Fecal microbiota transplantation prevents *Candida albicans* from colonizing the gastrointestinal tract

Kenzo Matsuo[1*], Akira Haku[1, 2*], Beibei Bi[1], Hiroki Takahashi[3], Nobuhiko Kamada[4], Takashi Yaguchi[3], Shinobu Saijo[1], Mitsutoshi Yoneyama[1], Yoshiyuki Goto[1, 5]

[1] Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8673, Japan

[2] Division of Innate Immunity, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo

[3] Division of Bio-resources, Medical Mycology Research Center, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8673, Japan

[4] Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

[5] Division of Mucosal Symbiosis, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

*These authors contributed equally.

Correspondence

Yoshiyuki Goto

Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8673, Japan

Tel: +81 43 226 2490, Fax: +81 43 226 2486

E-mail: y-gotoh@chiba-u.jp

Short Running Title

Resident bacteria block fungi infection

ABSTRACT

Gut microbes symbiotically colonize the gastrointestinal (GI) tract, interacting with each other and their host to maintain GI tract homeostasis. Recent reports have shown that gut microbes help protect the gut from colonization by pathogenic microbes. Here, we report that commensal microbes prevent colonization of the pathogenic fungus, *Candida albicans*, in the GI tract. Wild-type specific pathogen-free (SPF) mice are resistant to *C. albicans* colonization in the GI tract. However, administering certain antibiotics to SPF mice enables *C. albicans* colonization. Quantitative kinetics of commensal bacteria are inversely correlated with the number of *C. albicans* in the gut. Here, we provide further evidence that fecal microbiota transplantation effectively prevents *Candida* colonization in the GI tract. These data demonstrate the importance of commensal bacteria as a barrier of the GI tract surface, and highlight the potential clinical applications of commensal bacteria for preventing pathogenic fungal infections.

Key Words

Antibiotics, *Candida albicans*, Commensal bacteria, Fecal microbiota transplantation

Introduction

Many microbes, including bacteria, fungi, viruses, and parasites, symbiotically colonize the gastrointestinal (GI) tract (1). These microbes dynamically interact with each other and their host to maintain homeostasis. Dysbiosis, a disruption in the homeostasis of the gut microbiota, is postulated to cause various host diseases, including inflammatory bowel diseases, metabolic syndrome, cancer, and infection (1, 2). Gut microbes play important roles in resisting colonization and protecting the host from infection by pathogenic microbes (2, 3). For example, commensal bacteria prevent infection by enteropathogenic bacteria such as *Listeria monocytogenes*, *Citrobacter rodentium*, *Salmonella typhimurium*, vancomycin-resistant *Enterococcus*, and *Clostridium difficile* (3-7). Based on the commensal bacterial characteristics that protect the host from pathogen colonization, fecal microbiota transplantation (FMT), a therapeutic

strategy using commensal bacteria, has been applied and has cured patients infected with *C. difficile* (8).

Recent reports have shown that fungi are also major components of the human microbiota (9, 10). In healthy people, fungi reside in the oral cavity and GI and urogenital tracts; however, these fungi infect immunocompromised hosts, including patients in intensive care units, those infected with human immunodeficiency virus, patients treated with anticancer drugs and antibiotics, and patients that underwent allograft transplantation (11). In addition to superficially infecting the skin or mucous membranes, fungi invade the bloodstream and disseminate to the internal organs, infecting the deep tissues and causing invasive candidiasis, a severe infectious disease (11). *Candida albicans* is highly prevalent and constant in human feces, suggesting that *C. albicans* is a commensal fungus in the GI tract (9, 10). However, *C. albicans* colonization in the GI tract is thought to trigger invasive candidiasis (11). Thus, the mechanism of *C. albicans* for steadily colonizing the GI tract must be defined. Previous studies have shown that multiple antibiotics affect the colonization of *C.*

albicans in the GI tract (12, 13). Although these reports suggest that commensal bacteria resist *C. albicans* colonization in the GI tract, detailed mechanisms and bacterial species responsible for this colonization resistance have yet to be established.

Antifungal drug-resistant *Candida* has recently emerged as a public health problem that can be life-threatening to humans (14). In addition to conventional antifungal drugs, alternative strategies are needed to prevent *Candida* infections. Here, we investigate the role of commensal bacteria in regulating *C. albicans* colonization in the GI tract. We apply novel sequencing and polymerase chain reaction approaches for the investigation of the microbiota present during treatment with various antibiotics. We also examine the commensal bacteria's potential as therapeutic targets for *C. albicans* infections.

Materials and Methods

Mice

Six- to ten-week-old C57BL/6 and BALB/c specific pathogen-free (SPF) mice were purchased from CLEA Japan (Tokyo, Japan). All animals were

maintained with a gamma ray-sterilized diet, sterile water, and autoclaved wood chip bedding in the experimental animal facility at the Medical Mycology Research Center. The Animal Care and Use Committee of the Chiba University approved the experiments.

