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Research Article

Gut Commensal Fungi Promote Th17 Polarization Through Dectin-1/Syk Signaling, Thereby Exacerbating the Immunopathology of Inflammatory Bowel Disease

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Abstract

Although inflammation in colitis is attributed to intestinal fungal dysbiosis, we have not yet investigated how commensal fungi affect immune system immunopathology. To answer the question if fecal fungi promote an inflammation in intestinal mucosa by promoting Th17 cell polarisation via Dectin-1/Syk signalling, we study the immunopathological role of fungi in colritis using Dextran sulphate sodium (DSS) induced acute and chronic colitis animal models along with broad-spectrum antifungal clearance and specific symbiotic fungus (Candida albicans) recolonisation. We used Flow cytometry, immunofluorescence, fluorescence in situ hybridisation and 16S/ITS2 rRNA gene sequencing to study colonic lamina propria Th17 cells (IL-17A^{+}CD4^{+}) and intestinal mucosal fungal load and composition using Clec7a$^{-/-}$ (Dectin1 knockout) mice and Syk-specific inhibitor R788. The direct effect of fungal ligand binding on Th17 development was confirmed in vitro by co-culturing naïve CD4: T cells and bone marrow-derived dendritic cells (BMDCs). The gut mucosa of IBD patients and colitis mice is significantly increased by a large amount of fungel load and contents, while Candida growth is observed. Th17 infiltration, pathology and colon inflammation caused by DSS are all significantly reduced by the inclusion of funcolium clearance, but recolonised by C. This effect is reversed by albican. In vivo mechanistic studies showed that in vivo Clec7a$^{-/-}$ and R7888 groups have significantly lower colitis, lower Th17 propria and mesenteric lymph nodes, and lower expression of IL-6 and IL-23 compared to wild-type mice. Fungal β-glucan stimulate BMDCs to produce IL-4 and IL-23 by activating Dectinus-1 / Syk/CARD9 signalling. Fitted innate immune cells promote th17 cell polarization through the Decttin-1 signaling pathway, and alter intestinal immune system by reprogramming innate immune cell

responses via DCTN. In vitro mechanistic experiments suggest that Clec 822 and R8788 groups suffer much less serious colitis disorders, smaller Th17-type mouse proportions and lower expressions of IL6 and the IL-22 compared to a wild-like.

Keywords

Inflammatory bowel disease, Intestinal fungal microbiota, Dectin-1, Syk, Th17 cells, Immunopathology

1. Introduction

Crohn's disease (CD), ulcerative colitis (UC), and other chronic recurring intestinal inflammatory illnesses together referred to as inflammatory bowel disease (IBD) are caused by a combination of environmental factors, gut microbiota, dysregulated mucosal immune responses, and genetic vulnerability. One of the primary processes causing intestinal tissue damage at the immunopathological level is thought to be imbalance among CD4 T cell subsets, namely disturbance of the equilibrium between pro-inflammatory Th17 cells and regulatory Treg cells. By secreting effector chemicals such interleukin-17A (IL-17A), IL-17F, and GM-CSF, Th17 cells attract neutrophils and amplify the pro-inflammatory response of epithelial cells. Overactivation of the IL-23/Th17 axis is strongly linked to IBD severity and susceptibility, according to both preclinical models and genome-wide association studies (GWAS) (Duerr et al., 2006; McGovern et al., 2015). Antibodies that specifically target IL-17A, however, are not very effective in treating Crohn's disease and may even make the condition worse in some patients (Hueber et al., 2012). This suggests that there is still much to learn about the delicate upstream regulatory network of Th17 polarisation, one of which is how microbial-derived signals influence Th17 responses.

Gut microbiome research has traditionally focused on bacterial communities. In recent years, it has increasingly been clear how important the fungus population (mycobiome), an important component of the gut microecosystem, is to the pathophysiology of IBD. Clinical cohort studies based on ITS2 rRNA gene sequencing have shown that the composition of gut fungus in IBD patients is significantly dysregulated (Sokol et al., 2017). The main signs are an increase in Candida species and a decrease in symbiotic fungi such as Saccharomyces cerevisiae. Inflammatory marker levels and disease activity indices are correlated with changes in gut fungus diversity. Animal studies have shown that oral antifungal drugs can significantly lessen chemically induced colitis (Iliev et al., 2012), although re-colonization with particular symbiotic fungus might rekindle intestinal inflammation (Chiaro et al., 2017; Jawhara et al., 2008). These findings strongly suggest that gut symbiotic fungi are active participants in the immunopathology of IBD rather than passive colonisers. The specific host recognition mechanisms by which symbiotic fungi convert symbiotic signals into detrimental Th17 immune responses, however, are still poorly understood.

The C-type lectin receptor Dectin-1 (encoded by the Clec7a gene) is the primary receptor for identifying fungal cell wall β-glucans. The host immune system uses pattern recognition receptors (PRRs) to monitor fungal components. Following its binding to its ligand, Dectin-1 uses its intracellular ITAM motif to recruit and activate spleen tyrosine kinase (Syk). It then uses the CARD9-Bcl10-MALT1 signalling complex to stimulate the production of important cytokines like IL-6 and IL-23 that support Th17 differentiation in macrophages and dendritic cells (DCs) (LeibundGut-Landmann et al., 2007; Robinson et al., 2009). Dectin-1 signalling has a role in maintaining the mucosal barrier and antifungal defence in intestinal homeostasis, but its disruption is also linked to the pathophysiology of intestinal inflammation. Notably, Dectin-1 defective animals have diverse symptoms in colitis models, and mutations in the CLEC7A gene are strongly linked to a refractory UC subtype (Iliev et al., 2012). The Dectin-1/Syk axis may play distinctly different immunoregulatory roles at different stages of colitis or under the influence of different dominant fungi, according to some studies that have demonstrated increased susceptibility to DSS-induced colitis (Biagioli et al., 2016; Iliev et al., 2012), while others have found that blocking Syk can reduce intestinal inflammation (Taylor et al., 2007). There is currently insufficient systematic evidence to establish that commensal fungi in the gut directly cause pathogenic Th17 polarisation via the Dectin-1/Syk pathway, aggravating IBD.

Given the aforementioned research scenario, we have proposed the following scientific hypothesis: By activating the Dectin-1/Syk signalling axis, the overabundance of symbiotic fungi in the inflammatory intestinal microenvironment—such as the genus Candida—reprograms intestinal innate immune cells, promotes aberrant polarisation of Th17 cells, and ultimately exacerbates the immune pathology of IBD. By methodically analysing the causal mechanism of the fungi-Dectin-1/Syk-Th17 axis in the aggravation of colitis using a DSS-induced colitis model, fungal clearance/recolonization, Clec7a knockout mice, and pharmacological intervention of Syk, this study seeks to provide a theoretical basis for novel IBD treatment strategies that target the fungi-host recognition pathway.

2. Literature Review

Th17 cells are a subset of CD4 T cells that secrete the effector cytokines IL-17A, IL-17F, and IL-22 and express the transcription factor RORγt. They are both harmful and preserve homeostasis in intestinal mucosal immunity. The IL-23/Th17 pathway is linked to several risk loci found by genome-wide association studies (GWAS) in the context of IBD (Duerr et al., 2006; McGovern et al., 2015). These loci include IL23R, IL12B, STAT3, and JAK2, and it has been demonstrated that this system plays a crucial role in disease susceptibility. Further research on animals demonstrated that whereas Treg cells block Th17-mediated excessive inflammation by secreting IL-10, IL-23-driven Th17 cells develop encephalitogenic and colitogenic effector phenotypes by generating GM-CSF and IFN-γ. However, in clinical trials for Crohn's disease, the monoclonal antibody Secukinumab, which targets IL-17A, failed

to achieve the primary endpoint (Hueber et al., 2012). In fact, a trend of disease worsening emerged. This unexpected outcome led the academic community to re-examine the role of upstream regulatory signals in shaping Th17 pathogenicity. Subsequent studies have focused heavily on how particular microbial signals modify the polarisation threshold and effector functions of Th17 via innate immune cells.

High-throughput sequencing technology has uncovered the abnormalities in the gut fungal microbiota in IBD during the last ten years. Sokol et al. (2017) discovered a significant reduction in gut fungal diversity in IBD patients through ITS2 sequencing of faecal samples from CD and UC patients. The ratio of Basidiomycota to Ascomycota is inverted, with a decrease in Saccharomyces cerevisiae abundance and an increase in pro-inflammatory fungi like Candida albicans, Candida tropicalis, and Debaryomyces hansenii. The increase of Candida is strongly connected with the IBD disease activity index, C-reactive protein levels, and endoscopic severity, and the fungal composition tends to return to normal following anti-TNF-α treatment, according to subsequent multicenter cohort studies. In terms of mechanism investigation, oral broad-spectrum antifungal medications (like fluconazole) can considerably reduce colonic IL-17A^{+}cell infiltration and alleviate colitis caused by DSS and TNBS in mice (Iliev et al., 2012). On the other hand, intestinal colonisation with Malassezia restricta or supplementation of Candida albicans via gavage can reactivate or worsen intestinal inflammation (Bussel et al., 2018; Chiaro et al., 2017; Jawhara et al., 2008). According to these research, the fungal microbiota is now considered a “active participant” rather than a “bystander” in the immunopathology of IBD. It should be noted, though, that the majority of research is still in the descriptive correlation stage, and it is still unclear how fungi use host immune recognition pathways to trigger pathogenic T cell responses.

