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A member of the gut mycobiota modulates host purine metabolism exacerbating colitis in mice

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Abstract

The commensal microbiota has an important impact on host health, which is only beginning to be elucidated. Despite the presence of fungal, archaeal, and viral members, most studies have focused solely on the bacterial microbiota. Antibodies against the yeast *Saccharomyces cerevisiae* are found in some patients with Crohn's disease (CD), suggesting that the mycobiota may contribute to disease severity. We report that *S. cerevisiae* exacerbated intestinal disease in a mouse model of colitis and increased gut barrier permeability. Transcriptome analysis of colon tissue from germ-free mice inoculated with *S. cerevisiae* or another fungus, *Rhodotorula aurantiaca*, revealed that *S. cerevisiae* colonization affected the intestinal barrier and host metabolism. A fecal metabolomics screen of germ-free animals demonstrated that *S. cerevisiae* colonization enhanced host purine metabolism, leading to an increase in uric acid production. Treatment with uric acid alone worsened disease and increased gut permeability. Allopurinol, a clinical drug used to reduce uric acid, ameliorated colitis induced by *S. cerevisiae* in mice. In addition, we found a positive correlation between elevated uric acid and anti-yeast antibodies in human sera. Thus, yeast in the gut may be able to potentiate metabolite production that negatively affects the course of inflammatory bowel disease.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal (GI) tract that includes Crohn's disease (CD) and ulcerative colitis (UC). Unrestricted inflammation at intestinal sites leads to malabsorption of nutrients, severe abdominal pain, and increased chance of developing colorectal cancers (1). Although current therapies such as immunosuppression, intestinal resections, and antibiotics ameliorate symptoms, there is no cure for this disease. Emerging evidence supports a role for the microbiota in the modulation of IBD. Multiple studies have demonstrated that both community membership and abundance within the microbiota in patients with IBD are different from those of healthy individuals (2, 3). Moreover, probiotic treatment during experimental colitis can alleviate disease (4–6). The human microbiota is a complex ecosystem composed of bacteria, fungi, archaea, and viruses; however, most studies to date have focused solely on bacterial members and their involvement in IBD (2, 3).

Fungi comprise a diverse kingdom of eukaryotic organisms composed of yeasts, molds, and mushrooms. Several yeast species have been identified in the GI tract, where they are estimated to make up 0.1% of the intestinal microbiota (7). Common members include *Candida*, *Saccharomyces*, *Aspergillus*, *Cryptococcus*, and *Rhodotorula*, which indicate the existence of a diverse resident fungal community (8–14). Fungi are large, complex organisms known to act opportunistically during immune-mediated and antibiotic therapy (15–17). There are several clinical and experimental indications that yeast might influence intestinal inflammation. The first was the discovery of elevated anti–*Saccharomyces cerevisiae* antibodies (ASCAs) in the serum of CD patients (16, 18), which suggests that an aberrant immune response to yeast might be involved in IBD progression. Patients suffering from IBD are often prescribed antibiotics to kill bacteria thought to drive the chronic inflammatory response (19–22). Because yeasts are not targets of commonly used antibiotics, a well-documented side effect of extended antibiotic use is the overgrowth of fungal species (23–25). Moreover, yeasts are a common component of many foods, which might provide daily exposure to these organisms. Polymorphisms in genes such as *CARD9* and *CLEC7A* (dectin-1) that function in fungal recognition by the host have been described in patients who suffer from increased fungal infections (26–28). Deletion of these genes in mice leads to worsened intestinal disease (29, 30). Thus, there is evidence in both mouse models and humans that supports a role for fungi in intestinal disease, yet the mechanisms by which this occurs remain poorly defined. Here, we investigate a role for *S. cerevisiae* in the pathogenesis of colitis.

The study of resident fungal communities is still in its infancy; however, there are a handful of papers that have surveyed fungal populations in patients with IBD (8, 12, 31). Several of these studies have reported a loss of bacterial diversity with a concomitant increase in fungal variety and load during IBD (8, 32). Similarly, the most recent and largest patient survey of fungal communities identified a higher fungal-to-bacterial ratio in patients with IBD (12), further supporting the hypothesis that increases in fungal load might be associated with disease. Several species of fungi have now been identified from both colitogenic and healthy individuals, including *S. cerevisiae*, *Candida albicans*, *Penicillium italicum*, *Rhodotorula aurantiaca*, and *Malassezia sympodialis* (8, 12, 14). These species belong to two fungal

phyla, the Ascomycota and Basidomycota, which dominate the intestinal fungal community. To investigate how different yeast species identified in the human GI tract influence intestinal disease, we chose one organism from each of these fungal phyla that are readily available, easily cultured, and present in individuals with IBD—*S. cerevisiae*, a member of the Ascomycota, and *R. aurantiaca*, a member of the Basidomycota. *S. cerevisiae* represents one of the most highly abundant and most commonly detected fungal members in the human GI tract and is also found in food and the environment (12, 14), making it highly relevant to human biology. Moreover, we studied a wild, prototrophic, diploid strain that represents one of the more common strains found in the human gut. *R. aurantiaca* is commonly found within the environment and is a member of a genus that is beginning to emerge as a new class of opportunistic pathogens (33).

RESULTS

S. cerevisiae, but not *R. aurantiaca*, exacerbates experimental colitis

C57Bl/6 mice were orally gavaged with either *S. cerevisiae* or *R. aurantiaca* and subsequently challenged with two different reagents, 2,4,6-trinitrobenzenesulfonic acid (TNBS) or dextran sulfate sodium (DSS), which yielded two chemical models of colitis (34). Because animals are resistant to stable yeast colonization without prior treatment with antibiotics (35), a phenomenon termed colonization resistance, animals were orally gavaged with 10^6 colony-forming units (CFU) every day for 7 days. This treatment mimics consumption of food enriched with yeast products. Animals treated with *S. cerevisiae* consistently developed worsened disease, as measured by hematoxylin and eosin (H&E) staining of paraffin-embedded sections of the colon. Animals treated with *S. cerevisiae* had enhanced epithelial damage, greater crypt loss, and increased cellular infiltration when compared to either vehicle- or *R. aurantiaca*–treated animals (Fig. 1, A to D). This observation suggests that the presence of *S. cerevisiae*, but not *R. aurantiaca*, exacerbates intestinal disease. To determine whether live *S. cerevisiae* was required to worsen colitis or whether a host reaction to fungal cell wall constituents was the cause, we fed either live or heat-killed *S. cerevisiae* to C57Bl/6 mice and subsequently induced colitis with DSS. Whereas live *S. cerevisiae* induced aggravated colitis, heat-killed *S. cerevisiae* failed to elicit heightened disease. Colonic shortening occurs during the course of disease and is used as an indicator of disease severity. Animals treated with live *S. cerevisiae* had the shortest colons, whereas control and heat-killed groups had similar colon lengths (Fig. 1E). Moreover, heat-killed *S. cerevisiae* resulted in histology scores similar to those of control animals (Fig. 1F). Therefore, worsened disease was not simply a reflection of a host response to fungal components; rather, it required a metabolically active organism.

Inflammatory responses are known to play a critical role during colitis. Therefore, we analyzed the mesenteric lymph nodes (MLNs) for inflammatory interferon-γ (IFN-γ)– and interleukin-17 (IL-17)–producing T helper 1 (T_H1) and T_H17 cells, respectively. We did not detect significant differences in inflammatory T cell subsets in *S. cerevisiae*– or *R. aurantiaca*–treated animals compared to controls (fig. S1, A to C). In addition, we did not detect transcriptional changes in the colon of commonly elevated inflammatory cytokines such as IL-6 or tumor necrosis factor–α (fig. S1, D and E). These data imply that worsened

disease in *S. cerevisiae*–treated mice was not the result of a greater inflammatory response and suggest that exposure to *S. cerevisiae*, but not *R. aurantiaca*, had the potential to exacerbate the course of intestinal disease.

The prominent commensal fungus *S. cerevisiae* diminishes intestinal barrier function

We next sought to better understand how commensal yeast could have such disparate outcomes during the course of intestinal inflammation using an unbiased approach. To this end, we performed transcriptome analysis on colonic RNA isolated from either *S. cerevisiae* or *R. aurantiaca* monoassociated germ-free animals. Whereas *S. cerevisiae* stably colonized germ-free animals (fig. S2A), *R. aurantiaca* could not be detected in feces of germ-free animals after a single oral gavage of 10^6 CFU. Because these organisms are commonly found in the environment and food and to ensure equal exposure of animals to yeast, we placed *S. cerevisiae* and *R. aurantiaca* in the drinking water of germ-free animals. Yeast maintained equal viability in drinking water.