Antibiotic treatment

Mice were given the following broad-spectrum antibiotics in their drinking water: ampicillin (1 g/L; Nacalai Tesque, Kyoto, Japan), vancomycin (0.5 g/L; Shionogi, Osaka, Japan), neomycin (1 g/L; Nacalai Tesque), metronidazole (1 g/L; Nacalai Tesque), penicillin (1.5 g/L; Meiji Seika Pharma, Tokyo, Japan), streptomycin (2 g/L; Nacalai Tesque), gentamycin (0.1 g/L; Nacalai Tesque), and colistin (1 g/L; MP Biomedicals, Santa Ana, CA, USA) (15-17). These antibiotic treatments were continued throughout the experiments. For the quantitative kinetic experiments, mice were orally administered 0.5 mg ampicillin 1 day before injection with *C. albicans*.

Fecal bacterial DNA isolation

The method for isolating fecal bacterial DNA was performed as previously reported with modifications (18). Mouse feces were collected, weighed,

added to tubes containing glass beads, and suspended in 500 μL of DNA extraction buffer (200 mM Tris-HCl, 20 mM EDTA, 200 mM NaCl; pH8.0), 210 μL of 10% sodium dodecyl sulfate, and 500 μL of buffer-saturated phenol. The mixtures were agitated vigorously for 60 sec using a FastPrep-24 5G homogenizer (MP Biomedicals). After centrifugation at 14,000 rpm for 5 min, 400 μL of supernatant were collected into a new tube. Subsequently, 500 μL of buffer-saturated phenol and 100 μL of DNA extraction buffer were added to the suspension, and the mixture was vortexed vigorously. After centrifugation at 14,000 rpm for 5 min, 400 μL of supernatant was collected into the new tube. Bacterial DNA was then obtained by isopropanol precipitation. Bacterial DNA was pelleted by centrifugation at 14,000 rpm for 5 min and washed with 70% ethanol. Finally, DNA was suspended in 100 μL of Tris-EDTA buffer.

Real-time qPCR Fecal DNA was used to analyze the bacterial groups quantitatively. Quantitative real-time PCR was performed using a LightCycler 96 system (Roche Diagnostics, Mannheim, Germany). 16S rRNA genes were amplified by bacterial group-specific primers. Primers

were UniF340 5’-ACTCCTACGGGAGGCAGCAGT-3’ and UniR514 5’-ATTACCGCGGCTGCTGGC-3’ for total bacteria, UniF338 5’-ACTCCTACGGGAGGCAGC-3’ and C.cocR491 5’-GCTTCTTAGTCAGGTACCGTCAT-3’ for Clostridiales, LabF362 5’-AGCAGTAGGGAATCTTCCA-3’ and LabR677 5’-CACCGCTACACATGGAG-3’ for Lactobacillaceae, BactF285 5’-GGTTCTGAGAGGAAGGTCCC-3’ and UniR338 5’-GCTGCCTCCCGTAGGAGT-3’ for Bacteroidales, and Uni515F 5’-GTGCCAGCAGCCGCGGTAA-3’ and Ent826R 5’-GCCTCAAGGGCACAACCTCCAAG-3’ for Enterobacteriaceae (19). All reactions were performed using the KAPA SYBR Fast qPCR kit (KAPA BIOSYSTEMS, Wilmington, MA, USA).

16S rRNA gene amplicon sequencing

Extracted fecal bacterial DNA was used to analyze the gut microbiota composition based on 16S rRNA genes. 16S rRNA gene amplicon sequencing was performed per the Illumina 16S Metagenomic Sequencing Library Preparation protocol with modifications. Briefly, the corresponding

sequences were amplified using primers specific for the V3-V4 region of the 16S rRNA genes. For the PCR reaction, KAPA HiFi DNA polymerase (KAPA BIOSYSTEMS) was used, and the PCR product was purified using AMPure XP Beads (Beckman Coulter Inc., Brea, CA, USA). A second PCR was performed using the purified PCR product as a template and the Nextera DNA Index kit (Illumina, San Diego, CA, USA). After purifying the second PCR products, the DNA concentration was measured using a Qubit 4 fluorometer (Thermo Fischer Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Combined samples at a concentration of 10 pM were mixed with the PhiX Control kit v3 (Illumina) and loaded onto a MiSeq Reagent Kit v2 500 cycle cartridge (Illumina) and the MiSeq sequencing platform (Illumina). The 250-bp paired-end reads were merged using PANDAseq (ver. 2.8) with the default parameters set to other than "-l 400" (20). The merged sequences were analyzed by the pick_open_reference_otus.py workflow of QIIME (ver. 1.9.1) using the default parameters (21). The samples' alpha- and beta-diversities were calculated using the core_diversity_analyses.py workflow of QIIME.