The main C-type lectin receptor on the surface of myeloid cells that identifies β-1,3-glucans in the fungal cell wall is called Dectin-1 (encoded by CLEC7A). Src family kinases phosphorylate its intracellular ITAM motif upon ligand binding, attracting and activating Syk. Syk then triggers the NF-KB and MAPK pathways through the CARD9-Bcl10-MALT1 complex, causing the expression of pro-inflammatory factors like TNF-a, IL-6, and IL-23, which form the microenvironmental basis for Th17 differentiation. Dendritic cells activated by Candida albicans or pure β-glucan have been shown in vitro to enhance naïve T cell polarisation towards Th17 in a Dectin-1/Syk-dependent manner (LeibundGut-Landmann et al., 2007; Robinson et al., 2009). Dectin-1 signalling plays a highly context-dependent role in intestinal immunity. A common polymorphism of the CLEC7A gene (Y238X) is linked to severe subtypes of ulcerative colitis, suggesting that this pathway is essential for maintaining intestinal immune homeostasis. On the one hand, Clec7a$^{-/-}$mice exhibit increased susceptibility to intestinal infection by C. albicans (Iliev et al., 2012; Powrie et al., 1994), suggesting that Dectin-1 plays a protective role in antifungal defence. However, Dectin-1’s role in non-infectious colitis models varies: some studies report

that Clec7a$^{-/-}$mice are more susceptible to DSS colitis, attributing it to mucosal barrier damage and fungal overgrowth; other studies have found that T cell transfer colitis can be alleviated by using the Syk inhibitor Fostamatinib (R788) (Taylor et al., 2007). And lower the percentage of Th1 and Th17 cells in the innate layer. This phenotypic heterogeneity raises the possibility that distinct dominant fungi may cause essentially different downstream immunological effects through Dectin-1/Syk, either harmful Th17 polarisation or beneficial fungal clearance responses, under preexisting inflammatory circumstances. In a colitis model powered by symbiotic fungi, this crucial question has not yet been directly and methodically verified.

According to the aforementioned literature, the current research has the following primary limitations: the full evidence chain of "commensal fungi → host recognition receptors → Th17 polarisation → exacerbation of colitis" is still lacking, although the causal relationship between fungal dysbiosis and the aggravation of IBD has been tentatively established in animal models. The majority of research still only correlates alterations in fungal composition with markers of inflammation. The lack of control over the fungal microbiota background makes it difficult to determine whether the Dectin-1/Syk signalling pathway's function in colitis models is a direct result of commensal Candida in the gut or an indirect result of secondary fungal infection. Although Dectin-1/Syk can enhance DCs' capacity to polarise Th17 cells in vitro, there is currently insufficient experimental evidence using conditional gene knockout or selective signal blockade to determine whether this pathway directly controls Th17 cell differentiation in the mucosal lamina propria and influences the course of colitis in vivo.

The following are the novel contributions made by this study to fill the previously noted gaps: The Dectin-1/Syk signalling pathway was identified in vivo for the first time as the crucial pathway through which commensal fungi drive pathogenic Th17 polarisation with the aid of Clec7a systemic knockout mice and pharmacological Syk blockade; by combining in vivo and in vitro experiments, the study clarifies the causal regulatory mechanism of the fungal-Dectin-1/Syk-CARD9-IL-6/IL-23 axis, offering a new theoretical foundation and possible target for treating the fungal-host recognition interface.

3. Research Methods

Male SPF-C57BL/6J WT mice 6–8 weeks old. Each mouse was free from food and water, 12 hours light-dark and 32±1°C. IBD (CD and UC) and healthy subjects had colonoscopies of sigmoid or ascending colon mucosal biopsy samples. The clinical, endoscopic and pathology criteria were used in order to diagnose the mice. Patients taking antifungal drugs during the previous three months did not participate. Biopsy samples were either stored in 4% paraformaldehyde for histology, or put right out in RNAlater for RNA extraction. Mice were split six to ten randomly. Drinking water with 2.5% (w/v) dextran sulphate sodium (DSS, molecular weight 36-50 kDa, MP Biomedicals) induced acute colitis

seven days at once; 3 runs with 2% DSS in drinking water and 10 days of normal drinking water induced chronic colitis. Syk inhibition experiment: One day before DSS modelling, R788 (Fostamatinib, Selleck) was diluted in 0.5 % sodium carboxymethylcellulose and administered orally at 80 mg/kg/day. A third amount of solvent was administered to the control group. Fluconazole (0.5 mg/mL, Sigma-Aldrich) was given by drinking water in both at night and throughout during DSS. The medication was changed every two days. Faecal ITS region qPCR and culture was used to confirm fungal eradication effectiveness.

We used male C57BL/6 mice. Based on fluconazole decontamination, 200 μL of a 1×108CFU/mL solution of Candida albicans (strain SC5314) was gavaged every day until the experiment's conclusion, beginning three days before to DSS modelling. An equivalent volume of sterile PBS was gavaged into the control animals. After being cultivated in YPD liquid medium at 30°C with shaking until the log phase, C. albicans was washed three times with PBS, resuspended, and counted.

Himatoxylin-eosin (H&E) stained colon tissue fixed in 4% paraformaldehyde, paraffin and staining at 5 m. Two pathologists (left to right), with respect to the grouping criterion, scored on three criteria: ulcer area (0–4 points), crypt structural damage, inflammatory cells infiltration. The overall histological damage score of colon tissue was estimated by taking the three score into account. To remove the epithelial layer, colon tissue were opened with PBS, cut into 5 mm pieces, immersed in HBSS with 5 mM EDTA and agitated at 37 c. After washing the tissue with RPMI 1640 for 45 min with 0.5 mg/mL collagenase IV (Sigma) and 0.1 mg/L DNase I (Roche), Lamina propria mononuclear cells (LPMCs) were enriched with Percoll density gradient centrifugation (40/80% interface) as they were filtered through 70 ns mesh screen.After 4-5 hours of culture at 37C (PMA (50 ng/mL), ionomycin (1 μg/mL) and Brefeldin A (5 μg/L), dead cells were extracted using Fixable Viability Dye eFluor 780. After fixation and permeabilization, IL-17A and IFN-γ were stained after surface labelling for CD45, CD3 and CD4. BD LSRFortessa was used for flow cytometry, FlowJo v10 was used. Supplementary Table 1 summarizes antibody data（Table 1）.

Table 1

Serial number	Target antigen	Coupling / Labeling	Clone number	Host / Isotype	Supplier	Item Number	Application and dilution ratio
1	CD45	APC-Cy7	30-F11	Rat IgG2b, κ	BD Biosciences	557659	Flow cytometry (1:200)
2	CD3e	FITC	145-2C11	Armenian Hamster IgG	BD Biosciences	553062	Flow cytometry (1:100)

3	CD4	PerCP-Cy5.5	RM4-5	Rat IgG2a, κ	BD Biosciences	550954	Flow cytometry (1:200)
4	IL-17A	PE	TC11-18H10	Rat IgG1, κ	BD Biosciences	559502	Intracellular flow cytometry(1:100)
5	IFN-γ	APC	XMG1.2	Rat IgG1, κ	BioLegend	505810	Intracellular flow cytometry (1:100)
6	Fixable Viability Dye	eFluor 780	-	-	Thermo Fisher (eBioscience)	65-0865-14	Flow cytometry (1:1000)
7	CD4	Purification (unlabeled)	RM4-5	Rat IgG2a, κ	BioLegend	100502	Immunofluorescence(1:50)
8	IL-17A	Purification (unlabeled)	Polyclone	Rabbit IgG	Abcam	ab79056	Immunofluorescence (1:100)
9	Phospho-Syk (Tyr525/526)	Purification (unlabeled)	Polyclone	Rabbit IgG	Cell Signaling Technology	2710S	Western blot(1:1000)
10	Syk	Purification (unlabeled)	Polyclone	Rabbit IgG	Cell Signaling Technology	2712S	Western blot (1:1000)
11	CARD9	Purification (unlabeled)	Polyclone	Rabbit IgG	Abcam	ab133560	Western blot (1:1000)
12	β-actin	HRP direct labeling	Polyclone	Rabbit IgG	Cell Signaling Technology	5125S	Western blot (1:5000)
13	Goat anti-Rabbit IgG (H+L)	HRP	Polyclone	Goat	Jackson ImmunoResearch	111-035-144	Western blot(1:10000)
14	Goat anti-Rat IgG (H+L)	Alexa Fluor 488	Polyclone	Goat	Thermo Fisher (Invitrogen)	A-11006	Immunofluorescence (1:500)

15	Goat anti-Rabbit IgG (H+L)	Alexa Fluor 594	Polyclone	Goat	Thermo Fisher (Invitrogen)	A-11012	Immunofluorescence(1:500)

Note. Every antibody was diluted and incubated in accordance with the guidelines provided by the manufacturer. Fixable Viability Dye eFluor 780 was used in flow cytometry investigations to eliminate dead cells. Secondary antibodies were utilised to boost the signal in immunofluorescence investigations, and their species specificity matched that of the original antibodies' host.