The density of yeast is highest in the colon; thus, we focused our initial analysis at this site (29). Colonization with yeast did not induce a host transcriptional response as large as that reported for some bacterial strains (36, 37). Monoassociation with either yeast strains led to maximum gene expression changes of just 10-fold. Although the host gene expression profile induced by either yeast species was very similar, several genes were differentially regulated between the two strains (fig. S2, B and C). KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis of the microarray data revealed that yeast colonization most significantly influenced host metabolic pathways and genes involved in maintaining the intestinal barrier (fig. S2, B to C). With the exception of changes in antimicrobial peptides, there were few changes in immune-mediated pathways identified by this analysis. A secondary analysis using Ingenuity software also confirmed that intestinal barrier pathways were highly influenced in response to fungal colonization. Some of the most significant changes in gene expression between the two species were for genes involved in controlling bacterial populations and maintaining intestinal epithelial integrity (Fig. 2, A and B). Many antimicrobial peptides and genes involved in cellular tight junction formation were more highly up-regulated by the presence of *R. aurantiaca* compared to *S. cerevisiae*, suggesting that the presence of *S. cerevisiae* supported epithelial barrier integrity less strongly (Fig. 2, A and B). Consistent with these observations, quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis from colonic tissue of monoassociated animals revealed that multiple genes involved in forming epithelial junctions in the intestine were more highly up-regulated by *R. aurantiaca*, including claudin-2, Zonula occludens (ZO)-2, and claudin-7 (Fig. 2, C to F, and fig. S2D).

Given these results, we sought to functionally test how fungal organisms influenced intestinal barrier function. Animals received a daily oral gavage of *S. cerevisiae* or *R. aurantiaca* and were placed on 2.5% DSS in drinking water. Seven days after disease induction, animals were orally gavaged with fluorescein isothiocyanate (FITC)–dextran, and blood was collected 4 hours later to determine the level of intestinal leakage of FITC-dextran in the systemic compartment. Animals treated with *R. aurantiaca* had similar levels of intestinal penetration when compared to control animals (Fig. 2G). However, animals

treated with *S. cerevisiae* demonstrated a significant increase in the amount of FITC-dextran detected in their blood (Fig. 2G). These data indicate that the presence of *S. cerevisiae* led to greater intestinal leakage.

To determine whether *S. cerevisiae* could directly influence permeability of the intestinal epithelium, we developed an in vitro intestinal permeability assay (fig. S2E). Yeast strains were incubated with a confluent layer of MODE-K cells, a cell line derived from mouse small intestine. Penetration of FITC-dextran into the bottom of a Transwell was used as a measure of permeability. Intestinal epithelia cocultured with *S. cerevisiae* showed significantly enhanced, albeit modest, permeability when compared to cells incubated with medium alone or *R. aurantiaca*–treated cells (fig. S2F), suggesting that *S. cerevisiae* can directly act on intestinal epithelia to influence permeability. Maintaining intestinal epithelial barrier integrity has been shown to be a critical component to prevent intestinal disease in mouse models and humans (38, 39). Thus, our data suggest that a common member of the intestinal and environmental mycobiota, *S. cerevisiae*, can worsen intestinal disease by enhancing gut epithelial leakage.

S. cerevisiae enhances degradation of purine produced by intestinal epithelia

Other major gene expression pathways influenced by yeast colonization in germ-free mice were host metabolic pathways (fig. S2, B and C). The microbiota contributes important functions to their mammalian hosts, including colonization resistance and induction of immune responses; however, one of the most well-known roles for the microbiota is the breakdown of dietary components for use by host metabolism (40). How commensal fungi influence metabolism is largely unknown. Therefore, we generated a fecal metabolomic profile using gas chromatography–mass spectrometry (GC-MS) from SPF, germ-free, and *S. cerevisiae* or *R. aurantiaca* monoassociated animals to determine how yeast colonization might influence metabolism. SPF animals are markedly different from the other three groups, such that yeast monoassociated animals cluster more closely with germ-free animals (Fig. 3A). Despite the similarity between the metabolomic profile of yeast monocolonized and germ-free animals, there were 20 metabolites that were influenced by yeast colonization (Fig. 3B). Many of the metabolites found to be elevated in germ-free mice were sugars such as ribitol and mannitol (fig. S3, A and B). These sugars were depleted upon monoassociation with either yeast species and likely indicated the use of these sugars by colonizing yeast.

Of the few metabolites that differed between *S. cerevisiae* and *R. aurantiaca*, five were part of the purine degradation pathway (Fig. 3C and fig. S3C) and included adenosine, adenine, xanthine, hypoxanthine, and uric acid. Uric acid is the oxidative product of hypoxanthine and xanthine through the action of the enzyme xanthine oxidase (XO) (41). Most yeast species lack XO; rather, they convert hypoxanthine to xanthine via xanthine dioxygenase. However, *S. cerevisiae* lacks all of the enzymes necessary to catabolize purines (42). These observations support the idea that increased levels of uric acid are a host response to monoassociation with *S. cerevisiae* rather than the metabolic activity of the yeast. Supporting this notion, uric acid cannot be detected in the growth medium of *S. cerevisiae*. To determine whether *S. cerevisiae* can elevate serum uric acid in animals with an intact microbiota during disease, we treated mice with *S. cerevisiae* or *R. aurantiaca* and

subsequently induced DSS colitis. Treatment with *S. cerevisiae*, but not *R. aurantiaca*, elevated uric acid above controls (Fig. 3D). Moreover, treatment of MODE-K small intestinal epithelial cells with *S. cerevisiae* in vitro directly stimulated uric acid production, whereas heat-killed and chemically killed yeast failed to do so (Fig. 3E and fig. S3D), indicating that live yeast was necessary for induction of uric acid production by cells. Last, production of uric acid from intestinal epithelia was specific to *S. cerevisiae* because both *R. aurantiaca* and *C. albicans* did not induce this response (Fig. 3E). Together, these data establish that *S. cerevisiae* can elevate host uric acid production by the intestinal epithelia, even in animals with a complete microbiota. Thus, exposure to *S. cerevisiae* in the gut led to enhanced purine metabolism and promoted increased uric acid.

Host immune reactions to commensal yeast positively correlate with elevated uric acid production in humans

Elevated titers of ASCAs are often used as a diagnostic tool to differentiate CD from UC (18, 43). About 60 to 70% of CD patients have ASCA immunoglobulin G (IgG) and 35% have ASCA IgA, but the presence of both IgA and IgG at high levels is highly specific for CD (44). However, ASCAs are often found in healthy patients as well as in people with other forms of intestinal inflammation such as celiac disease. ASCAs react to components of fungal cell walls, specifically phosphopeptidomannan structures, common in multiple types of yeast (45). Although the role of ASCAs during intestinal inflammation remains unclear, elevated titers suggest that an immune response toward yeast or yeast products might influence the disease course.

To determine whether there is a connection between uric acid and immune responses to yeast in humans, we obtained serum from 168 individuals (84 men and 84 women) who did not report any symptoms of disease and were not currently on any medications. These samples were tested for ASCA IgG, IgA, and uric acid. We discovered a positive correlation between levels of ASCA IgG or IgA and uric acid in these serum samples (Fig. 4, A to C). To determine the specificity of the relationship between ASCAs and uric acid, we performed an analysis of anti-chromatin antibodies, which are used as a diagnostic marker for another autoimmune disease, systemic lupus erythematosus. There was no significant correlation between anti-chromatin antibodies and uric acid (fig. S4, A and B), ruling out the possibility that elevated uric acid is related to all autoimmune diseases. Furthermore, a comparison of ASCAs in individuals with normal or elevated uric acid revealed that subjects whose uric acid levels were above the range for average adults had more ASCA IgA (Fig. 4, D to F). Our analysis suggests that individuals with greater immune reaction to fungal species in the GI tract also have elevated uric acid. These data validate our findings in animal models that members of the mycobiota have effects on human metabolism.

Worsened intestinal disease induced by *S. cerevisiae* is mediated by uric acid

Uric acid is most commonly recognized as the etiological agent of gout, a disorder of purine metabolism, resulting in the deposition of monosodium urate crystals in joints (46); it is also the cause of uric acid nephrolithiasis (kidney stones). Patients with IBD, compared to healthy individuals, are more prone to develop uric acid nephrolithiasis. Moreover, uric acid is known to bind the NLRP3 (NALP3) inflammasome and is thus associated with

inflammatory disorders (46). These clinical studies suggest that elevated uric acid in the intestine during an inflammatory response could exacerbate intestinal disease.

