015). However, no study has been conducted to determine whether *C. sinensis* changes the gut microbiome or whether such changes are of immunological significance. In this study, we evaluated the effect of *C. sinensis* infection on the gut microbiome at specific time points: before infection (0 days post-infection (PI)), early stage (10 days PI), adult worm stage (20 and 30 days PI), and post-worm-expulsion stage (50 days PI). We used C57BL/6, a mouse strain that is relatively susceptible to *C. sinensis* infection; the worm recovery rate is 10% at 4 weeks PI (Uddin et al. 2012). This strain of mouse has been widely used in gut microbiome research. Finally, we performed 16S rRNA amplicon analysis to investigate the gut microbiome using high-throughput sequencing technology.

Materials and methods

Parasites and experimental design

C. sinensis metacercariae, used for infecting mice in this study, were collected from *Pungtungia herzi* caught at Gunwi-gun, Gyeongsangbuk-do, Korea. To standardize the intestinal bacteria, 6-week-old C57BL/6 mice were co-housed in one cage for 2 weeks. At 8 weeks of age, the mice were infected orally with 30 metacercariae. Seven mice were used for both infection and control groups. After metacercariae infection, each mouse was raised in a different cage. Stool from the mice was collected every 10 days until sacrifice at 50 days after infection. All animal studies were approved by the Department of Laboratory Animal Resources Committee of Yonsei University College of Medicine (No. 2015–0339). Animal experiments were carried out in animal biosafety level-3 (ABL-3) facilities in accordance with standard management practices.

High-throughput sequencing of 16S rRNA gene amplicons

DNA from the stool of each mouse was extracted using the QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany). The V3–V4 region of the 16S rRNA gene was amplified by PCR using the bacterial universal primer pair (forward primer: 5′- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; reverse primer: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′). A limited-cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters. The libraries were normalized, pooled, and sequenced using the MiSeq platform (Illumina MiSeq V3 cartridge [600 cycles]; Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

Bioinformatics

Bioinformatic analyses were performed as follows (Kim et al. 2018a, b). Raw reads were processed through a quality check, and low-quality (< Q25) reads were filtered using Trimmomatic 0.32 (Bolger et al. 2014). Paired-end sequence data were then merged using PandaSeq (Masella et al. 2012). Primers were trimmed using the ChunLab in-house program (ChunLab, Inc., Seoul, Korea), applying a similarity cut-off of 0.8. Sequences were denoised using the Mothur pre-clustering program, which merges sequences and extracts unique sequences, allowing up to two differences between sequences (Schloss et al. 2009). The EzBioCloud database (https://www.ezbiocloud.net/) (Yoon et al. 2017) was used for taxonomic assignment using BLAST 2.2.22, and pairwise alignments were generated to calculate similarity (Altschul et al. 1990; Myers and Miller 1988). The UCHIME algorithm and non-chimeric 16S rRNA database from EzTaxon were used to detect chimeric sequences for reads with a best hit similarity rate of < 97% (Edgar et al. 2011). Sequence data were then clustered using CD-Hit and UCLUST (Edgar 2010; Fu et al. 2012).

Statistical analysis

The following analyses were performed with BIOiPLUG, a commercially available ChunLab bioinformatics cloud platform for microbiome research (https://www.bioiplug.com/) (Kim et al. 2018b). The reads were normalized to 40,000 to perform the analyses. We computed the Shannon index (Shannon et al. 1948) and performed principal coordinate analysis (PCoA) (Gower 1966) and permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) based on the UniFrac distance (Lozupone and Knight 2005). The Wilcoxon rank-sum test was used to test the difference between groups in the number of operational taxonomic units (OTU), Shannon index, and relative abundances of specific taxa. $p < 0.05$ and false discovery rate (FDR)–adjusted p value < 0.1 were considered significant. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was used to identify significantly different taxa between the groups (Segata et al. 2011).

Histopathology

Hematoxylin and eosin staining

Mice were sacrificed by CO_2 asphyxiation at pre-determined times, and livers were collected and fixed in 10% formalin for

24 h. Whole liver specimens were embedded in paraffin. The middle part of the embedded livers was transversely cut into 4-μm-thick sections and processed for routine hematoxylin and eosin (H&E) staining. The slides were examined by light microscopy.