After dewaxing and antigen retrieval, paraffin sections were incubated overnight at 4°C with rabbit anti-mouse IL-17A (Abcam) and mouse anti-CD4 (BioLegend). Fluorescently labelled secondary antibodies were then used for detection. DAPI was used as a counterstain for nuclei. Using a Cy5-labeled pan-fungal 18S rRNA probe (5-CTCTGGCTTCACCCTATTC-3), fungal fluorescence in situ hybridisation (FISH) was carried out. Following two hours of hybridisation with tissue slices at 50°C, the sections were cleaned and mounted. A Leica SP8 confocal microscope was used to capture the images.

The QIAamp PowerFecal DNA Kit was used to extract DNA from colonic contents and faeces. Using universal primers for the 18S rRNA gene, the total fungal load was measured by SYBR Green real-time PCR and normalised to bacterial 16S rRNA gene primers. ITS2 area amplicon sequencing was used to analyse the makeup of the gut fungal population; primers ITS3F/ITS4R were used for amplification, and the Illumina NovaSeq PE250 platform was used for sequencing. ASV clustering and taxonomic annotation based on the UNITE database were applied to the raw sequencing data using the QIIME2 methodology. In order to rule out major disruptions to the bacterial community brought on by fungal intervention, sequencing of the bacterial 16S V3-V4 region was done concurrently.

BMDCs grown for 7 days in RPMI 1640 (20 ng/mL GM-CSF) from bone marrow cells of femurs and tibias of WT and Clec7a$^{-/-}$ mice. BMDCs were placed at a density of 1×106/mL and either with either (2 ng/ml, Sigma) or (3 μg/mL, MOI=5) heat-inactivated Candida albicans yeast, one was pretreated with R788 (1M). Cell supernatants were obtained for ELISA detection of IL-6 and IL-23 after 6 hours. stimulated BMDC. Stimulated BMDC with magnetically sorted initial T cells CD4^{+}CD62L^{+} Miltenyi were grown at 1:5 in CD3 (1 μg/ml), and TGF-β (2g/mL). T cells were collected for analysis of IL17A expression using flow cytometry. Biologist, K., K., Nograult, A, X-Comand, and B, were utilized. The BMDC was lysed in RIPA (phosphatase and protease inhibitors). SDS-PAGE separated equal amounts of proteins. They were transferred to PVDF membranes, blocked with 5% BSA, and incubated overnight at 4°C with primary antibodies to p-Syk (Tyr525/526), Syk, CARD9 and β-actin and then they were grown with ECL, measured greyscale using ImageJ, and combined with secondary antibodies associated with HRP. (Commercial ELISA kits eBioscience/BD) of 4, 15, 4 and 8 pg/mL for

test of IL6, IL-22, IL17 A, and TNF-α in cell cultures of granular and colon tissue. We used the following two groups. Using our CQ9, we used the Mann-Whitney U test or unpaired t-test. One-way/2-way ANOVA was used to compare groups, and Tukey/Bonferroni post hoc tests were used. Kaplan-type tests.

4. Experimental Results

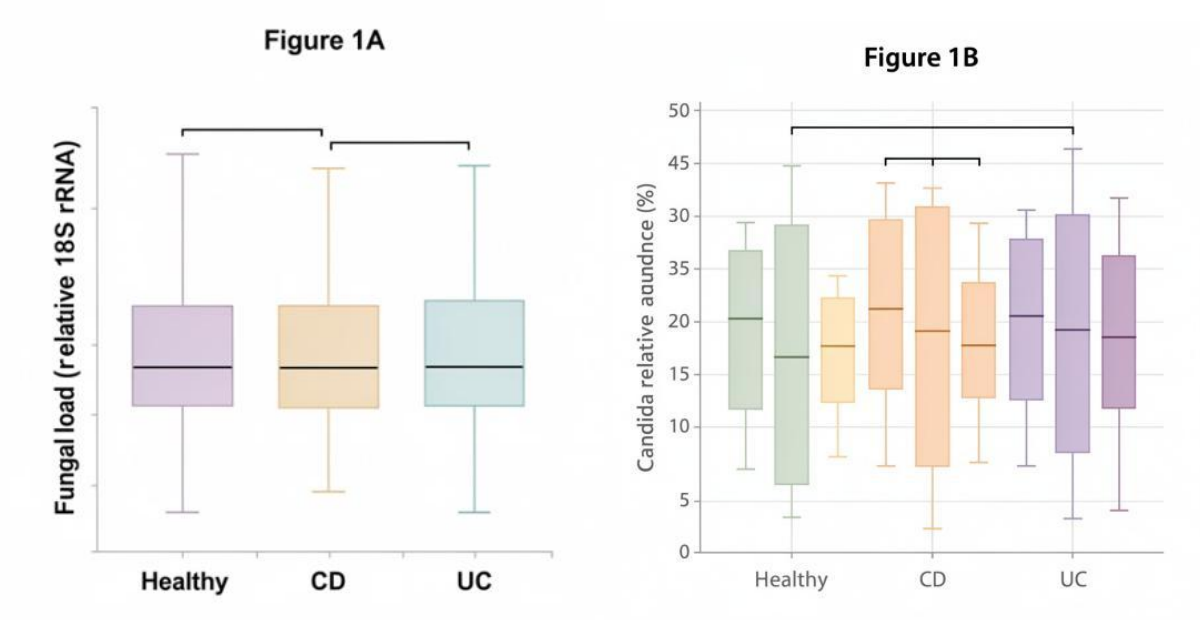
4.1. Dysbiosis of intestinal fungi in IBD patients in active stage and colitis mice accompanied by enhanced Th17 immunity

Initially, we examined the fungal load in colonic mucosal biopsy tissues from patients with ulcerative colitis (UC, n = 16), active Crohn's disease (CD, n = 18), and healthy controls (n = 15). Results from 18S rRNA qPCR and fluorescence in situ hybridisation (FISH) revealed that the mucosal fungal burden was 2.9 times higher in UC patients and 3.8 times higher in CD patients when compared to controls ($P < 0.01$, Fig. 1A). ITS2 amplicon sequencing showed that a notable growth of the genus Candida, with a proportion of C, was the primary characteristic of the fungal communities in IBD patients. Albicans increased in patients from 11.3% to 34.7% ($P < 0.001$, Fig. 1B). According to immunofluorescence co-staining, patients with active IBD had significantly more IL-17A$^+$CD4$^+$Th17 cells in their lamina propria than controls ($P < 0.001$, Fig. 1C), and there was a positive correlation between the density of Th17 cells and mucosal fungal load ($r = 0.62$, $P < 0.001$, Fig. 1D).