To test this hypothesis, we orally gavaged animals with uric acid and subsequently induced experimental colitis with DSS. Oral treatment with uric acid increased fecal uric acid by only 1.5-fold, elevating it to the same levels as seen with *S. cerevisiae* colonization of germ-free animals (Fig. 5A). Uric acid supplementation during colitis induced worsened disease, as evidenced by greater intestinal crypt loss and epithelial damage (Fig. 5, B and C). In addition, increased uric acid alone also caused greater intestinal permeability, as measured by leakage of FITC-dextran into the blood (Fig. 5D). Consistent with this finding, treatment of small intestinal epithelial cell lines in vitro with uric acid also resulted in increased permeability of FITC-dextran across the cell monolayer (fig. S5A). Because uric acid is known to bind to the NLRP3 inflammasome, we investigated a role for NLRP3 in eliciting exacerbated colitis during *S. cerevisiae* treatment. To this end, $NLRP3^{-/-}$ or wild-type (WT) mice were orally treated with *S. cerevisiae*, and DSS colitis was induced. There were no differences in colon length between the two groups, and $NLRP3^{-/-}$ animals actually had slightly elevated histology scores when compared to WT controls (fig. S5, B to D), demonstrating that heightened disease induced by *S. cerevisiae* is not dependent on NLRP3. Collectively, these data demonstrate that uric acid production induced by *S. cerevisiae* can enhance intestinal permeability and increase disease.

To demonstrate whether aggravated colitis induced by *S. cerevisiae* was dependent on the induction of purine metabolism, we sought to block this pathway using a clinical inhibitor of XO, allopurinol (41). *S. cerevisiae*–colonized animals were orally gavaged with allopurinol (10 mg/kg) during DSS-induced colitis. Administration of allopurinol reduced uric acid levels (Fig. 5E) and decreased intestinal disease compared to those animals that received only *S. cerevisiae* or DSS (Fig. 5, F to H). Colons from *S. cerevisiae*–treated animals were shorter compared to control groups, whereas colons from allopurinol-treated animals were longer than those from animals that received *S. cerevisiae* alone (Fig. 5F). Moreover, animals treated with allopurinol had less pathology when compared to *S. cerevisiae*–treated animals (Fig. 5, G and H). Together, these data demonstrate that certain members of the mycobiota can influence purine metabolism of the host and modulate disease pathology.

DISCUSSION

During hominid evolution, the loss of urate oxidase (uricase) activity led to higher levels of uric acid found in the blood of humans compared to other vertebrate species (47). Although a certain level of uric acid is thought to be beneficial as an antioxidant, excessive uric acid is associated with several disorders, including gout, vascular disease, and kidney stone formation. The increased incidence of developing uric acid nephrolithiasis during IBD is consistent with higher uric acid levels in people with intestinal disorders. Allopurinol has been used in patients with CD to increase the efficacy of the IBD medications 6-mercaptopurine and azathioprine (48), and many patients who received adjunctive allopurinol therapy were reported to have major clinical improvement (48). Our results suggest that some of the improvement might come from preventing yeast-induced uric acid

buildup in the intestine. Thus, allopurinol treatment in some IBD patients with adverse reactions to yeast and high uric acid might be of therapeutic benefit and should be explored.

The presence of ASCAs in CD patients suggests fungal involvement, but how these antibodies or the presence of yeast functionally influence IBD remains undefined. Using genetic and metabolic approaches, we experimentally connected these two clinical phenotypes by showing that a common intestinal commensal, *S. cerevisiae*, induced uric acid production and caused worsened intestinal disease by increasing intestinal permeability. A study performed 45 years ago demonstrated that feeding yeast to healthy young individuals elevated serum uric acid, corroborating our metabolic profiling experiments in germ-free mice (49). Our results suggest that uric acid testing combined with ASCA testing in patients with CD would represent an easy clinical assay to better inform therapeutic intervention regimens.

The mechanism by which *S. cerevisiae* increases intestinal permeability and exacerbates disease remains unclear. Uric acid is a ligand of the NLRP3 inflammasome that induces inflammatory mediators such as IL-1β and IL-18 (46); therefore, we tested a role for this protein complex in increased disease severity in response to *S. cerevisiae*. However, we found that *S. cerevisiae* induced similar colitis in NLRP3$^{-/-}$ animals, ruling out a role for NLRP3 in this setting. This finding is also consistent with our data that enhanced immune responses were not detected in animals colonized with *S. cerevisiae* when compared to control animals. The observation that heat-killed *S. cerevisiae* did not elicit worsened disease or elevate uric acid in mouse small intestinal epithelial cell lines suggests that the yeast must be metabolically active to induce this host response. Therefore, *S. cerevisiae* either produces a metabolite or influences the expression or function of a gene involved in purine metabolism. Future studies will be necessary to identify this mechanism and can take advantage of all the genetic tools available for this organism.

Patients with IBD are often treated with antibiotics to kill the resident bacteria that are eliciting chronic gut inflammation. Because fungi are not affected by most antibiotics, a common side effect of antibiotic therapy is an expansion of these organisms. Thus, the use of antibiotics in people with IBD might not only kill bacteria that are promoting healthy immune responses but also create an environment with little competition and allow for overgrowth of fungal populations. Even after cessation of antibiotic therapy, environmental fungal species might continue to colonize the host in greater numbers and disrupt the normal microbiota from recolonizing to the same levels. Supporting this hypothesis, two studies have demonstrated that patients with IBD have a greater diversity of fungi in their stool (8, 12). Although more studies are required, our findings suggest that anti-yeast therapy or avoidance of foods with live yeast might be therapeutically beneficial for patients with IBD. Note that not all yeasts are capable of exacerbating intestinal disease. Persistent exposure to *R. aurantiaca*, a common environmental fungus, did not alter the course of IBD. In addition, *Saccharomycopsis fibuligera* appeared to ameliorate intestinal disease in a mouse model of IBD (29), and *Saccharomyces boulardii* is often used as a probiotic, underscoring an important role for fungi in human health.

One limitation of our study is that numerous *S. cerevisiae* strains from a multitude of environmental, industrial, and clinical sources are known to exist (50, 51). Although *S. cerevisiae*, like most yeast species, is generally considered nonpathogenic, it can cause disease in immunocompromised individuals (52). In the current study, we chose to use an environmentally isolated diploid strain (RM11) to represent how exogenous yeast, acquired or encountered through diet, can influence the course of IBD (53). Although vaginal *S. cerevisiae* has been isolated and therefore could be passed vertically to offspring, *S. cerevisiae* has not been reported to date to be a common vaginal commensal (54, 55). Therefore, it is likely that most of the *S. cerevisiae* found in the GI tract is acquired initially from the environment, making use of an environmentally isolated strain appropriate. Further studies will be needed to address how different strains of *S. cerevisiae* behave in vivo. Investigating the transcriptional, proteomic, and metabolomic profiles of *S. cerevisiae* as a member of a complex microbiota, during both homeostatic and inflammatory states in the GI tract, will be instrumental in understanding its biology, its contribution to mammalian commensalism, and its ability to potentiate disease.

Because there are numerically more bacteria than fungi in the gut, it is likely that these organisms interact with one another. Consistent with this, a recent study showed correlations between the abundance of certain bacterial and fungal members of the human microbiota (12). Many bacterial and fungal species are able to degrade uric acid; thus, *S. cerevisiae*–induced uric acid production could influence the biology of bacterial and other fungal members of the microbiota. However, little is known about these interkingdom interactions in the gut, further highlighting the complexity of host-microbe symbiosis and its role in human health and disease.

MATERIALS AND METHODS

Study design

The aim of this study was to explore how commensal yeast species influence the course of murine experimental colitis. Preliminary experiments demonstrated that *S. cerevisiae* exacerbated colitis, as measured by histology and colon length, in the absence of traditional inflammatory markers, that is, T_H1 and T_H17 cells and corresponding cytokines, as assayed by flow cytometry of MLNs and qRT-PCR from colonic tissue. Microarray data of colonic RNA from germ-free mice monoassociated with *S. cerevisiae* or *R. aurantiaca* pointed to disrupted barrier integrity and multiple host metabolic pathways being affected. This led us to functionally investigate barrier disruption during experimental colitis, using FITC-dextran as a marker of intestinal permeability, as well as to characterize the metabolic profile of feces from animals that had been monoassociated with *S. cerevisiae* or *R. aurantiaca*, using GS-MS. Our metabolic profiling highlighted components of the purine degradation pathway, including uric acid. As ASCAs are used as a diagnostic marker of CD, we wanted to know whether there was a correlation between ASCAs and uric acid in human patients. Human subjects were chosen at random, with male and female subjects represented equally. A blinded pathologist performed histology: Slides were assigned random numbers that had no relevance to the experiment. After grading, histology scores were sent back to the investigator via email, and scores were decoded and assigned to their experimental group.