C. albicans injection and detection

C. albicans (IFM 60662, ATCC 18804) from the Medical Mycology Research Center of Chiba University's culture collection were routinely cultivated in potato dextrose broth at 37°C before oral administration. A total of 5×10^7 *C. albicans* were orally administered to mice treated with or without antibiotics. Feces were collected at the indicated time points, diluted, and plated on potato dextrose agar (PDA) containing chloramphenicol (0.05 g/L; Nacalai Tesque), penicillin (0.6 g/L), and streptomycin (0.167 g/L). Systemic tissues (spleen, kidney, and liver) were isolated from ampicillin-treated mice, homogenized using a TissueRuptor (QIAGEN, Hilden, Germany), and plated on PDA. Colonies were counted after incubation for 2 days. Feces were collected 7 days after *C. albicans* injection to detect fungal DNA in the feces. Primer sets specific for internal transcribed spacer (ITS) rDNA sequences, ITS1 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2 5'-GCTGCGTTCTTCATCGATGC-3', were used (10). ITS sequences were amplified using EmeraldAmp PCR Master Mix (TaKaRa, Shiga, Japan).

Fecal microbiota transplantation (FMT) and antifungal drug treatment

Mice treated with ampicillin were orally administered *C. albicans*. After we confirmed *C. albicans* colonization in feces of these mice by plating fecal diluents on PDA containing chloramphenicol, penicillin, and streptomycin, these mice were treated with fluconazole (0.5 g/L; LKT Laboratories), 5-fluorocytosine (2 g/L; TCI Chemicals), and amphotericin B (0.1 g/L; Nacalai Tesque) mixed with drinking water containing ampicillin (22, 23). For the FMT, drinking water containing ampicillin was changed to sterile water 1 day before the FMT. Donor feces were obtained from SPF C57BL/6 mice and subjected to FMT. After homogenizing 1–2 fecal pellets from donor mice in 1 mL of water, the supernatants were orally administered to one recipient mouse colonized with *C. albicans*. For the control mice without FMT treatment, drinking water containing ampicillin was changed to sterile water at the same time as FMT mice. Feces were then collected from these recipient mice at the indicated time points, and the *C. albicans* was counted.

Statistical analysis

Statistical analyses were conducted using Microsoft Excel and GraphPad Prism software (San Diego, CA, USA). Results were compared using two-tailed Student's *t*-tests and Fisher's exact test. Data were plotted as means ± standard deviation (s.d.).

Results

Experimental mice were resistant to *C. albicans* colonization in the gut

Based on a previous report demonstrating that commensal fungi endogenously colonized experimental mouse guts (10), we first investigated whether the mice had commensal fungi in their GI tracts by culture- and DNA-based approaches. In contrast to the previous report, fungi were undetected in the feces of experimental mice maintained at our facility (Fig. 1a and b). Therefore, we attempted to colonize *C. albicans*, a commensal and pathogenic fungus, through oral administration to fungus-free, wild-type C57BL/6 mice. We detected *C. albicans* in the feces immediately after oral administration; however, the number of fungi decreased gradually, and

eventually, *C. albicans* were not detected in these mice (Fig. 1c). After detecting no *C. albicans* in the feces, we further confirmed that *C. albicans* were not detected in the luminal contents of the GI tract (e.g., stomach, small intestine, cecum, and colon) (Fig. 1d), indicating that *C. albicans* had been completely cleared from the GI tract. We also confirmed that BALB/c mice were resistant to *C. albicans* colonization in the GI tract (data not shown). These data indicate that wild-type mice are resistant to *C. albicans* colonization in the GI tract.

β-lactam antibiotic treatment allowed *C. albicans* to colonize in the gut

We next examined why the wild-type C57BL/6 mice were resistant to *C. albicans* colonization in the gut. Because several environmental factors in murine guts may affect *Candida* colonization, we hypothesized that commensal microbes in the murine GI tract might protect against *Candida* colonization. To determine whether commensal microbes impacted *C. albicans* colonization, we treated SPF mice with multiple antibiotics. After antibiotic treatment, we collected feces from these mice and plated them on PDA for detection of *C. albicans*. As a result, significant numbers of *C.*

albicans colonies were detected in the feces of mice treated with ampicillin or penicillin, both of which are β-lactam antibiotics (Fig. 2a). Conversely, mice treated with the other antibiotics (vancomycin, metronidazole, neomycin, colistin, streptomycin, and gentamycin) had nearly the same number of *C. albicans* in their feces as the wild-type mice (Fig. 2a). We next addressed whether *C. albicans* would be detected in the luminal contents from the GI tracts of ampicillin-treated mice. In ampicillin-treated mice, we detected significant and comparable numbers of *C. albicans* in the luminal contents from the stomach to the colon, indicating that *C. albicans* successfully colonized the GI tracts of these mice (Fig. 2b). These *C. albicans* levels were maintained for at least 3 months after administration in the ampicillin-treated mice (data not shown). These data indicate that *C. albicans* colonized the GI tracts of the ampicillin-treated mice. We also confirmed that *C. albicans* colonized the GI tracts of ampicillin-treated BALB/c mice as with the C57BL/6 mice (Fig. 2c, d).