Figure 1

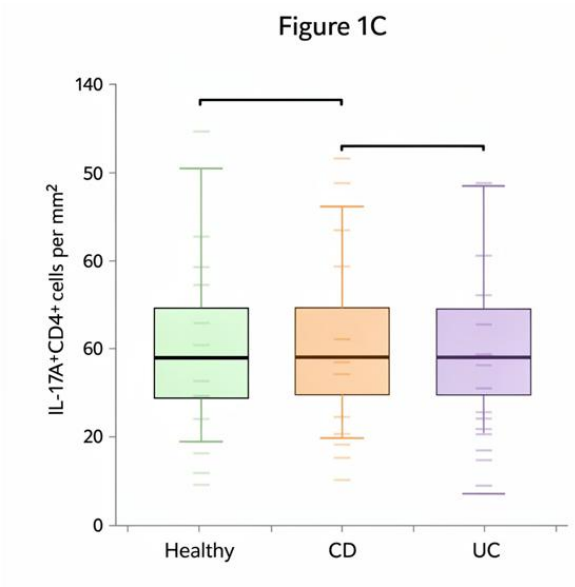
A. Mucosal fungal load by qPCR

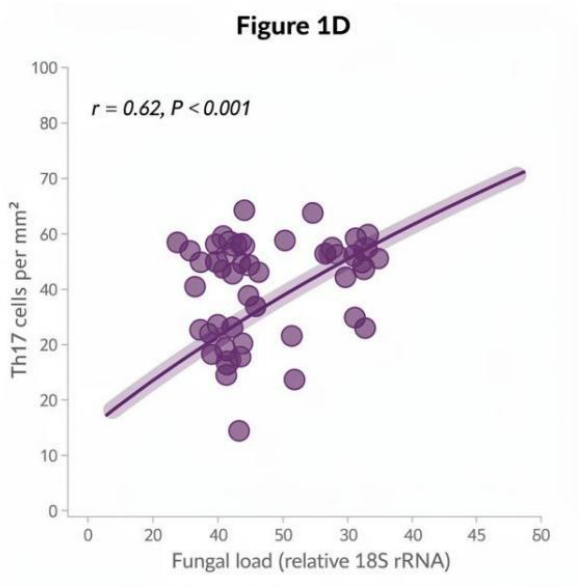
B. Candida abundance by ITS2 sequencing



C. Lamina propria Th17 cells

D. Correlation: fungal load vs Th17



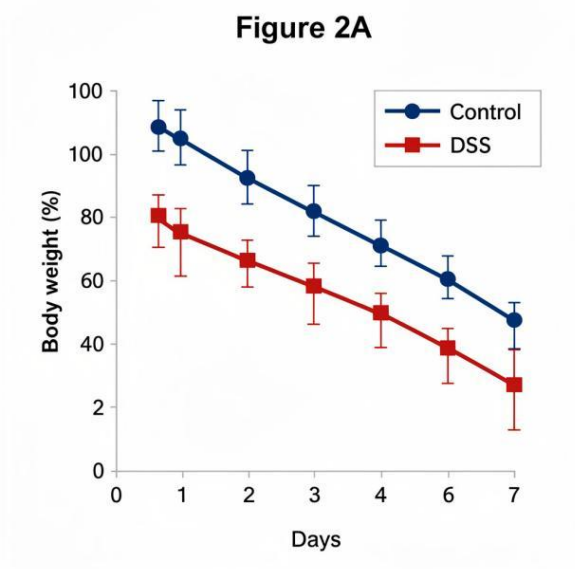


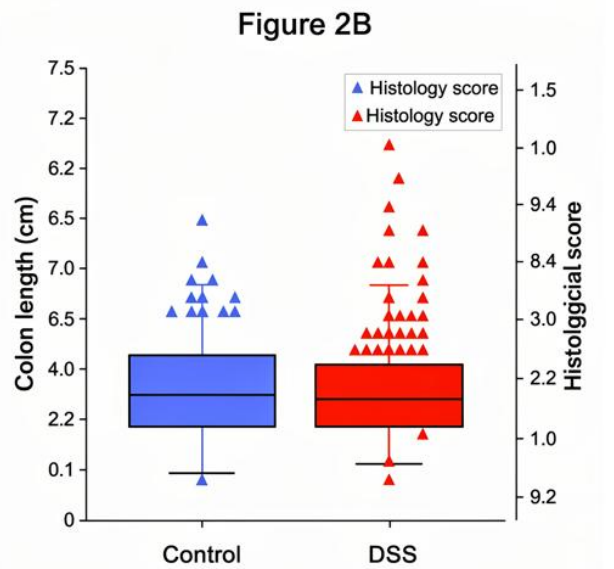
Both acute and chronic mouse colitis models caused by DSS showed a similar pattern. The relative abundance of the genus Candida in the microbial community increased from a baseline of 2.8% to 28.5% in acute colitis mice (DSS 7 days), and the faecal fungal load increased considerably by day 5, peaking at 12.4 times that of the control group ($P < 0.01$). The percentage of $CD45^+$ $CD3^+$ $CD4^+$ IL-$17A^+$ Th17 cells rose from 1.8±0.3% in the control group to 5.7±0.6% ($P < 0.01$) according to flow cytometry analysis of colonic lamina propria immune cells, while the percentage of IFN-γ^+ $CD4^+$ Th1 cells did not differ statistically significantly (Figure 2A–D). In the chronic colitis model, there was significant crypt structural degradation and inflammatory infiltration along with a high number of Th17 cells.

Figure 2

A. Body weight change (DSS vs Control)

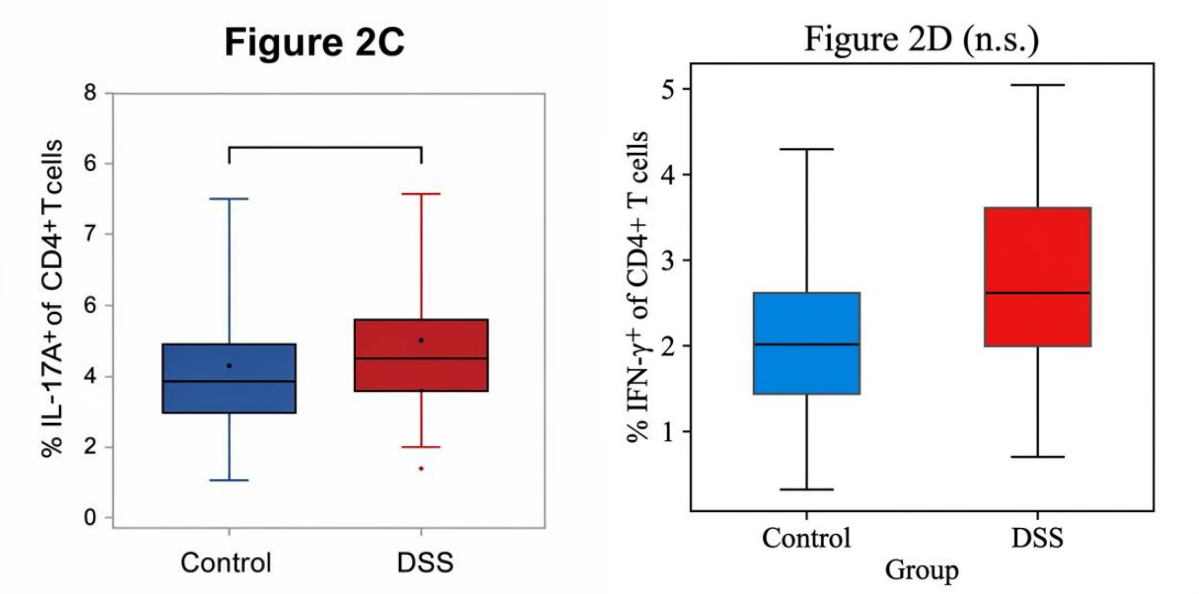
B. Colon length & histology score





C. Th17 cells in lamina propria (DSS vs Control)

D. Th1 cells (n.s.)



4.2. Clearing intestinal fungi can alleviate the severity of colitis and inhibit Th17 cell infiltration

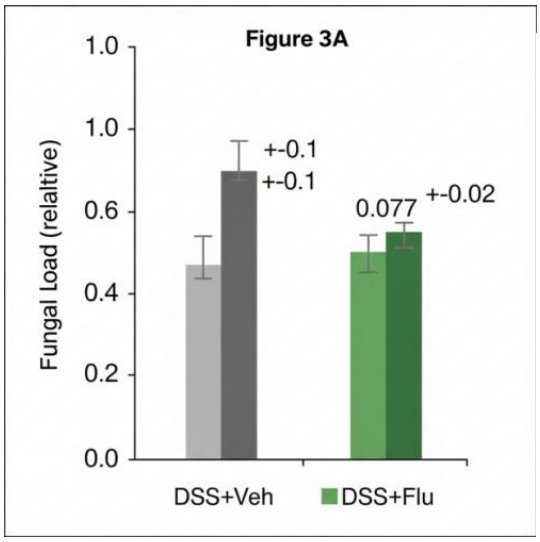
We eliminated intestinal fungus in the DSS acute colitis model by adding fluconazole (Flu) to the drinking water in order to ascertain if the fungal microbiome directly causes intestinal inflammation. Flu therapy decreased faecal fungal load by 92.3% ($P < 0.001$, Figure 3A), according to qPCR verification. Mice in the DSS+Flu group lost significantly less body weight (Day 7: -8.2% vs -18.5%, $P < 0.01$), their colon length shrank less (6.8±0.3 cm vs 5.2±0.4 cm, $P < 0.01$), and their overall histopathological score dropped from 9.2±1.1 to 4.5±0.8 ($P < 0.01$, Figure 3B–D).Flu intervention decreased the percentage of Th17 cells in the colonic lamina propria from 5.9±0.5% to 2.4±0.3% ($P < 0.001$) according to flow cytometry analysis, getting closer to the level of the normal control group (Figure 3E). Colon tissue homogenates showed a significant ($P < 0.01$) decrease in IL-17A protein levels as well as varied degrees of reduction in pro-inflammatory factors such TNF-α and IL-6. These findings suggest that DSS-induced Th17-type intestinal inflammation can be successfully reduced by lowering the fungal load.