Experimental replicates are cited in the figure legends. A statistician was consulted before the study to determine the minimum number of animals that would be required for a study based on published literature using a power analysis. All experiments were repeated two to four times as indicated in the figure legends.

Animals

All experiments were performed on age-matched, 8- to 10-week-old male mice on a C57Bl/6 background. C57Bl/6 (WT controls) and NLRP3$^{-/-}$ animals were purchased from the Jackson Laboratory and housed under SPF conditions. C57Bl/6 germ-free mice were bred at the University of Utah, and sterility was checked by anaerobic and aerobic microbial plating and PCR. All animal use was in compliance with federal regulations and guidelines set by the University of Utah's Institutional Animal Care and Use Committee (protocol #14-05009).

Yeast strains

S. cerevisiae used in all experiments was a prototrophic diploid strain on an RM11 background provided from the laboratory of D. Stillman. *S. cerevisiae* was grown in yeast peptone, adenine, and dextrose (YPAD) at 30°C. *R. aurantiaca* was obtained from the American Type Culture Collection (ATCC 10655) and was grown in Sabouraud dextrose agar at room temperature. *C. albicans* was provided by the laboratory of S. Nobel and was a 50:50 mixture of WT:WOR1 mutant grown in YPAD at 30°C. Heat-killed *S. cerevisiae* (HKY) at a concentration of 10^7/ml was incubated in a water bath (70°C) for 1 hour. HKY was struck out on YPAD agar plates and incubated for 48 hours at 30°C to verify no growth. HKY stocks were stored at −80°C.

Microarrays

RNA was collected from the indicated samples using a Qiagen miRNA RNA isolation kit. RNA was then provided to the University of Utah Microarray/High Throughput Genomics Core using the Agilent platform. RNA was analyzed by an Agilent Bioanalyzer, labeled with modified nucleotide samples (Cy dyes or biotin), hybridized, and scanned using standard procedures.

ASCA/uric acid quantification in human sera

Human serum samples were collected and processed at ARUP Laboratories and approved under Institutional Review Board protocol #7740. Sera from deidentified females and males aged 20 to 66 years were collected in plasma serum separator tubes. Patients were not currently on any therapies at the time of sample collection. Human serum was analyzed for the presence of ASCA IgG and IgA using a U.S. Food and Drug Administration–approved enzyme-linked immunosorbent assay (Inova Diagnostics). Uric acid from human serum was detected using quantitative spectrometry.

Flow cytometry

MLNs were gently pushed through a 40-μM filter to obtain lymphocytes. Surface staining for lymphocytes was done in sterile 1× Hank's balanced salt solution (HBSS) (Corning)

supplemented with10 mMHepes (Cellgro), 2 mM EDTA (Cellgro), and 0.5% (v/v) fetal bovine serum (FBS) (Gibco BRL) for 20 min at 4°C. Cells were then washed twice in supplemented 1× HBSS and enumerated via flow cytometry. The following antibodies were used: anti-CD4 (FITC) and anti-CD3 (Pacific Blue).

For intracellular staining, cells were first stimulated with ionomycin (500 ng/ml), phorbol 12-myristate 13-acetate (5 ng/ml), and brefeldin A (5 mg/ml; BioLegend) for 4 hours at 37°C. Cells were surface-stained, washed, and then permeabilized and fixed in 100 ml of Perm/Fix buffer (eBioscience) overnight at 4°C. Cells were washed twice in Perm/Wash buffer (eBioscience) and then stained for intracellular cytokines with the following antibodies: anti–IFN-γ (phycoerythrin) and anti–IL-17A (allophycocyanin). These data were collected with a BD LSRFortessa and analyzed with FlowJo software.

Quantitative PCR

For inflammatory cytokine and tight junction transcript analysis, a 1-cm cut of colon tissue about 1 cm proximal to the cecum was washed in sterile PBS and placed in QIAzol (Qiagen) and stored at −20°C until extraction. A standard phenol-chloroform extraction was performed to isolate RNA. RNA samples were deoxyribonuclease-treated (AMPD-1KT, Sigma-Aldrich) before reverse transcription (qScript, cDNA SuperMix, Quanta Biosciences Inc.) following the manufacturer's instructions. qPCR was performed with a LightCycler LC480 instrument (Roche) under the following cycling parameters: for tight junctions, 45 cycles: denaturation for 15 s at 95°C, annealing for 1 min at 60°C, and elongation for 1 min at 72°; for inflammatory cytokines, 45 cycles: denaturation for 15 s at 95°C, annealing for 30 s at 60°C, and elongation for 30 s at 72°. Primer sets are shown in table S1.

TNBS colitis

For induction of TNBS colitis, C57Bl/6 mice were anesthetized with isoflurane, and 50:50 (v/v) ethanol (EtOH)/TNBS was administered intrarectally. Mice were then held tail up for 30 s to allow the solution to adsorb. Animals were weighed daily and sacrificed 5 days after TNBS administration.

DSS colitis

For induction of DSS colitis, WT C57Bl/6 or NLRP3$^{-/-}$ mice were provided 2.5% DSS in drinking water for 8 days. Animals were sacrificed on day 8. DSS water was changed every other day.

Oral gavage

Before and during colitis, mice were supplemented with daily doses (~1.0×10^6 CFU/mouse per dose) of either *S. cerevisiae* or *R. aurantiaca*. Uric acid [molecular weight (MW), 168.1, Sigma-Aldrich] was suspended in sterile PBS at a concentration of 100 mg/ml. Before each mouse was orally gavaged, the suspension was shaken vigorously to obtain a homogeneous mixture. One hundred microliters (10 mg) of the mixture was administered twice a day for 10 days. Allopurinol (10 mg/kg) (MW, 136.11; Sigma-Aldrich) was orally gavaged every other day during DSS colitis.

Histology

Whole colons were removed and washed in sterile PBS before being fixed in 10% formalin. Colons were then paraffin-embedded, sectioned, and stained with H&E for pathology assessment. Colons were scored by a blinded pathologist using the following scoring system: 0, no crypt loss; 1 to 2, mild crypt loss; 3 to 4, medium crypt loss; and 5 to 6, severe crypt loss. The amount of colon affected was also taken into consideration: 1, 1 to 10%; 2, 10 to 20%; 3, 20 to 40%; 4, 40 to 60%; and 5, >60%. The level of inflammation was also assessed: 1 to 2, mild; 3, medium; and 4, severe.

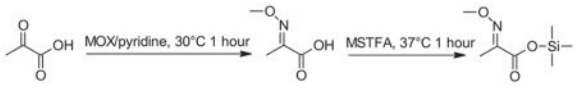
Metabolomic analysis

Metabolite extraction from pellet—Mouse fecal pellets were extracted using a modified method derived from Canelas *et. al* (56). To each cell pellet, we added 5 ml of boiling 75% EtOH (aqueous), followed by vortex mixing, and then incubated it at 90°C for 5 min. The EtOH solution contained a mixture of U-^{13}C/U-^{15}N stable isotope-labeled amino acids (CNLM-6696-1, Cambridge Isotope Laboratories) at 5 μg per sample, and d_4-succinate was added at 1 μg per sample. Cell debris was removed by centrifugation at 5000*g* for 3 min. The supernatant was removed to new tubes and dried en vacuo.

GC-MS analysis

All GC-MS analysis was performed with a Waters GCT Premier Mass Spectrometer fitted with an Agilent 6890 Gas Chromatograph and a GERSTEL MPS2 Autosampler. Dried samples were suspended in 100 μl of *O*-methoxylamine hydrochloride (40 mg/ml) in pyridine and incubated for 1 hour at 30°C. To the autosampler vials, we added 25 μl of this solution. Ten microliters of *N*-methyl-*N*-trimethylsilyltrifluoracetamide was added automatically via the auto-sampler and incubated for 60 min at 37°C with shaking. After incubation, 3 μl of a fatty acid methyl ester standard solution was added via the autosampler, and 1 μl of the prepared sample was then injected to the gas chromatograph inlet in the split mode, with the inlet temperature held at 250°C. Two GC-MS runs were performed: one at a 10:1 split ratio to detect low-level metabolites and the second at a 100:1 split ratio to accurately measure high-concentration metabolites, which saturate the detector at the 10:1 split ratio. For the 10:1 split ratio analysis, the gas chromatograph had an initial temperature of 95°C for 1 min, followed by a 40°C/min ramp to 110°C, and a hold time of 2 min. This was followed by a second 5°C/min ramp to 250°C, a third ramp to 350°C, and then a final hold time of 3 min. For the 100:1 split ratio analysis, the gas chromatograph had an initial temperature of 95° C for 1 min, followed by a 40°C/min ramp to 110°C, and a hold time of 2 min. This was followed by a second 25°C/min ramp to 330°C. A 30-m Phenomenex ZB-5 MSi column with a 5-m-long guard column was used for chromatographic separation. Helium was used as the carrier gas at 1 ml/min.