Commensal microbiota composition was changed in ampicillin-treated mice

We next examined the bacterial compositions of ampicillin-treated and -untreated mice. We isolated feces from these mice and analyzed the bacterial compositions based on 16S rRNA gene amplicon sequencing. Gut microbiota compositions and bacterial diversity changed in the ampicillin-treated mice (Fig. 3a, b, c; Supplementary Fig. 1, 2; and Supplementary Table 1). We also examined the bacterial composition of other antibiotic-treated mice (Supplementary Fig. 3, 4, 5; and Supplementary Table 1). Each antibiotic induced specific dysbiosis of fecal microbiota. We further investigated the quantity of major bacterial genera in the feces of ampicillin-treated mice by qPCR. Quantitative analysis showed that the numbers of Lactobacillaceae, Bacteroidales, Clostridiales, and Enterobacteriaceae were markedly reduced in the ampicillin-treated mice (Fig. 3d).

Commensal bacteria and *C. albicans* numbers were inversely correlated in the gut

In addition to specific bacterial groups, total commensal bacterial numbers were dramatically reduced in the ampicillin-treated mice (Fig. 4a), indicating that ampicillin treatment effectively induced dysbiosis in murine GI tracts. We next investigated the quantitative kinetics of commensal bacteria and *C. albicans* in the gut after ampicillin treatment. Single oral administration of ampicillin drastically reduced commensal bacterial numbers, but the effect was transient. Luminal bacterial numbers recovered 4 days after injecting ampicillin (Fig. 4b). To examine the relationship between *C. albicans* numbers and commensal bacteria, we orally injected ampicillin into mice, then administered *C. albicans*. *C. albicans* numbers increased greatly immediately after the injection, and were maintained for 4 days. Subsequently, the *C. albicans* numbers were reduced, and the colonization levels were comparable to those of the ampicillin-untreated mice at 7 days after *C. albicans* injection (Fig. 4c). These data suggest that commensal

bacterial colonization is inversely correlated with *C. albicans* in the GI tract, and commensal bacteria likely inhibit *C. albicans* colonization.

FMT prevented *C. albicans* colonization in murine GI tracts

We next investigated whether commensal bacteria have therapeutic potential against *C. albicans* colonization in the GI tract. FMT is a commensal bacteria-based therapeutic approach for treating pathogenic bacterial infections in the gut (8). Therefore, we attempted FMT in *C. albicans*-colonized mice. After the FMT, *C. albicans* numbers in the feces were drastically and immediately reduced compared with mice without the FMT (Fig. 4d). We confirmed that total numbers of commensal bacteria were restored 4 days after FMT (Fig. 4e). To compare the effects of FMT and conventional antifungal drugs against *C. albicans* colonization in the GI tract, we treated *C. albicans*-colonized mice with the antifungal reagents, fluconazole, 5-Fluorocytosine, and amphotericin B. Amphotericin B effectively depleted *C. albicans* in the gut, while fluconazole and 5-Fluorocytosine did not (Fig. 4f). This indicates that FMT effectively prevented *C. albicans* from colonizing the murine GI tract.

Discussion

In this study, we showed that murine GI microbiota contain potential therapeutic targets for *C. albicans* colonization in the GI tract. Wild-type experimental mice were resistant to *C. albicans* colonization in the GI tract, while *C. albicans* successfully colonized the GI tracts of mice treated with antibiotics such as ampicillin, inducing dysbiosis in the gut microbiota. FMT prevented *C. albicans* from colonizing the GI tract. These data highlight that murine commensal bacteria function as barriers and play roles in protecting the host from *C. albicans* colonization.

The mechanism by which commensal bacteria inhibit *C. albicans* colonization remains unclear. Several reports have shown that the protective effects of commensal bacteria against pathogenic bacteria are mediated by differentiation and activation of host innate and acquired immune cells in the gut (18, 24). Commensal bacteria, especially segmented filamentous bacteria (SFB), induce Th17 cells, which protect against *Candida* infection by producing IL-17A and subsequently activating neutrophils (25). However, SFB and Th17 cells were unlikely to affect *C.*

albicans colonization in the gut, because vancomycin treatment—which effectively eliminates SFB and Th17 cells—did not allow *C. albicans* colonization in the gut (24, 26) (Fig. 2a). Further studies are necessary to determine whether a commensal bacterial-immune cell-axis affects *C. albicans* colonization in the gut.

Another possible mechanism is that commensal bacteria directly protect against *C. albicans* colonization in the GI tract. Commensal bacteria may compete with *C. albicans* for oxygen, pH, and nutrients in this environmental niche. Commensal bacteria produce various metabolites in the gut lumen, which may impact *C. albicans* colonization and proliferation.