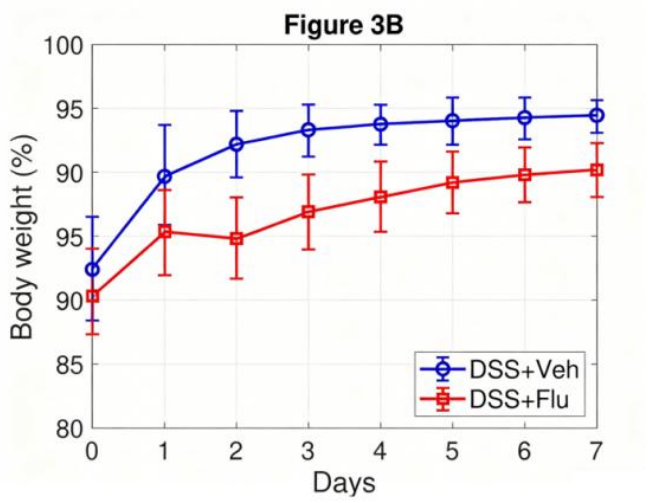
Figure 3

A. Fungal clearance (qPCR)

B. Weight change (Flu vs Veh)

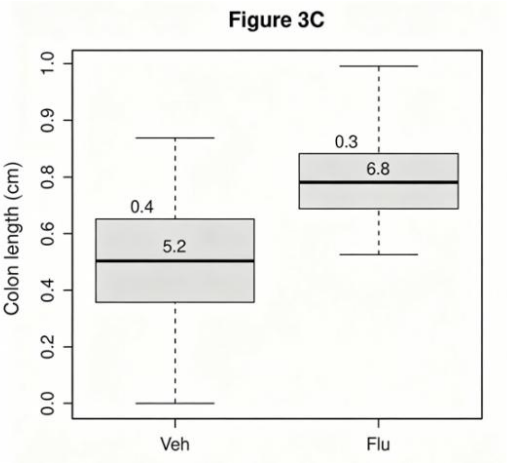
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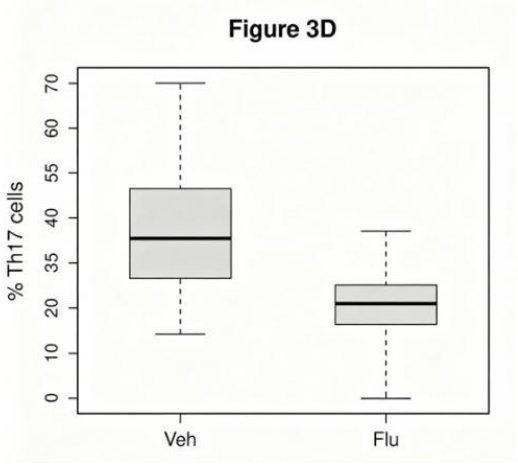




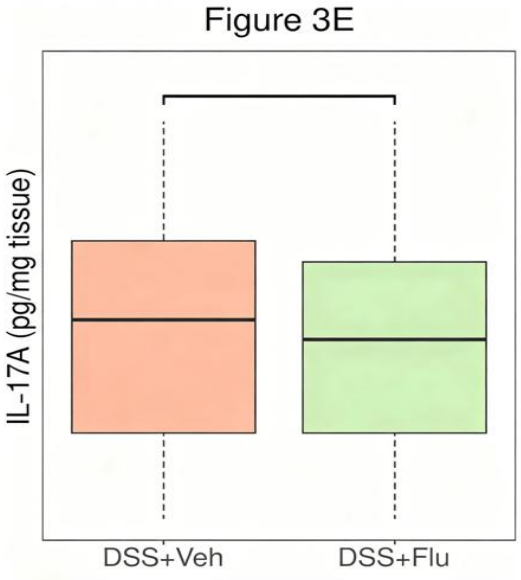
C. Colon length (Flu vs Veh)

D. Th17 infiltration (Flu vs Veh)





E. Colonic IL-17A protein (ELISA) after fungal clearance



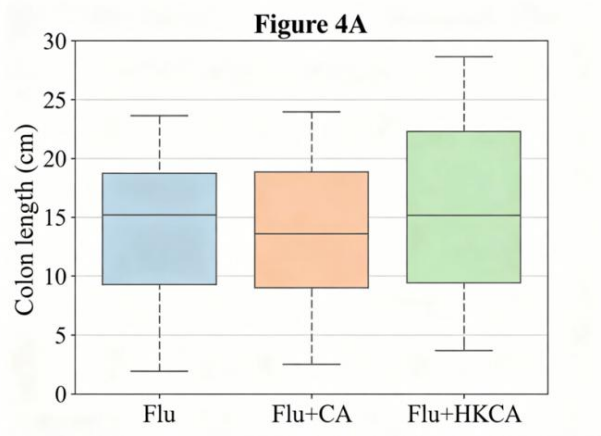
4.3. Recolonization of candida albicans reactivates fungi-dependent Th17 inflammation

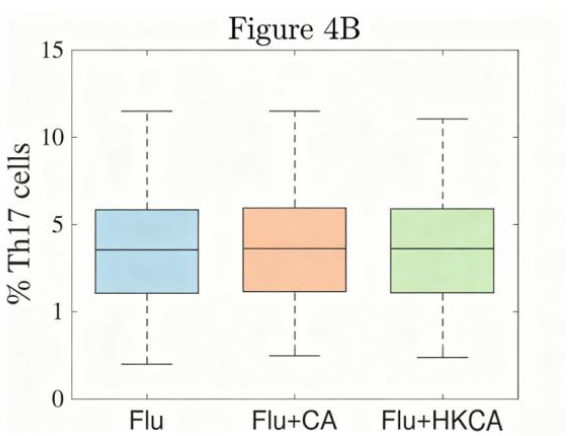
We carried out daily gavage of Candida albicans (CA) for re-colonization based on Flu-mediated fungal clearance in order to confirm the pathogenic involvement of the genus Candida. Re-colonization increased the relative abundance of Candida albicans in faeces to 21.6%, according to ITS sequencing. Mice in the DSS+Flu+CA group had more severe colitis than those in the DSS+Flu group. They lost more body weight (day 7: -16.4% vs -8.2%, $P < 0.01$), had a shorter colon (5.6±0.3 cm vs 6.8±0.3 cm, $P < 0.01$), and had higher histological scores (7.8±0.9, $P < 0.01$). Lamina propria Th17 cell recovery increased from 2.4±0.3% to 5.1±0.4% ($P < 0.001$, Fig. 4A–D).accompanied with increased expression of IL-6 and IL-17A in the colon. Re-colonization with an equivalent amount of heat-killed S. cerevisiae or Candida albicans, on the other hand, did not significantly aggravate colitis or increase Th17 cells, suggesting that pathogenic Th17 responses require live fungus and their hyphal-associated signals. These findings demonstrate a causal connection between Th17-mediated intestinal inflammation and commensal Candida overgrowth.

Figure 4

A. Colon length (Candida recolonization)

B. Th17 cells (Candida recolonization)

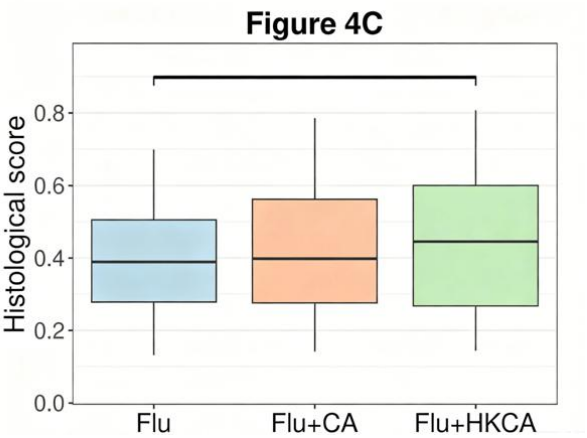


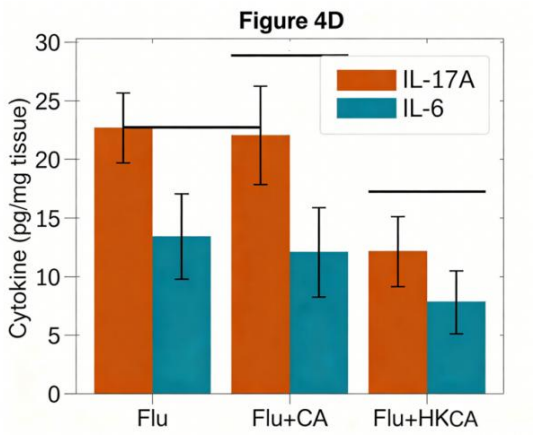


C. Histological score (Candida recolonization)

D. Colonic cytokine levels (IL-17A and IL-6)

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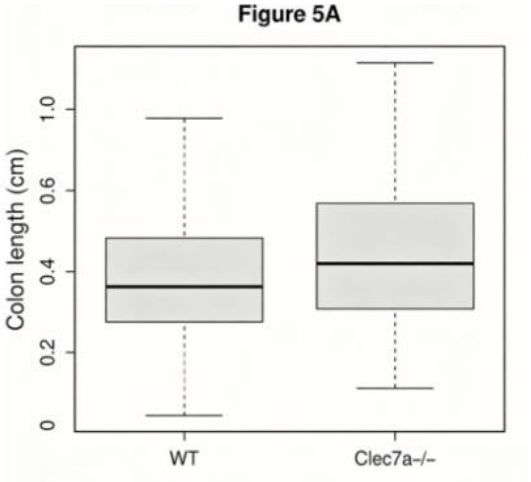
4.4. Dectin-1 deficiency can inhibit Th17 polarization and alleviate colitis