Below is a description of the two-step derivatization process used to convert nonvolatile metabolites to a volatile form amenable to GC-MS. Pyruvic acid is used here as an example.



Analysis of GC-MS data

Data were collected using MassLynx 4.1 software (Waters). A two-step process was used for data analysis, targeted followed by nontargeted analysis. For the targeted approach, known metabolites were identified, and their peak area was recorded using QuanLynx. Data were normalized to the internal standard d_4-succinate, and the pellet was weighed.

For the nontargeted approach, peak picking and analysis were performed using MarkerLynx and the freely available online software www.metaboanalyst.ca. Chemical entities were identified using MarkerLynx, followed by the generation of an XML file. This file was formatted to an Excel format, and the peak areas were normalized to weight. These data were analyzed in MetaboAnalyst version 2.0 (57). PCA and volcano plots were generated.

Metabolite identity was established using a combination of an in-house metabolite library developed using pure purchased standards and commercially available NIST library. When reporting each metabolite, those with absolute identity are not qualified, whereas those that are identified using the NIST library are noted using a percentage of certainty produced by the NIST software. Metabolites, which are completely unknown, are labeled as unRTm/z, where un is the unknown, RT is the retention time, and m/z is the mass-to-charge ratio. An example of this is an unknown metabolite that elutes at 15.22 min and has a characteristic mass of 247 would be written as un15.22_247. Only those unknowns that are statistically relevant were recorded.

FITC-dextran permeability assay

For assessment of intestinal permeability, animals were fasted for 4 hours before receiving an oral gavage of 60 mg/100 g of FITC-dextran 4 (MW, 3000 to 5000; Sigma-Aldrich). Animals were then fasted for another 4 hours before sacrificing. Blood was taken via cardiac puncture and collected in serum separator tubes. Blood was allowed to sit at room temperature for 30 min before being centrifuged at 1300g for 10 min. One hundred microliters of serum was then assayed for the presence of FITC fluorophores using a microplate reader at 488-nm excitation and 519-nm emission (Biotek Synergy H1).

Uric acid measurement

Serum was collected as previously described. To remove interfering substances, we filtered serum in 10,000-MW filters and spun it at 14,000g for 20 min. Filtered serum was then used in the QuantiChrom Uric Acid Assay Kit (DIUA-250, BioAssay Systems) or Amplex Red Uric Acid/Uricase Assay Kit (Life Technologies) following the manufacturer's guidelines. For uric acid measurement in feces, mouse fecal pellets were weighed and then homogenized in 200 to 300 μl of sterile PBS. Feces were filtered as described above, and filtrates were used to detect uric acid levels using the same detection kit. Uric acid levels were then normalized to fecal weight in grams.

In vitro experiments using mouse intestinal epithelial cells (MODE-K cells)

Mouse intestinal epithelial cells were maintained in Dulbecco's modified Eagle's medium (DMEM), with L-glutamine and sodium pyruvate. DMEM was supplemented with 10% FBS, 1% (v/v) glutamine, penicillin-streptomycin, and 1% Hepes. For uric acid

determination, a confluent monolayer of cells was incubated with different MOIs for 24 hours. Medium was then assayed for the presence of uric acid as previously described. For Transwell experiments, FITC-dextran was added to a confluent monolayer in the apical compartment for a final concentration of 25 mg/ml. After 24 hours, the medium in the basolateral compartment was assayed for FITC fluorophores as previously described.

Statistics

Statistics were carried out using Prism 6.0 (GraphPad) and JMP9.0 (SAS). Two-tailed unpaired Student's *t* test was used for all pairwise statistical comparisons unless otherwise noted. Nonparametric Mann-Whitney *t* test was used on human data. Error bars in all figures represent means ± SEM. Before data analysis, outliers were identified and excluded using the ROUT method in Prism 6.0. Sample size, number of replicates, and statistical test are reported in all figure captions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES AND NOTES

1. Wlodarska M, Kostic AD, Xavier RJ. An integrative view of microbiome-host interactions in inflammatory bowel diseases. Cell Host Microbe. 2015; 17:577–591. [PubMed: 25974300]
2. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Jin Song S, Yassour M, Morgan XC, Kostic AD, Luo C, González A, McDonald D, Haberman Y, Walters T, Baker S, Rosh J, Stephens M, Heyman M, Markowitz J, Baldassano R, Griffiths A, Sylvester F, Mack D, Kim S, Crandall W, Hyams J, Huttenhower C, Knight R, Xavier RJ. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe. 2014; 15:382–392. [PubMed: 24629344]
3. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA. 2007; 104:13780–13785. [PubMed: 17699621]
4. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008; 453:620–625. [PubMed: 18509436]
5. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. T_{reg} induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013; 500:232–236. [PubMed: 23842501]

6. Dieleman LA, Goerres MS, Arends A, Sprengers D, Torrice C, Hoentjen F, Grenther WB, Sartor RB. *Lactobacillus GG* prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. Gut. 2003; 52:370–376. [PubMed: 12584218]
7. Tang J, Iliev ID, Brown J, Underhill DM, Funari VA. Mycobiome: Approaches to analysis of intestinal fungi. J Immunol Methods. 2015; 421:112–121. [PubMed: 25891793]
8. Ott SJ, Kühbacher T, Musfeldt M, Rosenstiel P, Hellmig S, Rehman A, Drews O, Weichert W, Timmis KN, Schreiber S. Fungi and inflammatory bowel diseases: Alterations of composition and diversity. Scand J Gastroenterol. 2008; 43:831–841. [PubMed: 18584522]
9. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, Schoenfeld D, Nomicos E, Park M, Kong HH, Segre JA. NIH Intramural Sequencing Center Comparative Sequencing Program. Topographic diversity of fungal and bacterial communities in human skin. Nature. 2013; 498:367–370. [PubMed: 23698366]
10. Scupham AJ, Presley LL, Wei B, Bent E, Griffith N, McPherson M, Zhu F, Oluwadara O, Rao N, Braun J, Borneman J. Abundant and diverse fungal microbiota in the murine intestine. Appl Environ Microbiol. 2006; 72:793–801. [PubMed: 16391120]
11. Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, Gillevet PM. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLOS Pathog. 2010; 6:e1000713. [PubMed: 20072605]
12. Sokol H, Leducq V, Aschard H, Pham H-P, Jegou S, Landman C, Cohen D, Liguori G, Bourrier A, Nion-Larmurier I, Cosnes J, Seksik P, Langella P, Skurnik D, Richard ML, Beaugerie L. Fungal microbiota dysbiosis in IBD. Gut. 2016:1–10.
13. Richard ML, Lamas B, Liguori G, Hoffmann TW, Sokol H. Gut fungal microbiota: The Yin and Yang of inflammatory bowel disease. Inflamm Bowel Dis. 2015; 21:656–665. [PubMed: 25545379]
14. Lewis JD, Chen EZ, Baldassano RN, Otley AR, Griffiths AM, Lee D, Bittinger K, Bailey A, Friedman ES, Hoffmann C, Albenberg L, Sinha R, Compher C, Gilroy E, Nessel L, Grant A, Chehoud C, Li H, Wu GD, Bushman FD. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's disease. Cell Host Microbe. 2015; 18:489–500. [PubMed: 26468751]
15. Underhill DM, Iliev ID. The mycobiota: Interactions between commensal fungi and the host immune system. Nat Rev Immunol. 2014; 14:405–416. [PubMed: 24854590]
16. Underhill D, Braun J. Current understanding of fungal microflora in inflammatory bowel disease pathogenesis. Inflamm Bowel Dis. 2008; 14:1147–1153. [PubMed: 18286647]
17. Huffnagle GB, Noverr MC. The emerging world of the fungal microbiome. Trends Microbiol. 2013; 21:334–341. [PubMed: 23685069]
18. Vermeire S, Joossens S, Peeters M, Monsuur F, Marien G, Bossuyt X, Groenen P, Vlietinck R, Rutgeerts P. Comparative study of ASCA (Anti-*Saccharomyces cerevisiae* antibody) assays in inflammatory bowel disease. Gastroenterology. 2001; 120:827–833. [PubMed: 11231936]
19. Ursing B, Alm T, Bárány F, Bergelin I, Ganrot-Norlin K, Hoevels J, Huitfeldt B, Järnerot G, Krause U, Krook A, Lindström B, Nordle O, Rosén A. A comparative study of metronidazole and sulfasalazine for active Crohn's disease: The cooperative Crohn's disease study in Sweden. II. Result. Gastroenterology. 1982; 83:550–562. [PubMed: 6124474]
20. Prantera C, Zannoni F, Scribano ML, Berto E, Andreoli A, Kohn A, Luzi C. An antibiotic regimen for the treatment of active Crohn's disease: A randomized, controlled clinical trial of metronidazole plus ciprofloxacin. Am J Gastroenterol. 1996; 91:328–332. [PubMed: 8607501]
21. Sutherland L, Singleton J, Sessions J, Hanauer S, Krawitt E, Rankin G, Summers R, Mekhjian H, Greenberger N, Kelly M. Double blind, placebo controlled trial of metronidazole in Crohn's disease. Gut. 1991; 32:1071–1075. [PubMed: 1916494]
22. Lal S, Steinhart AH. Antibiotic therapy for Crohn's disease: A review. Can J Gastroenterol. 2006; 20:651–655. [PubMed: 17066156]
23. Trenschel R, Peceny R, Runde V, Elmaagacli A, Dermoumi H, Heintschel von Heinegg E, Müller K-D, Schaefer UW, Beelen DW. Fungal colonization and invasive fungal infections following allogeneic BMT using metronidazole, ciprofloxacin and fluconazole or ciprofloxacin and