Although we investigated the microbiota in mice treated with multiple antibiotics in this study, the specific bacterial species that protected against colonization of *C. albicans* in the GI tract are still unknown. *C. albicans* colonized the GI tract of mice treated with ampicillin and penicillin, both of which dramatically reduced the number of total and specific commensal bacteria (Fig. 3d, 4a; data not shown). In contrast, mice treated with other antibiotics, inducing dysbiosis (Supplementary Fig. 3, 4, 5; and

Supplementary Table 1), were resistant to *C. albicans* colonization (Fig. 2a), suggesting that several bacterial species might be involved in the protection of *C. albicans* colonization. From 16s rRNA sequence analysis, we also found that vancomycin-treated mice predominantly harbored Lactobacillaceae (Supplementary Fig. 6; and Supplementary Table 1). Taken together with the data that vancomycin-treated mice were resistant to *C. albicans* colonization, these data suggest that Lactobacillaceae are one of the types of commensal bacteria responsible for protection against *C. albicans* colonization in vivo. Indeed, a previous report showed that *Lactobacillus* inhibited the expression of virulence factors of *C. albicans* in vitro (27). The specific kind of bacteria and the detailed mechanism of *Lactobacillus* protecting against *C. albicans* colonization in the GI tract require investigation in future studies.

Because commensal bacteria affect *C. albicans* colonization, the intestinal environment that influences the commensal population may be critical for *C. albicans* colonization in the GI tract. The sources that determine *C. albicans* colonization, including environmental factors such as

food, water, and bedding, and how these factors affect the commensal bacterial composition and subsequent *C. albicans* colonization in the GI tract remain unclear. Indeed, several fungi, including *Saccharomyces*, *Trichosporon*, and *Candida* species, colonized endogenously, even in SPF mice bred at another experimental facility (10). Conversely, other groups have reported fungus-free SPF mice (28).

C. albicans colonization of the GI tract is considered a trigger for invasive candidiasis (11). However, *C. albicans* were minimally detectable in the systemic tissues, even in ampicillin-treated *C. albicans*-colonized mice (Supplementary Fig. 7 and Supplementary Table 2). Conversely, many *C. albicans* were detected in the systemic tissues after intravenous injection, indicating that *C. albicans* can reach systemic compartments via the bloodstream (Supplementary Fig. 3 and Supplementary Table 2). These data suggest that the host's surface barrier system, particularly the gut epithelial cells and immune system, may prevent *C. albicans* from infiltrating the blood vessels, and commensal bacteria may be important to the surface barriers preventing *C. albicans* infections. In addition to life-threatening

systemic fungal infections in immunocompromised patients, fungal colonization of the GI tract exacerbates allergic airway inflammation (22). Thus, controlling fungal colonization may represent a strategy for regulating infections as well as inflammation and allergies. Targeted modification of commensal bacteria may provide novel approaches for controlling these diseases.

Acknowledgments

We thank the members of the Division of Molecular Immunology for their discussion and suggestion. This work was supported in part by grants from the following sources: a Grant-in-Aid for Young Scientists (A) grant (16H06229 to Y.G.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; the Japan Agency for Medical Research and Development (AMED) PRIME (JP18gm6010005 to Y.G.); the Practical Research Project for Allergic Diseases and Immunology (JP18ek0410029 to Y.G.); the Takeda Science Foundation (to Y.G.); the Yakult-Bioscience Foundation (to Y.G.); the Kato Memorial Bioscience Foundation (to Y.G.); the Joint Usage/Research Program of Medical Mycology Research Center,

Chiba University (17-25 to Y.G., N.K.); and the Joint Research Project of the Institute of Medical Science, the University of Tokyo (2016-3005, 2018-3047 to Y.G.); and Leading Research Promotion Program of Chiba University (to H.T., S.S., M.Y., Y.G.). We thank Katrina Krogh, MD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Disclosure

All authors of the manuscript declare no potential conflicts of interest.

References

1. Gilbert J.A., Blaser M.J., Caporaso J.G., Jansson J.K., Lynch S.V., Knight R. (2018) Current understanding of the human microbiome. *Nat Med* **24**: 392-400.

2. Blander J.M., Longman R.S., Iliev I.D., Sonnenberg G.F., Artis D. (2017) Regulation of inflammation by microbiota interactions with the host. *Nat Immunol* **18**: 851-60.

3. Buffie C.G., Pamer E.G. (2013) Microbiota-mediated colonization resistance against intestinal pathogens. *Nat Rev Immunol* **13**: 790-801.

4. Becattini S., Littmann E.R., Carter R.A., Kim S.G., Morjaria S.M., Ling L., Gyaltshen Y., Fontana E., Taur Y., Leiner I.M., Pamer E.G.. (2017) Commensal microbes provide first line defense against *Listeria monocytogenes* infection. *J Exp Med* **214**: 1973-89.

5. Caballero S., Kim S., Carter R.A., Leiner I.M., Susac B., Miller L., Kim G.J., Ling L., Pamer E.G. (2017) Cooperating Commensals Restore Colonization Resistance to Vancomycin-Resistant *Enterococcus faecium*. *Cell Host Microbe* **21**: 592-602 e4.

6. Rivera-Chavez F., Zhang L.F., Faber F., Lopez C.A., Byndloss M.X., Olsan E.E., Xu G., Velazquez E.M., Lebrilla C.B., Winter S.E., Baumler A.J. (2016) Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell Host Microbe* **19**: 443-54.