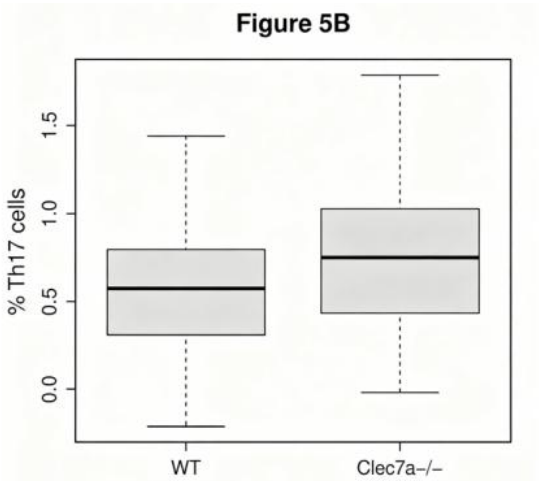
We employed Clec7a$^{-/-}$ mice to simulate DSS-induced colitis in order to study the primary receptor by which the host recognises fungus. The Clec7a$^{-/-}$ DSS group demonstrated significantly reduced body weight loss (day 7: -9.3% vs -18.5%, $P < 0.01$), longer colon length (6.4±0.4 cm vs 5.2±0.4 cm, $P < 0.01$), and histological scores decreased from 9.2±1.1 to 5.1±0.9 ($P < 0.01$, Figure 5A–C) in the absence of fluconazole-mediated fungal clearance. Significantly, the percentage of Th17 cells in the colonic lamina propria dropped from 5.9±0.5% to 2.1±0.4% ($P < 0.001$, Figure 5D), and the same trend was seen in the percentage of Th17 cells in lymphocytes from mesenteric lymph nodes. Th1 and Treg cell proportions did not significantly change, although IL-17A mRNA and protein levels were decreased in colon tissues. Interestingly, there was no discernible change between the faecal fungal load of Clec7a$^{-/-}$ mice and WT mice, indicating that Dectin-1 deficiency exclusively prevents downstream pro-inflammatory signals generated by fungi rather than affecting fungal burden. Immunoblotting revealed that while p-Syk signals were hardly noticeable in Clec7a$^{-/-}$ mice, DSS-induced colitis elevated Syk phosphorylation (p-Syk) levels in WT colon tissues (Figure 5E).

Figure 5

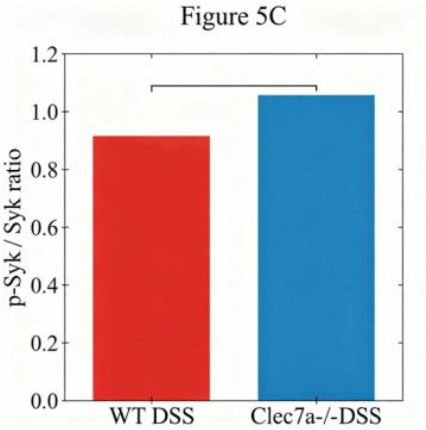
A. Colon length (WT vs Clec7a-/-)

B. Th17 cells (WT vs Clec7a-/-)



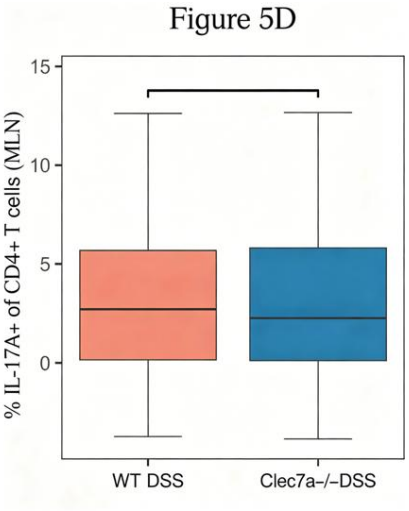


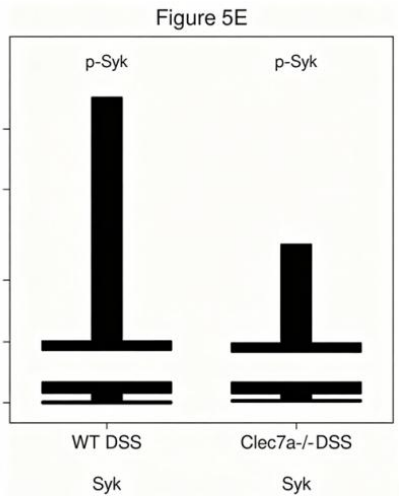
C. p-Syk activation



D. Th17 cells in mesenteric lymph nodes (WT vs Clec7a$^{-/-}$)

E. Representative p-Syk Western blot





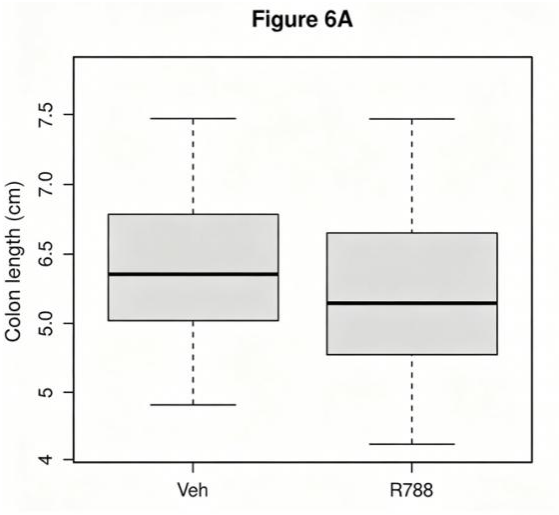
4.5. Syk pharmacological inhibition mimics the protective effect of Dectin-1 deficiency

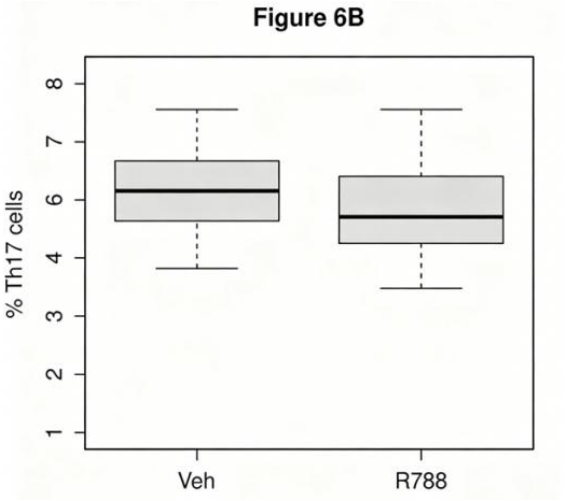
We administered the Syk inhibitor R788 to WT DSS mice in order to verify the involvement of the downstream Syk kinase of Dectin-1. The R788-treated group showed a protective phenotype that was very similar to Clec7a$^{-/-}$ mice when compared to the solvent control: decreased weight loss, increased colon length (6.6±0.3 cm vs 5.1±0.5 cm, $P < 0.01$), and lowered histology scores (4.9±0.8 vs 9.2±1.1, $P < 0.01$). Th17 cell percentages dropped from 5.7±0.6% to 2.3±0.3% ($P < 0.001$, Figure 6A,B). Following R788 intervention, protein levels of IL-6 and the IL-23 p19 subunit in colonic tissue homogenates decreased by 62% and 57%, respectively ($P < 0.01$), suggesting that Syk signalling is essential for preserving the pro-Th17 cytokine milieu in the mucosal lamina propria. Further evidence that this route is predominantly dependent on the Dectin-1/Syk axis comes from the fact that subsequent treatment of R788 in Clec7a$^{-/-}$ mice did not result in an additive protective effect.