fluconazole as intestinal decontamination. Bone Marrow Transplant. 2000; 26:993–997. [PubMed: 11100279]

24. Samonis G, Margioris AN, Toloudis P, Dassiou M, Maraki S, Tselentis Y. Murine yeast gut flora affected by tetracycline, metronidazole and norfloxacin. J Chemother. 1994; 6:408–411. [PubMed: 7699429]
25. Noverr MC, Noggle RM, Toews GB, Huffnagle GB. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. Infect Immun. 2004; 72:4996–5003. [PubMed: 15321991]
26. McGovern, Dermot PB., Gardet, A., Törkvist, L., Goyette, P., Essers, J., Taylor, KD., Neale, BM., Ong, Rick TH., Lagacé, C., Li, C., Green, T., Stevens, CR., Beauchamp, C., Fleshner, PR., Carlson, M., D'Amato, M., Halfvarson, J., Hibberd, ML., Lördal, M., Padyukov, L., Andriulli, A., Colombo, E., Latiano, A., Palmieri, O., Bernard, E-J., Deslandres, C., Hommes, DW., de Jong, DJ., Stokkers, PC., Weersma, RK., Sharma, Y., Silverberg, MS., Cho, JH., Wu, J., Roeder, K., Brant, SR., Phillip Schumm, L., Duerr, RH., Dubinsky, MC., Glazer, NL., Haritunians, T., Ippoliti, A., Melmed, GY., Siscovick, DS., Vasiliauskas, EA., Targan, SR., Annese, V., Wijmenga, C., Pettersson, S., Rotter, JI., Xavier, RJ., Daly, MJ., Rioux, JD., Seielstad, M. The NIDDK IBD Genetics Consortium. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet. 2010; 42:332–337. [PubMed: 20228799]
27. Franke A, McGovern DPB, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PCF, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet. 2010; 42:1118–1125. [PubMed: 21102463]
28. Ferwerda B, Ferwerda G, Plantinga TS, Willment JA, van Spriel AB, Venselaar H, Elbers CC, Johnson MD, Cambi A, Huysamen C, Jacobs L, Jansen T, Verheijen K, Masthoff L, Morré SA, Vriend G, Williams DL, Perfect JR, Joosten LA, Wijmenga C, van der Meer JW, Adema GJ, Kullberg BJ, Brown GD, Netea MG. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med. 2009; 361:1760–1767. [PubMed: 19864674]
29. Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, Strom SP, Brown J, Becker CA, Fleshner PR, Dubinsky M, Rotter JI, Wang HL, McGovern DPB, Brown GD, Underhill DM. Interactions between commensal fungi and the C-type lectin receptor dectin-1 influence colitis. Science. 2012; 336:1314–1317. [PubMed: 22674328]
30. Sokol H, Conway KL, Zhang M, Choi M, Morin B, Cao Z, Villablanca EJ, Li C, Wijmenga C, Yun SH, Shi HN, Xavier RJ. Card9 mediates intestinal epithelial cell restitution, T-helper 17 responses, and control of bacterial infection in mice. Gastroenterology. 2013; 145:591–601.e3. [PubMed: 23732773]
31. Mukhopadhya I, Hansen R, Meharg C, Thomson JM, Russell RK, Berry SH, El-Omar EM, Hold GL. The fungal microbiota of de-novo paediatric inflammatory bowel disease. Microbes Infect. 2015; 17:304–310. [PubMed: 25522934]
32. Ott SJ, Plamondon S, Hart A, Begun A, Rehman A, Kamm MA, Schreiber S. Dynamics of the mucosa-associated flora in ulcerative colitis patients during remission and clinical relapse. J Clin Microbiol. 2008; 46:3510–3513. [PubMed: 18701655]
33. Wirth F, Goldani LZ. Epidemiology of *Rhodotorula*: An emerging pathogen. Interdiscip Perspect Infect Dis. 2012; 2012:465717. [PubMed: 23091485]
34. Wirtz S, Neufert C, Weigmann B, Neurath MF. Chemically induced mouse models of intestinal inflammation. Nat Protoc. 2007; 2:541–546. [PubMed: 17406617]

35. Spees AM, Lopez CA, Kingsbury DD, Winter SE, Bäumler AJ. Colonization resistance: Battle of the bugs or Ménage à Trois with the host? PLOS Pathog. 2013; 9:e1003730. [PubMed: 24278012]
36. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009; 139:485–498. [PubMed: 19836068]
37. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. Science. 2001; 291:881–884. [PubMed: 11157169]
38. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann Intern Med. 1986; 105:883–885. [PubMed: 3777713]
39. Teshima CW, Dieleman LA, Meddings JB. Abnormal intestinal permeability in Crohn's disease pathogenesis. Ann N Y Acad Sci. 2012; 1258:159–165. [PubMed: 22731729]
40. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. Science. 2012; 336:1262–1267. [PubMed: 22674330]
41. Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: Renaissance half a century after the discovery of allopurinol. Pharmacol Rev. 2006; 58:87–114. [PubMed: 16507884]
42. Pantazopoulou A, Diallinas G. Fungal nucleobase transporters. FEMS Microbiol Rev. 2007; 31:657–675. [PubMed: 17784857]
43. Desplat-Jégo S, Johanet C, Escande A, Goetz J, Fabien N, Olsson N, Ballot E, Sarles J, Baudon JJ, Grimaud JC. Update on anti-Saccharomyces cerevisiae antibodies, anti-nuclear associated anti-neutrophil antibodies and antibodies to exocrine pancreas detected by indirect immunofluorescence as biomarkers in chronic inflammatory bowel diseases: Results of a multicenter study. World J Gastroenterol. 2007; 13:2312–2318. [PubMed: 17511029]
44. Ashorn S, Raukola H, Välineva T, Ashorn M, Wei B, Braun J, Rantala I, Kaukinen K, Luukkaala T, Collin P, Mäki M, Iltanen S. Elevated serum anti-Saccharomyces cerevisiae, anti-I2 and anti-OmpW antibody levels in patients with suspicion of celiac disease. J Clin Immunol. 2008; 28:486–494. [PubMed: 18496744]
45. Standaert-Vitse A, Jouault T, Vandewalle P, Mille C, Seddik M, Sendid B, Mallet JM, Colombel JF, Poulain D. *Candida albicans* is an immunogen for anti-*Saccharomyces cerevisiae* antibody markers of Crohn's disease. Gastroenterology. 2006; 130:1764–1775. [PubMed: 16697740]
46. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006; 440:237–241. [PubMed: 16407889]
47. Kratzer JT, Lanaspa MA, Murphy MN, Cicerchi C, Graves CL, Tipton PA, Ortlund EA, Johnson RJ, Gaucher EA. Evolutionary history and metabolic insights of ancient mammalian uricases. Proc Natl Acad Sci USA. 2014; 111:3763–3768. [PubMed: 24550457]
48. Govani SM, Higgins PDR. Combination of thiopurines and allopurinol: Adverse events and clinical benefit in IBD. J Crohns Colitis. 2010; 4:444–449. [PubMed: 21122542]
49. Edozien JC, Udo UU, Young VR, Scrimshaw NS. Effects of high levels of yeast feeding on uric acid metabolism of young man. Nature. 1970; 228:180. [PubMed: 5460023]
50. Danø Klingberg T, Lesnik U, Arneborg N, Raspor P, Jespersen L. Comparison of *Saccharomyces cerevisiae* strains of clinical and nonclinical origin by molecular typing and determination of putative virulence traits. FEMS Yeast Res. 2008; 8:631–640. [PubMed: 18355272]
51. de Llanos R, Llopis S, Molero G, Querol A, Gil C, Fernández-Espinar MT. In vivo virulence of commercial *Saccharomyces cerevisiae* strains with pathogenicity-associated phenotypical traits. Int J Food Microbiol. 2011; 144:393–399. [PubMed: 21081253]
52. de Hoog GS. Risk assessment of fungi reported from humans and animals. Mycoses. 1996; 39:407–417. [PubMed: 9144996]
53. Mortimer RK, Romano P, Suzzi G, Polsinelli M. Genome renewal: A new phenomenon revealed from a genetic study of 43 strains of *Saccharomyces cerevisiae* derived from natural fermentation of grape musts. Yeast. 1994; 10:1543–1552. [PubMed: 7725789]