Results and discussion

For the *C. sinensis* infection and control groups at all time points, the average read count was 77,067 and 88,437, respectively (Table S1). Three samples were not included in data analysis (I4 at 50 days PI due to low read counts, I7 at 30 days PI, and C3 at 50 days PI due to sequencing failure). After normalizing the reads, alpha diversity was assessed. The number of OTU and Shannon index were not significantly different between the two groups at all time points (Table S2), suggesting that *C. sinensis* infection did not significantly alter the number of bacterial species (richness) and bacterial diversity in the mouse gut microbiome. To assess the beta diversity, we performed PERMANOVA (Table S3). The result showed that *C. sinensis* infection was a significant factor in determining gut microbiome composition at 20 and 30 days PI, but the significance disappeared at 50 days PI. In PCoA, the samples at 20 and 30 days PI were organized well according to infection status (Fig. 1a), but the samples from the other time points were not (Fig. 1b).

At the genus level (Fig. 2), the two most abundant genera were *Lactobacillus* and *Bacteroides*. In *C. sinensis*–infected mice, the initial relative abundances of *Lactobacillus* and *Bacteroides* were 39.2% and 34.4%, respectively. After infection, the relative abundance of *Lactobacillus* increased until 30 days PI, reaching 50.5%, 2.5 times that of *Bacteroides*. At 50 days PI, the relative abundance of *Lactobacillus* decreased to 43.1%, with a ratio to *Bacteroides* of 1.06.

LEfSe analysis was performed to identify the differences in bacterial abundance between the groups at the 20 and 30 days PI (Fig. 3). The highest LDA score in the infection group was for *Lactobacillus*: 4.94 at 20 days PI and 4.79 at 30 days PI. In particular, the LDA score of the *Lactobacillus reuteri* group was 3.87 at 20 days PI and 3.63 at 30 days PI. The relative abundance of *Lactobacillus reuteri* at 20 days PI was 1.55% in the infection group and 0.06% in the control group, decreasing to 0.90% and 0.04% at 30 days PI, respectively. At 0, 10, and 50 days PI, the relative abundances of genus *Lactobacillus* and species of the *Lactobacillus reuteri* group were not different between the infection and control groups (Table S4).

The relative abundance of *Lactobacillus* was not different between groups at 0 and 10 days PI (Fig. S1). The relative abundance of *Lactobacillus* in the infection group increased over time and became significantly different from that of the control group at 20 and 30 days PI. Nonetheless, the abundance decreased at 50 days PI, and the significance of difference disappeared.

In this study, there was no significant difference in alpha diversity between the infection and control groups. However, the composition (beta diversity) of the mouse gut microbiome changed at 20 days and 30 days after *C. sinensis* infection, but this change disappeared at 50 days. This was mainly related to the change in relative abundance of *Lactobacillus*.

The change in composition of the microbiome over time might be related to the life cycle of *C. sinensis* in C57BL/6 mice. We confirmed that the adult worms had already been removed or passed from the body at 50 days PI. At that time,