7. Kamada N., Kim Y.G., Sham H.P., Vallance B.A., Puente J.L., Martens E.C., Nunez G. (2012) Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. *Science* **336**: 1325-9.

8. van Nood E., Vrieze A., Nieuwdorp M., Fuentes S., Zoetendal E.G., de Vos W.M., Visser C.E., Kuijper E.J., Bartelsman J.F., Tijssen J.G., Speelman P., Dijkgraaf M.G., Keller J.J. (2013) Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* **368**: 407-15.

9. Motooka D., Fujimoto K., Tanaka R., Yaguchi T., Gotoh K., Maeda Y., Furuta Y., Kurakawa T., Goto N., Yasunaga T., Narazaki M., Kumanogoh A., Horii T., Iida T., Takeda K., Nakamura S. (2017) Fungal ITS1 Deep-Sequencing Strategies to Reconstruct the Composition of a 26-Species Community and Evaluation of the Gut Mycobiota of Healthy Japanese Individuals. *Front Microbiol* **8**: 238.

10. Iliev I.D., Funari V.A., Taylor K.D., Nguyen Q., Reyes C.N., Strom S.P., Brown J., Becker C.A., Fleshner P.R., Dubinsky M., Rotter J.I., Wang H.L., McGovern D.P., Brown G.D., Underhill D.M. (2012) Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science* **336**: 1314-7.

11. Kullberg B.J., Arendrup M.C. (2015) Invasive Candidiasis. *N Engl J Med* **373**: 1445-56.

12. Kennedy M.J., Johnson A.M., Volz P.A., Neely A.N., Yancey R.J. (1992) Mechanisms of association of *Candida albicans* with intestinal mucosa. *J Med Microbiol*. **36**: 428-36.

13. Kennedy M.J., Volz P.A. (1985) Ecology of *Candida albicans* gut colonization: inhibition of Candida adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. *Infect Immun*. **49**:654-63.

14. Fisher M.C., Hawkins N.J., Sanglard D., Gurr S.J. (2018) Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science* **360**: 739-42.

15. Shankar J., Solis N.V., Mounaud S., Szpakowski S., Liu H., Losada L., Nierman W.C., Filler S.G. (2015) Using Bayesian modelling to investigate factors governing antibiotic-induced *Candida albicans* colonization of the GI tract. *Sci Rep* **5**: 8131.

16. Rakoff-Nahoum S., Paglino J., Eslami-Varzaneh F., Edberg S., Medzhitov R. (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**: 229-41.

17. Viaud S., Saccheri F., Mignot G., Yamazaki T., Daillere R., Hannani D., Enot D.P., Pfirschke C., Engblom C., Pittet M.J., Schlitzer A., Ginhoux F., Apetoh L., Chachaty E., Woerther P.L., Eberl G., Berard M., Ecobichon C., Clermont D., Bizet C., Gaboriau-Routhiau V., Cerf-Bensussan N., Opolon P., Yessaad N., Vivier E., Ryffel B., Elson C.O., Dore J., Kroemer G., Lepage P., Boneca I.G., Ghiringhelli F., Zitvogel L. (2013) The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **342**: 971-6.

18. Goto Y., Obata T., Kunisawa J., Sato S., Ivanov I.I., Lamichhane A., Takeyama N., Kamioka M., Sakamoto M., Matsuki T., Setoyama H., Imaoka A., Uematsu S., Akira S., Domino S.E., Kulig P., Becher B., Renauld J.C., Sasakawa C., Umesaki Y., Benno Y., Kiyono H. (2014) Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science* **345**: 1254009.

19. Bouskra D., Brezillon C., Berard M., Werts C., Varona R., Boneca I.G., Eberl G. (2008) Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* **456**: 507-10.

20. Masella A.P., Bartram A.K., Truszkowski J.M., Brown D.G., Neufeld J.D. (2012) PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics* **13**: 31.

21. Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Pena A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335-6.

22. Kim Y.G., Udayanga K.G., Totsuka N., Weinberg J.B., Nunez G., Shibuya A. (2014) Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE(2). *Cell Host Microbe* **15**: 95-102.

23. Wheeler M.L., Limon J.J., Bar A.S., Leal C.A., Gargus M., Tang J., Brown J., Funari V.A., Wang H.L., Crother T.R., Arditi M., Underhill D.M., Iliev I.D. (2016) Immunological Consequences of Intestinal Fungal Dysbiosis. *Cell Host Microbe* **19**: 865-73.

24. Ivanov I.I., Atarashi K., Manel N., Brodie E.L., Shima T., Karaoz U., Wei D., Goldfarb K.C., Santee C.A., Lynch S.V., Tanoue T., Imaoka A., Itoh K., Takeda K., Umesaki Y., Honda K., Littman D.R. (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**: 485-98.