54. Posteraro B, Sanguinetti M, D'Amore G, Masucci L, Morace G, Fadda G. Molecular and epidemiological characterization of vaginal *Saccharomyces cerevisiae* isolates. J Clin Microbiol. 1999; 37:2230–2235. [PubMed: 10364590]

55. Drell T, Lillsaar T, Tummeleht L, Simm J, Aaspõllu A, Väin E, Saarma I, Salumets A, Donders GGG, Metsis M. Characterization of the vaginal micro-and mycobiome in asymptomatic reproductive-age Estonian women. PLOS ONE. 2013; 8:e54379. [PubMed: 23372716]

56. Canelas AB, ten Pierick A, Ras C, Seifar RM, van Dam JC, van Gulik WM, Heijnen JJ. Quantitative evaluation of intracellular metabolite extraction techniques for yeast metabolomics. Anal Chem. 2009; 81:7379–7389. [PubMed: 19653633]

57. Xia J, Mandal R, Sinelnikov IV, Broadhurst D, Wishart DS. MetaboAnalyst 2.0—A comprehensive server for metabolomic data analysis. Nucleic Acids Res. 2012; 40:W127–W133. [PubMed: 22553367]

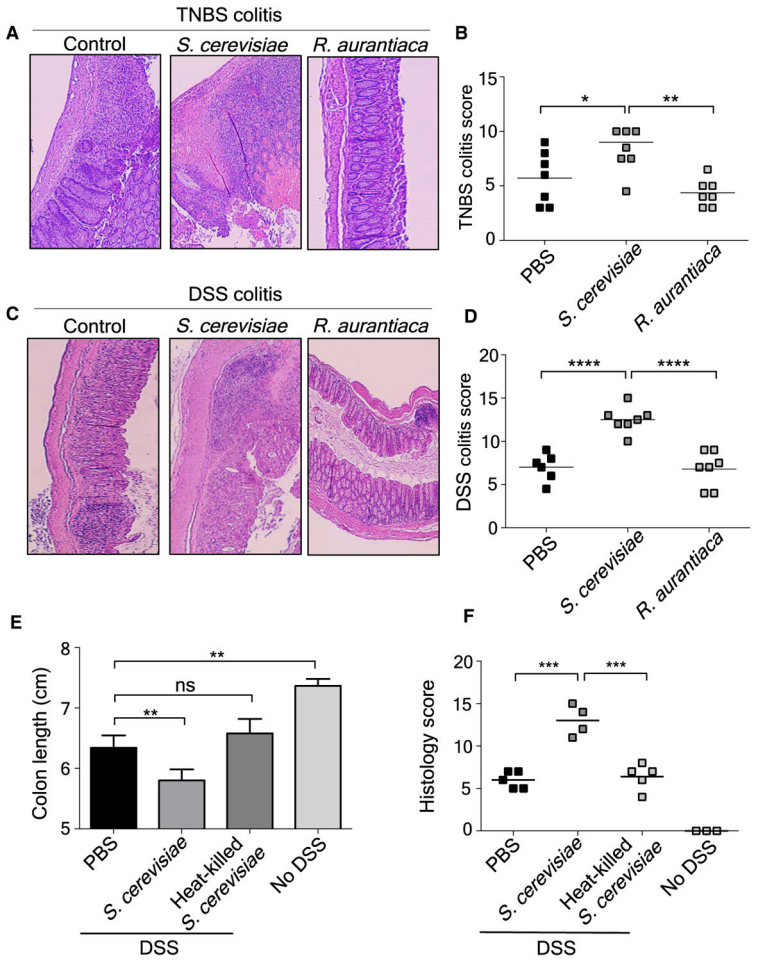

Fig. 1. *S. cerevisiae*, but not *R. aurantiaca*, exacerbates intestinal disease in two mouse models of colitis

(**A** and **B**) C57Bl/6 specific pathogen–free (SPF) mice were orally gavaged daily for 7 days with 10^6 of *S. cerevisiae* ($n = 7$) or *R. aurantiaca* ($n = 7$), and control mice were mock-gavaged with phosphate-buffered saline (PBS) ($n = 6$) each day for 1 week. Colitis was induced via administration of TNBS. These data are representative of two independent experiments with six to seven mice per group in each experiment. (**C** and **D**) C57Bl/6 SPF animals were treated as in (A) [*S. cerevisiae* ($n = 7$), *R. aurantiaca* ($n = 7$), and PBS ($n = 6$)], and colitis was induced by placing 2.5% DSS in drinking water. Animals were sacrificed 8 days after DSS induction, and colons were analyzed by histology for disease severity. These are representative of three independent experiments with six to seven mice per group in each experiment. (**E** and **F**) C57Bl/6 SPF animals were orally gavaged for 1 week with 10^6 live ($n = 4$) or heat-killed ($n = 5$) *S. cerevisiae*; controls ($n = 5$) were mock-gavaged with PBS each day for 1 week. Colitis was induced by placing 2.5% DSS in drinking water. Animals were sacrificed 8 days after DSS treatment, and colons were analyzed for disease severity. Colon length (E) and histology scores (F) are shown. These data are representative of two replicate experiments with four to six mice per group in each experiment. Each dot indicates an individual mouse in all panels. $*P < 0.05$; $**P < 0.01$; $***P < 0.005$; $****P < 0.001$, *t* test. ns, not significant.

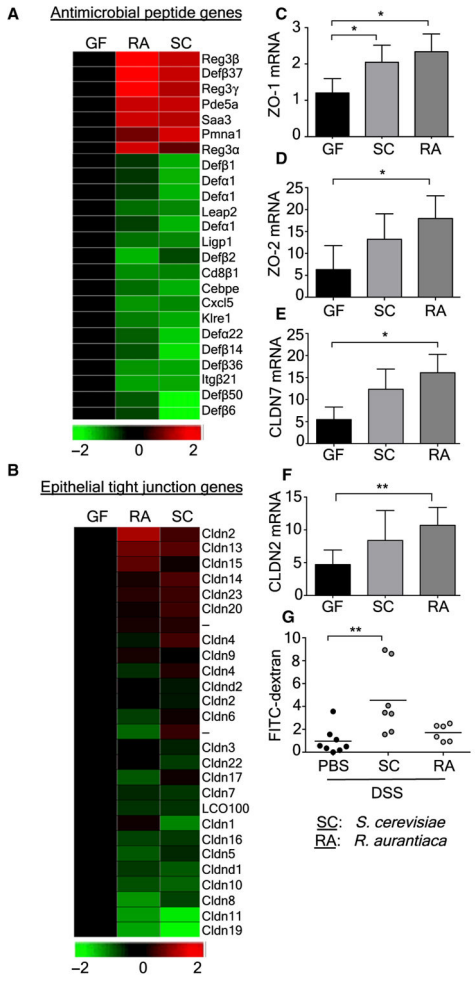

Fig. 2. *S. cerevisiae* colonization disrupts intestinal barrier function

(**A** and **B**) C57Bl/6 germ-free animals were colonized with *S. cerevisiae* or *R. aurantiaca* by placing 10^8 yeast in 200 ml of drinking water. RNA was collected from the colons of the indicated animals 1 month after colonization and used for microarray analysis. Genes shown were statistically significantly different ($P < 0.05$) with at least a 1.5-fold change. GF, germ-free ($n = 4$); SC, *S. cerevisiae* monoassociated germ-free animals ($n = 4$); RA, *R. aurantiaca* monoassociated germ-free animals ($n = 4$). (**C** to **F**) Some of the genes involved in intestinal epithelial tight junction formation were verified by qPCR; *y* axis values represent 10^6 gene transcripts per L32 copies. (**G**) C57Bl/6 SPF animals were treated as described in Fig. 1 [*S. cerevisiae* ($n = 6$), *R. aurantiaca* ($n = 8$), and DSS control (PBS, $n = 7$)]. Eight days after DSS treatment, animals were orally gavaged with FITC-dextran. Serum was collected and analyzed for the presence of FITC-dextran. Two independent experiments were performed and are shown here with three to four mice per group in each experiment. $*P < 0.05$; $**P < 0.01$, *t* test.