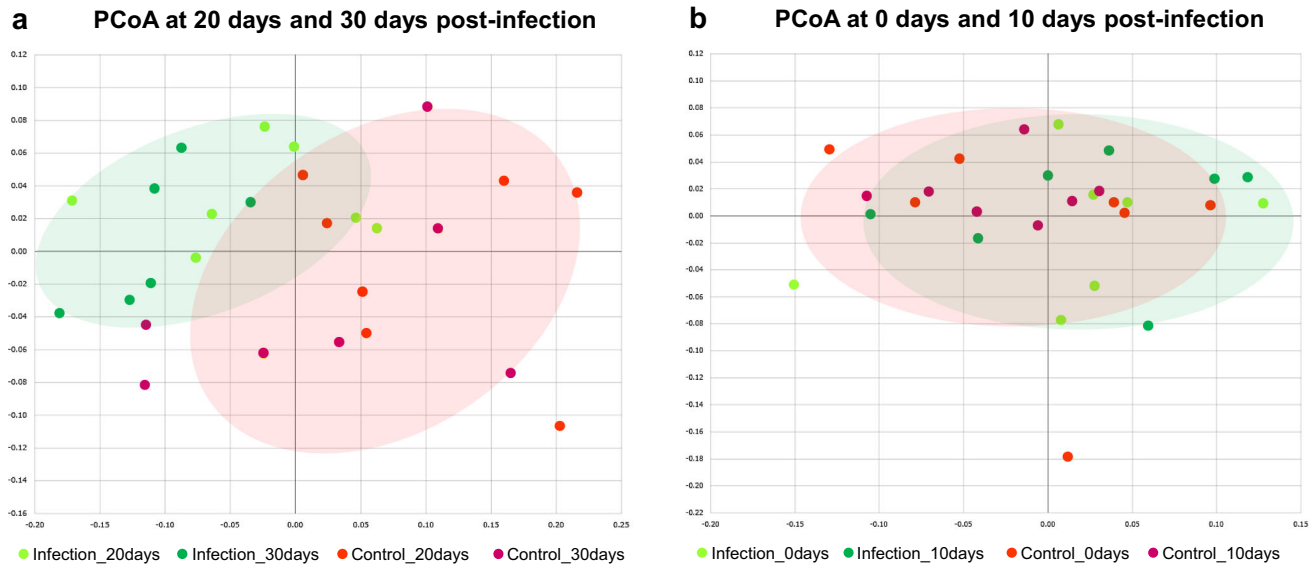

Fig. 1 PCoA of the gut microbiome of *C. sinensis*–infected (green circle) and control (red circle) mice at **a** 20 and 30 days post-infection and **b** 0 and 10 days post-infection. The samples at 20 and 30 days post-infection were organized well according to infection status, but the samples from 0 to 10 days post-infection were not