25. Saijo S., Ikeda S., Yamabe K., Kakuta S., Ishigame H., Akitsu A., Fujikado N., Kusaka T., Kubo S., Chung S.H., Komatsu R., Miura N., Adachi Y., Ohno N., Shibuya K., Yamamoto N., Kawakami K., Yamasaki S., Saito T., Akira S., Iwakura Y. (2010) Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against *Candida albicans*. *Immunity* **32**: 681-91.

26. Ivanov I.I., Frutos Rde L., Manel N., Yoshinaga K., Rifkin D.B., Sartor R.B., Finlay B.B., Littman D.R. (2008) Specific microbiota direct the

differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* **4**: 337-49.

27. Ribeiro FC, de Barros PP, Rossoni RD, Junqueira JC, Jorge AO. (2017) *Lactobacillus rhamnosus* inhibits *Candida albicans* virulence factors in vitro and modulates immune system in *Galleria mellonella*. *J Appl Microbiol*. **122**:201-11.

28. Tang C., Kamiya T., Liu Y., Kadoki M., Kakuta S., Oshima K., Hattori M., Takeshita K., Kanai T., Saijo S., Ohno N., Iwakura Y. (2015) Inhibition of Dectin-1 Signaling Ameliorates Colitis by Inducing Lactobacillus-Mediated Regulatory T Cell Expansion in the Intestine. *Cell Host Microbe* **18**: 183-97.

Figures

Figure 1. **Experimental mice are resistant to *C. albicans* colonization in the GI tract. (a)** Culture-based quantification of fungi of feces isolated from wild-type C57BL/6 mice and mice injected with *C. albicans* (n = 4). Feces were collected 0 and 7 days after orally injecting *C. albicans*. **(b)** Fungus-specific DNA was detected in feces isolated from mice injected with *C. albicans* but not in untreated C57BL/6 mice. M: marker, WT: wild-type, CA: *C. albicans*. **(c)** *C. albicans* numbers of feces isolated from C57BL/6 mice injected with *C. albicans* were counted at the indicated time points (n = 10). Error bars: s.d. **(d)** *C. albicans* numbers of GI contents of mice 45 days after orally injecting *C. albicans* (n = 3). ND: not detected, SI: small intestine. Data represent two to three independent experiments.

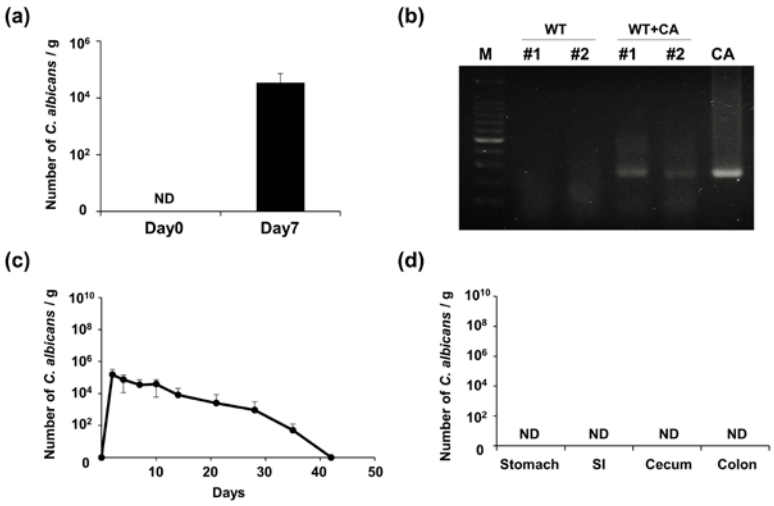

Figure 2. ***C. albicans*** **colonized ampicillin- and penicillin-treated mice (a)** C57BL/6 mice were treated with or without indicated antibiotics. *C. albicans* numbers of feces were counted 28 days after oral injection (n = 5). WT: wild-type, Amp: ampicillin, Vnc: vancomycin, Met: metronidazole, Neo: neomycin, Col: colistin, Pen: penicillin, Str: streptomycin, Gen: gentamycin. **(b)** *C. albicans* numbers of GI contents of C57BL/6 mice treated with ampicillin (n = 5). **(c)** *C. albicans* numbers of feces were counted 28 days after oral injection into BALB/c mice treated with or without ampicillin (n = 6). ND: not detected. **(d)** *C. albicans* numbers of GI contents from ampicillin-treated BALB/c mice (n = 6). SI: small intestine. Error bars, s.d. $^{***}P < 0.001$, $^{**}P < 0.01$ using Student's *t*-test. Data are representative of two independent experiments.