Figure 6

A. Colon length (R788 vs Veh)

B. Th17 cells (R788 vs Veh)



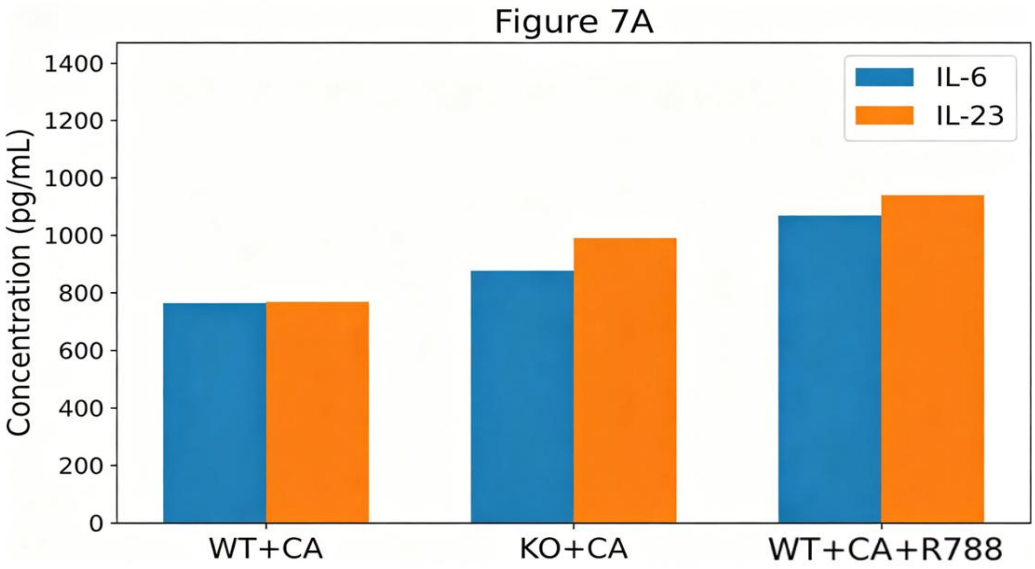


4.6. C. albicans drives dendritic cells to produce Th17-polarizing cytokines through the Dectin-1/Syk/CARD9 axis

We conducted additional in vitro validation of the mechanism. When WT mouse bone marrow-derived dendritic cells (BMDCs) were stimulated with either pure β-glucan or heat-killed Candida albicans yeast form, p-Syk rapidly increased (peaking 15–30 minutes after stimulation) and downstream CARD9 protein production was upregulated. Simultaneously, BMDCs released considerably greater amounts of IL-6 and IL-23: following C. albicans stimulation, IL-6 and IL-23 concentrations were 12.3-fold and 8.9-fold higher than those in the control group, respectively ($P < 0.001$, Figure 7A). The C. albicans-induced elevation of p-Syk and CARD9 was virtually eliminated in Clec7a$^{-/-}$ BMDCs, and the production of IL-6 and IL-23 dropped to essentially baseline levels (reduction >85%, $P < 0.001$). Similar dose-dependent suppression of IL-6 and IL-23 production was seen when WT BMDCs were pretreated with R788. Similar outcomes were obtained from CARD9 siRNA knockdown trials. These results verify that Dectin-1 stimulates the production of pro-Th17 cytokines through the Syk-CARD9 signalling axis after identifying fungus.

Figure 7

A. DC cytokine production



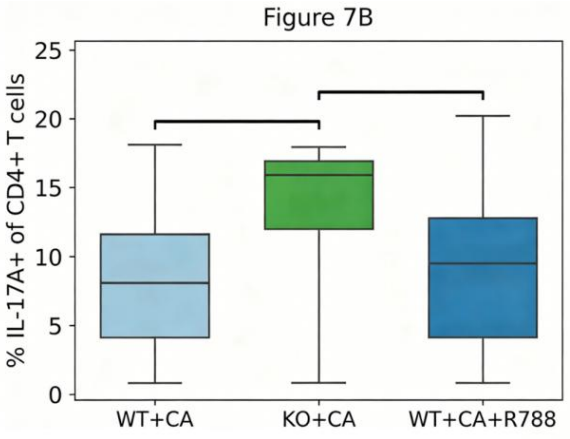
4.7. Dectin-1/Syk signaling is essential for fungus-induced Th17 differentiation in vitro

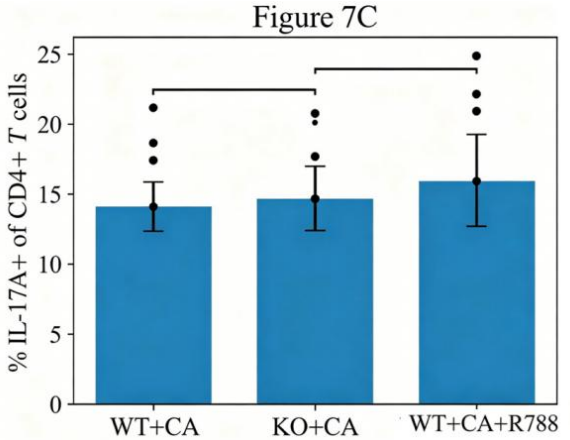
Non-polarizing (TGF-β and low-dose anti-CD3) BMDCs co-cultured naïve $CD4^+$ T cells with C. albicans. In C. bicans, IL-17A3 CD4's differentiation percentage decreased from 3.2 ± 0.4% to 18.7 ± 1.5% ($P < 0.001$). C. abicans eliminated most of its ability to stimulate Th17 polarisation with $Clec7a^{-/-}$ BMDC (4.1 ± 0.5% vs. 18. 7%, $P < 0001$). Th17 induction rate for Candida albican dropped to 5.3 ± 0.,6% after R788 on WT BMDC formulations (Figure 7B,C). The Th17 differentiation rate of $Clec7a^{-/-}$ BMDC group reduced when exogenous IL-6 and IL-23 were added to the co-culture system (Dectin-1/Syk-dependent DC cytokines are at the heart of Th17 polarization). $Clec7a^{-/-}$ abicans polarised in vitro Th17 cells by Candida, co-expressed TNF-α and GM-CSF with a pro-inflammatory effector phenotype.

Figure 7

B. Th17 polarization

C. Th17 polarization statistics (bar chart with individual points)



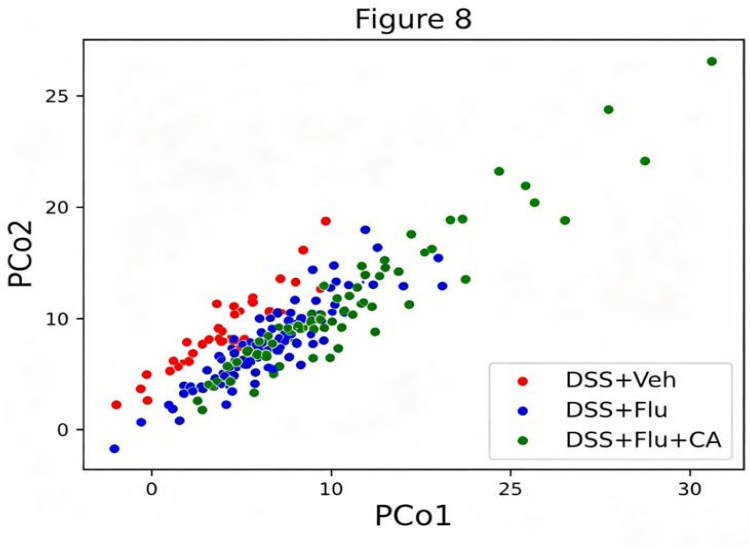


4.8. Intervention in the fungi-Dectin-1 axis does not affect the overall structure of the bacterial community

We used 16S rRNA gene sequencing on the faeces of the mice in each group to rule out the indirect impact of the aforementioned occurrences resulting from alterations in the bacterial flora. The DSS treatment itself significantly changed the structure of the bacterial community, according to principal coordinate analysis of β-diversity (Bray-Curtis). However, there was no significant clustering separation of bacterial communities between the DSS+Flu, DSS+Flu+CA, and Clec7a$^{-/-}$ DSS groups ($P>0.05$, PERMANOVA, Fig. 8). Additionally, systemic alterations linked to fungal intervention or genotype were not found by differential abundance analysis of dominant bacterial phyla and genera. These findings imply that rather than being indirectly controlled by the bacterial microbiota, the immunological and clinical phenotypes seen in this investigation are mostly driven by direct fungus-host interactions.

Figure 8

Bacterial community PCoA



All of the aforementioned findings show that the gut commensal fungus Candida stimulates lamina propria dendritic cells to secrete IL-6 and IL-23 by activating the Dectin-1/Syk signalling axis. This causes Th17 cells to proliferate pathologically and exacerbates intestinal inflammation. Th17-dependent immunopathology can be particularly reduced by targeting this pathway without increasing the fungal load.

5. Discussion

The gut commensal fungus Candida albicans causes pathological polarisation of Th17 cells in the intestinal lamina propria through activation of the Dectin-1/Syk signalling axis, aggravating the immunopathology of inflammatory bowel disease. This study systematically clarifies the entire causal

chain for the first time by combining clinical samples, conditional gene knockout mouse models, and in vitro mechanistic experiments. In addition to expanding our knowledge of how the fungal microbiome actively influences mucosal T cell immunity, our research offers a solid molecular foundation for treating IBD by focusing on the fungus-host recognition interface.

Three levels can be used to summarise the study's main conclusions. First, in both DSS-induced colitis mice and humans with active IBD, we consistently found elevated intestinal fungal load with a predominance of Candida species, and the degree of mucosal Th17 cell infiltration was directly connected with fungal load. Recolonisation with Candida albicans reproduced this pathogenic phenotype, demonstrating a causal link between symbiotic fungi and Th17-driven intestinal inflammation. In contrast, clearing fungi with fluconazole reduced colitis and inhibited the growth of Th17 cells. Second, we showed that inhibiting the Dectin-1/Syk signalling pathway may virtually eliminate fungal-induced Th17 cell growth and greatly reduce colitis severity without changing intestinal fungus load using Clec7a$^{-/-}$ mice and the Syk inhibitor R788. Lastly, in vitro BMDC-T cell co-culture experiments verified that C. albicans directly drives naïve T cells toward Th17 polarisation by stimulating dendritic cells to produce IL-6 and IL-23 via the Dectin-1/Syk/CARD9 pathway. This effect can be prevented by Syk inhibition or Dectin-1 deficiency.