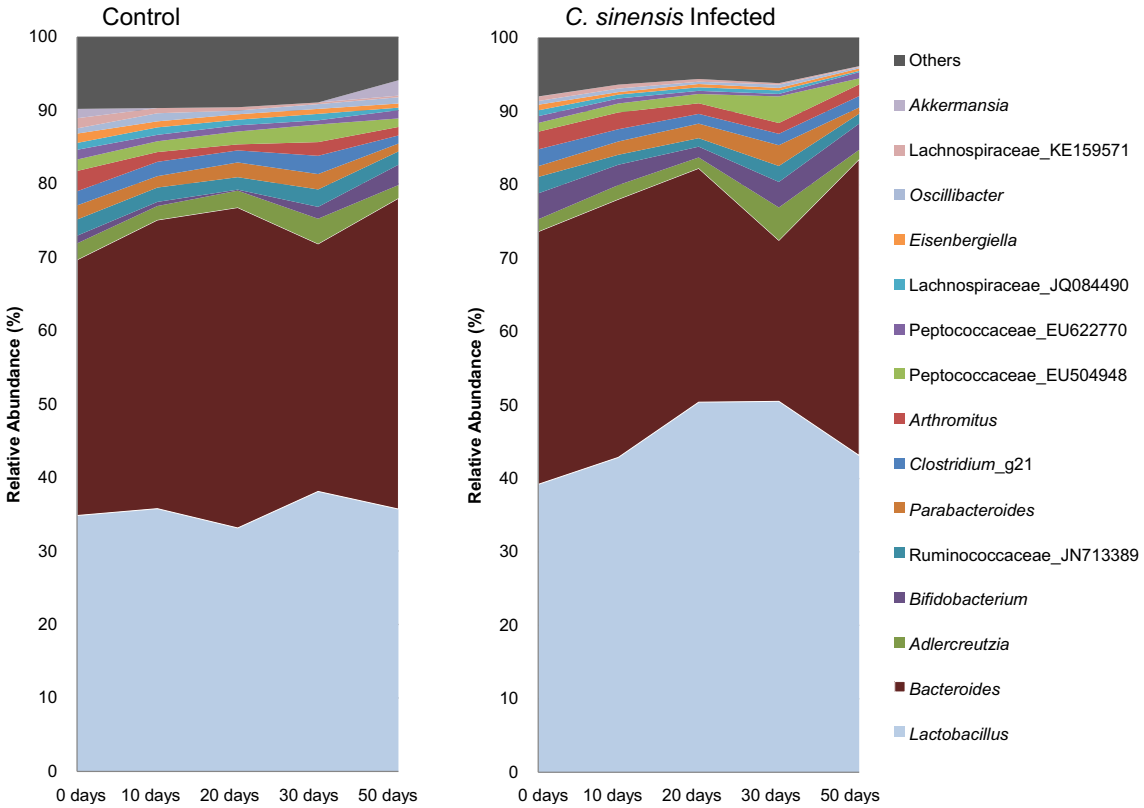

Fig. 2 Gut microbiome composition of *C. sinensis*–infected ($n = 7$) and control ($n = 7$) C57BL/6 mice over time at the genus level. Stools of mice were collected every 10 days and analyzed by 16S rRNA amplicon analysis using high-throughput sequencing technology. Genera representing more than 1% of reads are shown

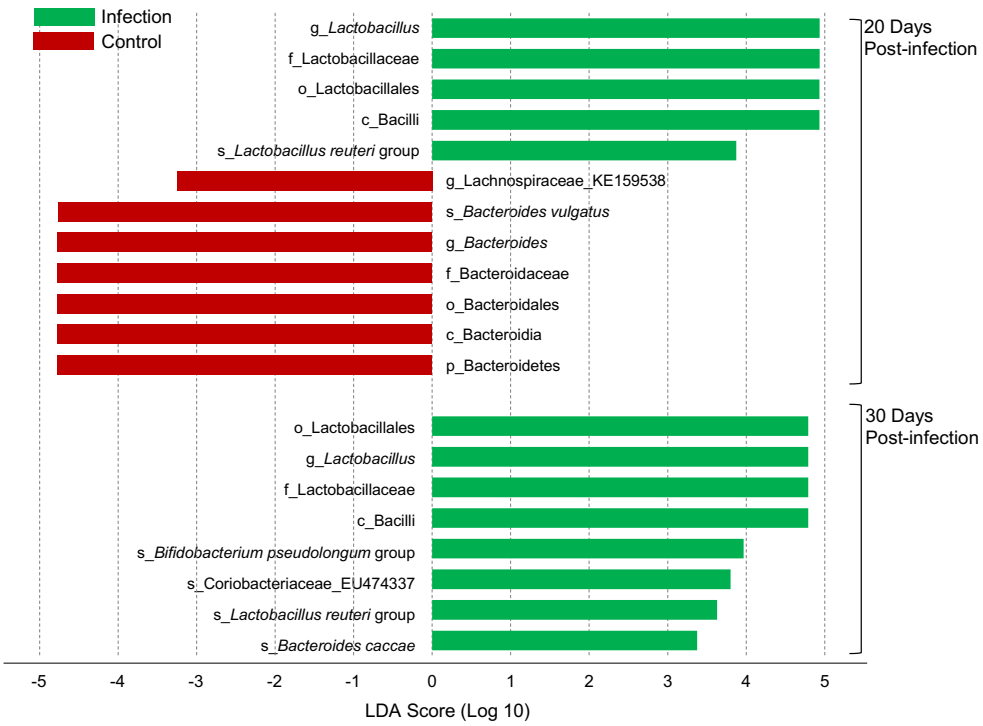

Fig. 3 LEfSe analysis of differentially abundant gut bacterial taxa between *C. sinensis*–infected (green) and control (red) groups at 20 and 30 days post-infection. Only taxa meeting and LDA significance threshold of > 3 are shown. p, phylum; c, class; o, order; f, family; g, genus; s, species

we did not find parasitic eggs microscopically in the mouse stool, nor did we detect adult worms in hepatobiliary tissue staining. However, the eggs of *C. sinensis* were trapped in hepatobiliary tissue of all infected mice (Fig. S2). Therefore, it was considered that the gut microbiome changed at 20 and 30 days when *C. sinensis* grew into adults and secreted eggs or excretory-secretory products into the bile and intestine, and this effect disappeared after adult worms were removed from the body.