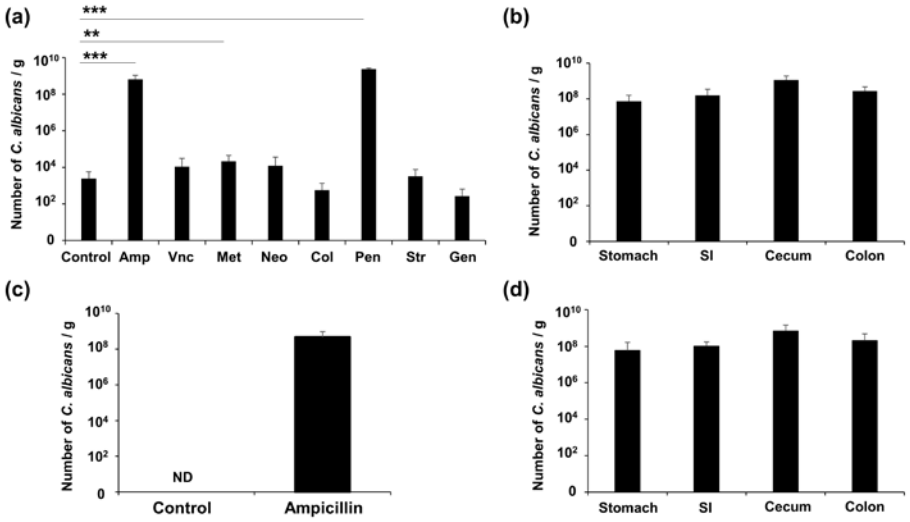

Figure 3. **Dysbiosis was induced in ampicillin-treated mice (a), (b), (c)** Based on 16S rRNA genes in feces from ampicillin-treated and -untreated mice, PCoA Plots of unweighted UniFrac distances (a), alpha refraction curve (b), and ratios of bacterial phyla (c) are represented. **(d)** Quantitative real-time qPCR analysis of bacterial groups in feces isolated from mice treated or untreated with ampicillin (n = 5). Error bars, s.d. $^{**}P <$ 0.01 using Student's *t*-test. Data are representative of three independent experiments.

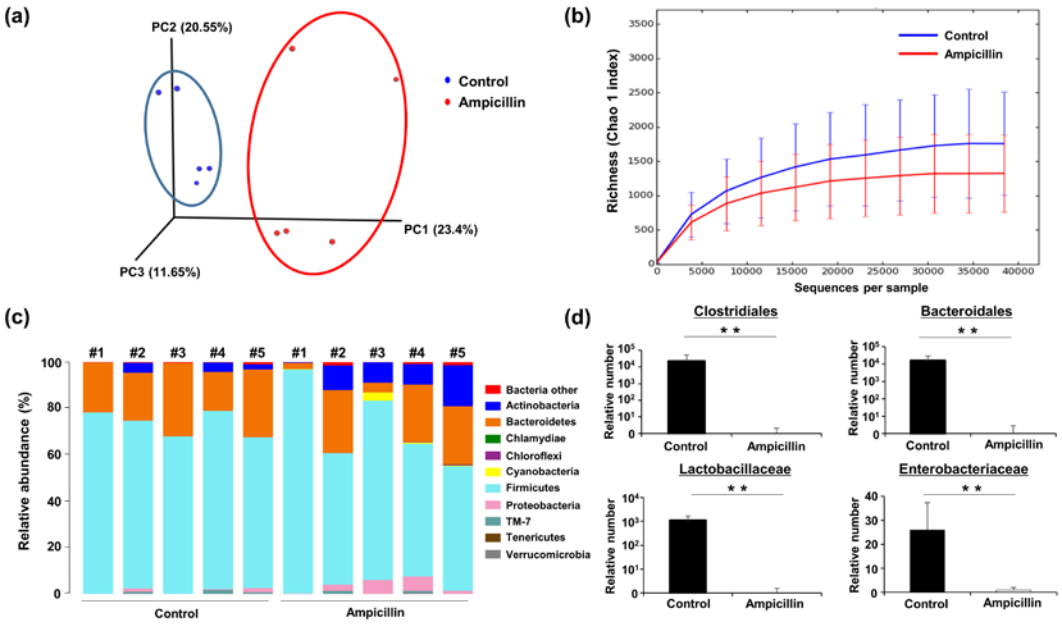

Figure 4. **FMT prevented *C. albicans* from entering the GI tract (a)** Real-time qPCR analysis of total bacteria in feces isolated from mice treated or untreated with ampicillin (n = 5). **(b)** Relative numbers of total fecal bacteria were analyzed using real-time qPCR before and after injecting ampicillin (n = 5). **(c)** *C. albicans* counts of feces from ampicillin-treated or -untreated mice (n = 5). **(d)** *C. albicans*-colonized mice received water or FMT after cessation of ampicillin treatment. *C. albicans* counts of feces from mice at 0, 7, or 28 days after administration (n = 3). FMT: fecal microbiota transplantation. **(e)** Relative numbers of total fecal bacteria from mice transferred with fecal microbiota were examined at the indicated time points (n = 3). **(f)** *C. albicans*-colonized mice treated with or without antifungal drugs. *C. albicans* counts of feces from mice at 0, 7, or 28 days after administration (n = 3). Cont: control, Fluc: fluconazole, 5-FC: 5-fluorocytosine, Amph: amphotericin B, ND: not detected. Error bars, s.d. $^{*}P < 0.05$, $^{**}P < 0.01$ using Student's *t*-test. Data are representative of two independent experiments.