The aforementioned results have several immunological ramifications. First, they offer a crucial microbial upstream signal that drives the aberrant Th17 cell proliferation in IBD. Our findings clearly include fungal components into the Th17 regulatory network, highlighting the fact that in the intestinal multi-kingdom microbial ecosystem, fungal signals can drive pathogenic Th17 responses independently of bacterial communities. Prior research has mostly concentrated on the induction of Th17 cells by bacterial communities (such as SFB). Second, the harmful role of the Dectin-1/Syk axis in the inflammatory intestinal milieu is clarified by this study. Although Dectin-1's antifungal immune activity has long been thought to be its primary function, our findings imply that the same signalling pathway becomes a crucial node amplifying immunological disease in the context of intestinal barrier failure and fungal overgrowth in IBD. This offers a molecular explanation for the correlation between severe subtypes of ulcerative colitis and CLEC7A gene polymorphisms: commensal fungi-driven, functionally intact Dectin-1 may stimulate inflammation instead of just serving a protective role. Third, the conclusion that the "fungal-Dectin-1 axis directly drives mucosal immune pathology," which has frequently been disregarded in earlier research, is supported by our data, which unequivocally rule out general disruptions to the bacterial community brought on by fungal intervention.

Our findings support the findings of the majority of clinical relevance studies and some animal tests, but they also provide light on a number of contentious issues in the area. The proliferation of Candida in IBD has been observed by Sokol and the multicenter cohort; this work advances this phenomena beyond correlation to causal processes. Early research on the intricate roles of Dectin-1 in

colitis revealed that Clec7a$^{-/-}$ mice are more prone to DSS-induced colitis, which was attributed to fungal overgrowth and compromised mucosal barriers. Our data does not refute these findings; rather, it emphasises the significance of context-dependence: Dectin-1 deficiency may result in uncontrolled fungal load and secondary harm in the absence of specialised fungal community management and with severely compromised intestinal barriers. However, because we employed a shorter DSS cycle model and the study goal was more concerned with immunological responses than fungal infection outcomes, the fungal burden of Clec7a$^{-/-}$ mice under DSS circumstances did not differ significantly from that of WT mice in our investigation. More significantly, heat-inactivated fungi are unable to replicate the pathogenic effect of C, as demonstrated by parallel validation with the Syk inhibitor R788.Therefore, all of our results point to the notion that, rather than being a straightforward loss of barrier protection, live fungal signals cause inflammation through Dectin-1. This variation also implies that Dectin-1 may play a biphasic role in various stages of IBD or under various dominant fungal backgrounds: promoting pathogenic Th17 responses during the inflammatory phase caused by fungal overgrowth, while maintaining antifungal defence during early homeostasis.

Furthermore, our research adds to the body of evidence supporting the in vitro Dectin-1/Syk/IL-6/IL-23-Th17 pathway in the intestinal environment. Although it has long been known that Dectin-1 stimulates Th17 differentiation in vitro, in vivo verification is crucial since Dectin-1 is simultaneously expressed by dendritic cells and macrophages in the intestine, and the intestinal milieu controls their activity. Our findings from Clec7a$^{-/-}$ and R788 treatments directly demonstrate that this pathway is equally essential for promoting Th17 growth in the colonic lamina propria in vivo and are in complete agreement with the IL-23/Th17 axis theory.

Establishing a full causal evidence chain of “commensal fungi-Dectin-1/Syk-Th17-colitis exacerbation,” which bridges the mechanistic gap from fungal dysbiosis to host immune recognition; concurrently employing gene knockout and pharmacological blockade, which provides dual genetic and chemical evidence and eliminates the confounding effect of developmental compensation; and eliminating the interference of bacterial flora, which clarifies that the immune effects caused by fungi are independent.

Nevertheless, there are some limitations to this study. First, even though the DSS-induced chemical colitis model is frequently employed in IBD research, it is unable to accurately replicate the chronic recurrent features and heterogeneity of human disease; further validation in T cell adoptive transfer models or spontaneous colitis models, such as IL-10 knockout, could improve the generalisability of the findings. Second, Dectin-1 signalling in CD103^{+} dendritic cells, CX3CR1^{+} macrophages, or intestinal stromal cells may not be the primary driver of Th17 polarisation. Further research employing conditional knockout mice may yield more accurate results. Our in vivo mechanistic experiments primarily relied on systemic Dectin-1 knockout and systemic Syk inhibition. Additionally, only one strain of C was the

subject of this investigation. albicans, but the human gut mycobiota is made up of dozens of fungus species; more research is needed to determine whether Malassezia and other Candida species affect immune responses via the same or distinct receptor pathways. Furthermore, the immunogenic distinctions between hyphal and yeast forms, as well as the dynamic metabolic signalling of live fungus in the gut, were not properly replicated in our in vitro tests using heat-killed yeast-form fungi and pure β-glucan. Lastly, even though this study demonstrated the crucial role of IL-6/IL-23 downstream of Dectin-1 in mediating Th17 polarisation, more research is needed to determine whether direct treatment with IL-6 or IL-23 receptor blockade can fully mimic the protective phenotype of Dectin-1/Syk deficiency and whether other Th17-driving factors (like IL-1β and TGF-β3) are involved.

This work offers a theoretical foundation for treating IBD by focusing on the fungal-Dectin-1/Syk pathway from the standpoint of translational medicine. It is feasible to carry out proof-of-concept clinical trials in IBD patients with Syk inhibitors, such as Fostamatinib, which have been approved for immune thrombocytopenia with mature safety data. However, for IBD patients who do not react to standard treatments, antifungal techniques that specifically eradicate dominant pathogenic fungi or alter the composition of the intestinal fungal community may also become supplemental choices. It is noteworthy that patient subgroups appropriate for antifungal or anti-Dectin-1 medication may be screened using CLEC7A polymorphism testing or study of faecal fungus load/composition.

Future studies could focus on the following areas: mapping the spatial distribution of immune cells linked to fungal colonisation and Dectin-1 signalling activation using single-cell transcriptomics and spatial transcriptomics technologies; determining which antigen-presenting cell subsets are the primary effector cells driving Th17 polarisation in vivo; assessing whether Dectin-1/Syk blockade and anti-IL-23 therapy have synergistic effects in chronic colitis models or T-cell transfer models; investigating the mechanisms that cause gut fungal dysbiosis, such as passive expansion brought on by intestinal barrier disruption or selective fungal growth under dietary or antibiotic pressure, to provide upstream intervention targets for preventing fungus-driven inflammation; carrying out prospective clinical cohort studies to confirm the relationship between gut fungal load and Dectin-1 pathway activation markers with the prognosis and response to treatment of IBD patients.

By demonstrating a novel mechanism by which gut symbiotic fungi activate pathogenic Th17 immune responses via the Dectin-1/Syk axis, this work concludes by elucidating the viability of this route as a potential target for IBD treatments. In addition to adding to the theoretical framework of “multi-kingdom microbe-host immune interactions,” this study provides an essential foundation for precision immunotherapy treatments for IBD based on fungal signalling.

6. Conclusion

In conclusion, by activating the Dectin-1/Syk signalling axis and causing dendritic cells to release

IL-6 and IL-23, the gut symbiotic fungus Candida albicans causes pathological polarisation of intestinal lamina propria Th17 cells and exacerbates immune pathology of inflammatory bowel disease. This study, for the first time, fully clarifies the causal mechanism. We consistently demonstrated in both in vitro and in vivo experiments that blocking the Dectin-1/Syk pathway can specifically inhibit Th17-type intestinal inflammation without causing fungal overgrowth using gene knockout and pharmacological blockade techniques. This effect is independent of changes in the gut bacterial microbiota. In addition to redefining the fungal microbiota from a “bystander” to a crucial active player in influencing intestinal T cell immunity, these discoveries offer precise molecular targets and a theoretical foundation for IBD treatment approaches that target the fungi-host recognition interface.

Future studies should concentrate on the following areas: determining whether Dectin-1 signalling in $CD103^+$ dendritic cells, $CX3CR1^+$ macrophages, or other intestinal stromal cells dominates in vivo Th17 polarisation using cell-specific conditional gene knockout tools; mapping the spatial interactions between immune cells during fungal colonisation and Dectin-1 signal activation using single-cell transcriptomics and spatial multi-omics technologies; assessing the long-term safety and efficacy of Syk inhibitors or selective antifungal strategies in chronic and spontaneous colitis models and investigating their possible synergy with anti-IL-23 therapy; validating the viability of employing intestinal fungal burden and Dectin-1 pathway activation indicators as biomarkers for stratified treatment and prognosis assessment in IBD patients through prospective clinical cohort studies. It is anticipated that as the aforementioned research advances, new precision intervention techniques based on fungus-host immunological interactions will provide IBD patients additional treatment alternatives.

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Author contributions

Conceptualization, Gang Liu; methodology, Jia Wang; validation, Alena Mikalaeuna Vasiukevich; formal analysis, Biao Feng and Liqiang An; investigation, Jia Wang and Biao Feng; resources, Gang Liu and Liqiang An; data curation, Alena Mikalaeuna Vasiukevich; writing—original draft preparation, Jia Wang; writing—review and editing, Gang Liu and Alena Mikalaeuna Vasiukevich; visualization, Biao Feng; supervision, Gang Liu; project administration, Liqiang An. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

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Consent for publication

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