In previous studies on *O. viverrini* and *O. felineus* infection, an increase in *Lactobacillus* in the feces and liver was reported, consistent with our finding of increased *Lactobacillus* in *C. sinensis*–infected mice. A study of the microbiome of hamster infected by *Opisthorchis viverrini*, another liver fluke prevalent in Southeast Asia, is in agreement with our results. During 6-week infection, *Lactobacillus* was increased in the feces of hamster (Plieskatt et al. 2013). A previous human study is also in line with our results. In bile microbiome examination of patients who underwent cholecystectomy for cholelithiasis, *Lactobacillus* was increased by *O. felineus* infection (Saltykova et al. 2016). Other studies have also demonstrated that nematode infections, including of *Heligmosomoides polygyrus* and *Trichinella spiralis*, in C57BL/6 mice significantly increased the abundance of Lactobacillaceae (Osborne et al. 2014; Walk et al. 2010).

Notably, the proportion of the *L. reuteri* group was increased by *C. sinensis* infection. This bacterium, currently used as a probiotic, has been reported to have anti-inflammatory effects. Oral treatment with *L. reuteri* attenuated allergic airway inflammation and induced regulatory T cells in a mouse model (Karimi et al. 2009). Additionally, *L. reuteri* attenuated necrotizing enterocolitis in a rat model, reducing TLR4 and NF-κB signaling and increasing regulatory T cells in the intestine (Liu et al. 2012, 2013). The results of recent studies that showed that allergic airway inflammation was decreased by treatment with various liver flukes such as *C. sinensis* and *Fasciola hepatica* might be associated with increased anti-inflammatory bacteria such as *Lactobacillus* (Finlay et al. 2017; Jeong et al. 2011).

Chronic *O. viverrini* infection promoted *Helicobacter pylori* growth in the hamster liver (Itthitaetrakool et al. 2016) and *H. pylori* was reported to be increased in the feces and liver of *O. viverrini*–infected hamster (Deenonpoe et al. 2015). Furthermore, recent studies reported that *Helicobacter* spp., associated with *O. viverrini* infection, is a possible risk factor of cholangiocarcinoma (Kaewpitoon et al. 2016). In our study, we could not confirm the correlation between *C. sinensis* infection and relative abundance of *H. pylori*, since we did not use a mouse that already had *H. pylori*. In this study, using mouse stool, sample 0daycontrol1 had two reads of *Helicobacter* spp. (0.002%), whereas 0dayinfection1 had two reads (0.002%), 20daycontrol7 had one read (0.001%), and the other 64 stool samples did not have any. Intermittently occurring one or two reads, from the approximately 80,000 reads, was negligible. We performed animal studies in animal biosafety level-3 (ABL-3) facilities using SPF mice, and *H. pylori* was considered not to be present initially in the mice. In addition, *Helicobacter* spp. was not detected by microbiome analysis of *C. sinensis* metaceracariae, which were initially introduced into the mice (Table S5). In previous studies with *O. viverrini*–infected hamster, control animals had *H. pylori* (Deenonpoe et al. 2015). If *C. sinensis* infects in presence of *H. pylori*, it may alter the relative abundance of *H. pylori* and also the inflammation and carcinogenesis pattern in the liver, since *O. viverrini* and *C. sinensis* both cause inflammation in the liver and can cause cholangiocarcinoma.

Some previous studies on microbiome change, caused by liver fluke infections (*O. viverrini* and *O. felineus*), had investigated the microbiome of the hepatobiliary system, focusing on the carcinogenic effect of altered microbiome on the system (Plieskatt et al. 2013; Saltykova et al. 2016). However, we focused on the anti-inflammatory effect of the altered microbiome, by parasite, and did not investigate the hepatobiliary bacteria. In addition, we used C57BL/6 mouse strain, which is widely used in immunological and microbiome studies. Although this strain is relatively more susceptible to *C. sinensis* than other mouse strains, in general, mouse is not susceptible to *C. sinensis*. In future, rabbit or hamster, which is more susceptible than mice, should be used for the study of microbiome changes caused by *C. sinensis* infection.