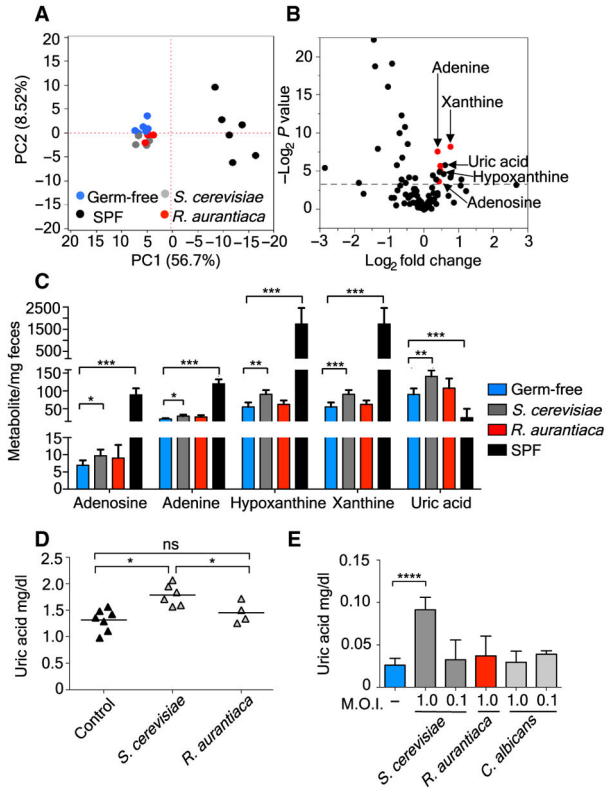

Fig. 3. *S. cerevisiae* colonization induces elevated purine metabolism in mouse gut

(**A** and **B**) C57Bl/6 germ-free animals were monoassociated with either *S. cerevisiae* or *R. aurantiaca* as described in Fig. 2. Fecal metabolites were analyzed using GC-MS ($n = 4$ to 6 in each group). Principal component analysis (PCA) of metabolic profiles between germ-free and yeast monoassociated animals (A). Volcano plot showing individual metabolites between yeast monoassociated and germ-free animals (B). (**C**) Pairwise comparisons of significantly changed metabolites involved in purine metabolism between the indicated groups. (**D**) Animals were treated as described in Fig. 1, and colitis was subsequently induced with DSS. Eight days after DSS induction, serum uric acid levels were measured in the indicated animals. (**E**) MODE-K cells were treated with the indicated fungal strains for 24 hours, and uric acid production in the medium was analyzed. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.001$, *t* test. MOI, multiplicity of infection.

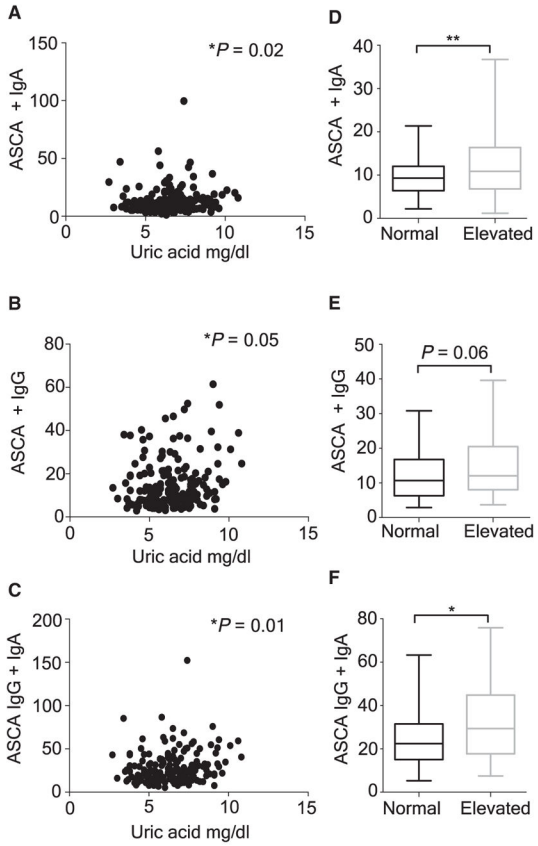

Fig. 4. A positive correlation between ASCAs and uric acid in human sera

(**A** to **C**) Human serum was obtained from healthy donors, and ASCA IgA (A), IgG (B), both ASCA IgG and IgA combined titers (C), and uric acid were determined. Spearman correlation analysis was performed on the data, and *P* values are provided. (**D** to **F**) Serum uric acid levels were divided into two groups. Each group was then evaluated for the presence of ASCA IgA (D), IgG (E), or combined antibody titers (F). Normal range for female and male uric acid is 2.5 to 6.0 mg/dl and 3.5 to 8.0 mg/dl, respectively (normal). Above these ranges, uric acid is considered elevated. $*P < 0.05$, $**P < 0.01$, *t* test.

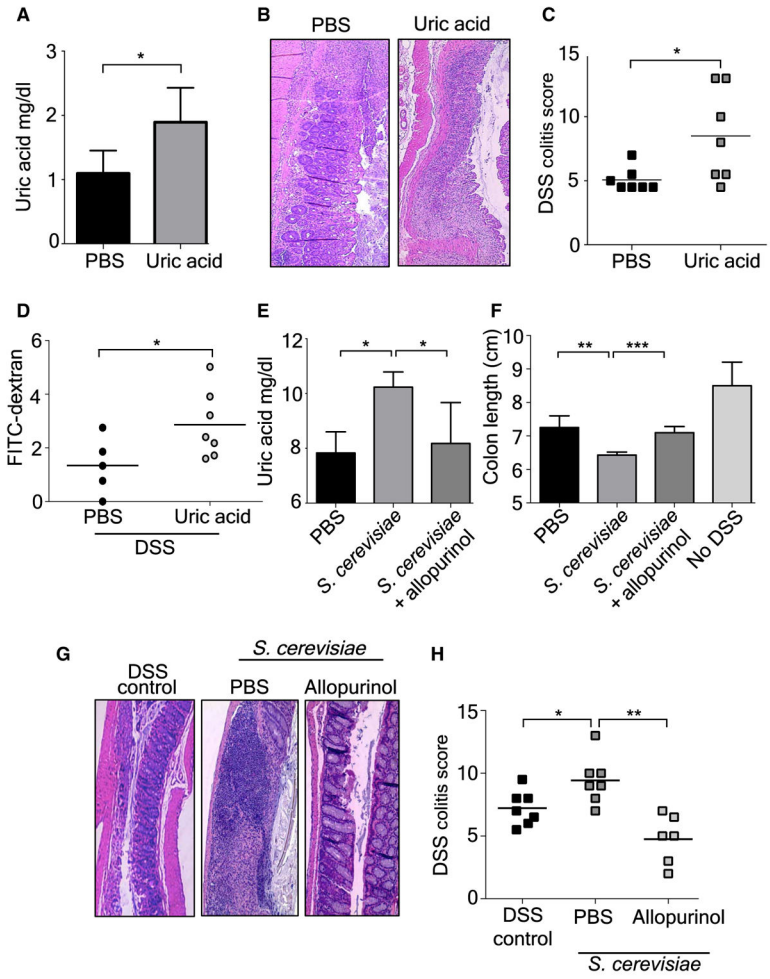

Fig. 5. *S. cerevisiae* colonization causes worsened colitis by inducing production of uric acid

(**A** to **D**) C57Bl/6 SPF mice were treated daily with uric acid 3 days before the induction of colitis with DSS. Eight days after colitis induction, animals were analyzed for fecal uric acid levels (A), disease severity were analyzed by histology (B and C), and intestinal barrier penetration were analyzed by FITC-dextran (D). These data are representative of three independent experiments with five to seven animals in each group per experiment. (**E** to **H**) C57Bl/6 animals were treated with *S. cerevisiae* ($n = 4$) as described in Fig. 1. Animals were also treated with allopurinol ($n = 4$) or vehicle control ($n = 2$). Eight days after colitis, animals were analyzed for serum uric acid levels (E), colon length (F), and colitis severity as determined by histology of H&E-stained colon sections (G and H). These data are representative of two independent experiments with five to seven mice per group in each experiment. *$P < 0.05$; **$P < 0.01$, ***$P < 0.005$, *t* test.