In conclusion, *C. sinensis* infection in mice changed the composition of the gut microbiome and increased the proportion of probiotic *Lactobacillus* species, which may act to modulate the immune system and alleviate immune-mediated diseases.

Funding information This study was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST; numbers NRF-2016R1A2B4016194 and 2011-0012166).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving animals All animal protocols were approved by the Institutional Animal Care and Use Committee at Yonsei University College of Medicine (No. 2015–0339). Animal experiments were carried out in animal biosafety level-3 (ABL-3) facilities in accordance with standard management practices.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Eco 26:32–46

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120

Broadhurst MJ, Ardeshir A, Kanwar B, Mirpuri J, Gundra UM, Leung JM, Wiens KE, Vujkovic-Cvijin I, Kim CC, Yarovinsky F, Lerche NW, McCune JM, Loke P (2012) Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the inflammatory signature and mucosal microbiota of the colon. PLoS Pathog 8: e1003000

Choi MH, Chang YS, Lim MK, Bae YM, Hong ST, Oh JK, Yun EH, Bae MJ, Kwon HS, Lee SM, Park HW, Min KU, Kim YY, Cho SH (2011) *Clonorchis sinensis* infection is positively associated with atopy in endemic area. Clin Exp Allergy 41:697–705

Choi YK, Yoon BI, Won YS, Lee CH, Hyun BH, Kim HC, Oh GT, Kim DY (2003) Cytokine responses in mice infected with *Clonorchis sinensis*. Parasitol Res 91:87–93

Cooper PJ, Chico ME, Rodrigues LC, Ordonez M, Strachan D, Griffin GE, Nutman TB (2003) Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. J Allergy Clin Immunol 111:995–1000

Deenonpoe R, Chomvarin C, Pairojkul C, Chamgramol Y, Loukas A, Brindley PJ, Sripa B (2015) The carcinogenic liver fluke *Opisthorchis viverrini* is a reservoir for species of Helicobacter. Asian Pac J Cancer Prev 16:1751–1758

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200

Feary J, Britton J, Leonardi-Bee J (2011) Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 66:569–578

Finlay CM, Stefanska AM, Coleman MM, Jahns H, Cassidy JP, McLoughlin RM, Mills KHG (2017) Secreted products of *Fasciola hepatica* inhibit the induction of T cell responses that mediate allergy. Parasite Immunol 39:Epub

Fu L, Niu B, Zhu Z, Wu S, Li W (2012) CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28: 3150–3152

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325–338

Hong ST (2003) Clonorchis sinensis. In: Miliotis MD, Bier JW (ed) International handbook of foodborne pathogens. Marcel Dekker, New York, Ch. 35

Itthitaetrakool U, Pinlaor P, Pinlaor S, Chomvarin C, Dangtakot R, Chaidee A, Wilailuckana C, Sangka A, Lulitanond A, Yongvanit P (2016) Chronic *Opisthorchis viverrini* infection changes the liver microbiome and promotes *Helicobacter* growth. PLoS One 11: e0165798

Jeong YI, Kim SH, Ju JW, Cho SH, Lee WJ, Park JW, Park YM, Lee SE (2011) *Clonorchis sinensis*-derived total protein attenuates airway inflammation in murine asthma model by inducing regulatory T cells and modulating dendritic cell functions. Biochem Biophys Res Commun 407:793–800

Kaewpitoon SJ, Loyd RA, Rujirakul R, Panpimanmas S, Matrakool L, Tongtawee T, Kootanavanichpong N, Pengsaa P, Kompor P, Chavengkun W, Kujapun J, Norkaew J, Ponphimai S, Padchasuwan N, Polsripradist P, Eksanti T, Phatisena T, Kaewpitoon N (2016) Helicobacter species are possible risk factors of cholangiocarcinoma. Asian Pac J Cancer Prev 17:37–44

Karimi K, Inman MD, Bienenstock J, Forsythe P (2009) *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. Am J Respir Crit Care Med 179:186–193

Kim EM, Kwak YS, Yi MH, Kim JY, Sohn WM, Yong TS (2017) *Clonorchis sinensis* antigens alter hepatic macrophage polarization in vitro and in vivo. PLoS Negl Trop Dis 11:e0005614

Kim JY, Yi MH, Hwang Y, Lee JY, Lee IY, Yong D, Yong TS (2018a) 16S rRNA profiling of the *Dermatophagoides farinae* core microbiome: *Enterococcus* and *Bartonella*. Clin Exp Allergy 48: 607–610

Kim JY, Kim EM, Yi MH, Lee J, Lee S, Hwang Y, Yong D, Sohn WM, Yong TS (2018b) Intestinal fluke *Metagonimus yokogawai* infection increases probiotic *Lactobacillus* in mouse cecum. Exp Parasitol 193:45–50

Li RW, Wu S, Li W, Navarro K, Couch RD, Hill D, Urban JF Jr (2012) Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. Infect Immun 80:2150–2157

Liu Y, Fatheree NY, Mangalat N, Rhoads JM (2012) *Lactobacillus reuteri* strains reduce incidence and severity of experimental necrotizing enterocolitis via modulation of TLR4 and NF-κB signaling in the intestine. Am J Physiol Gastrointest Liver Physiol 302:608–617

Liu Y, Fatheree NY, Dingle BM, Tran DQ, Rhoads JM (2013) *Lactobacillus reuteri* DSM 17938 changes the frequency of Foxp3+ regulatory T cells in the intestine and mesenteric lymph node in experimental necrotizing enterocolitis. PLoS One 8:e56547

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71: 8228–8235

Maizels RM, Yazdanbakhsh M (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 3: 733–744

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD (2012) PANDAseq: paired-end assembler for illumina sequences. BMC bioinformatics 13:31

Myers EW, Miller W (1988) Optimal alignments in linear space. Comput Appl Biosci 4:11–17

Osborne LC, Monticelli LA, Nice TJ, Sutherland TE, Siracusa MC, Hepworth MR, Tomov VT, Kobuley D, Tran SV, Bittinger K, Bailey AG, Laughlin AL, Boucher JL, Wherry EJ, Bushman FD, Allen JE, Virgin HW, Artis D (2014) Coinfection. Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. Science 345:578–582

Plieskatt JL, Deenonpoe R, Mulvenna JP, Krause L, Sripa B, Bethony JM, Brindley PJ (2013) Infection with the carcinogenic liver fluke *Opisthorchis viverrini* modifies intestinal and biliary microbiome. FASEB J 27:4572–4584

Rausch S, Held J, Fischer A, Heimesaat MM, Ku¨hl, AA, Bereswill S, Hartmann S (2013) Small intestinal nematode infection of mice is associated with increased enterobacterial loads alongside the intestinal tract. PLoS ONE 8:e74026

Rim HJ (2005) Clonorchiasis: an update. J Helminthol 79:269–281

Saltykova IV, Petrov VA, Logacheva MD, Ivanova PG, Merzlikin NV, Sazonov AE, Ogorodova LM, Brindley PJ (2016) Biliary microbiota, gallstone disease and infection with *Opisthorchis felineus*. PLoS Negl Trop Dis 10:e0004809

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60

Shannon C, Petigara N, Seshasai S (1948) A mathematical theory of communication. Bell Syst Tech J 27:379–423

Uddin MH, Li S, Bae YM, Choi MH, Hong ST (2012) Strain variation in the susceptibility and immune response to *Clonorchis sinensis* infection in mice. Parasitol Int 61:118–123

Walk ST, Blum AM, Ewing SA, Weinstock JV, Young VB (2010) Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. Inflamm Bowel Dis 16:1841–1849

Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M (2014) Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. Lancet Infect Dis 14:1150–1162

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617

Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, Piersigilli A, Menin L, Walker AW, Rougemont J, Paerewijck O, Geldhof P, McCoy KD, Macpherson AJ, Croese J, Giacomin PR, Loukas A, Junt T, Marsland BJ, Harris NL (2015) The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation. Immunity 43:998–1010

Zaiss MM, Harris NL (2016) Interactions between the intestinal microbiome and helminth parasites. Parasite Immunol 38:5–11