Mycobiota-induced IgA antibodies regulate fungal commensalism in the gut and are dysregulated in Crohn’s disease

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Secretory immunoglobulin A (sIgA) plays an important role in gut barrier protection by shaping the resident microbiota community, restricting the growth of bacterial pathogens and enhancing host protective immunity via immunological exclusion. Here, we found that a portion of the microbiota-driven sIgA response is induced by and directed towards intestinal fungi. Analysis of the human gut mycobiota bound by sIgA revealed a preference for hyphae, a fungal morphotype associated with virulence. Candida albicans was a potent inducer of IgA class-switch recombination among plasma cells, via an interaction dependent on intestinal phagocytes and hyphal programming. Characterization of sIgA affinity and polyreactivity showed that hyphae-associated virulence factors were bound by these antibodies and that sIgA influenced C. albicans morphotypes in the murine gut. Furthermore, an increase in granular hyphal morphologies in patients with Crohn’s disease compared with healthy controls correlated with a decrease in antifungal sIgA antibody titre with affinity to two hyphae-associated virulence factors. Thus, in addition to its importance in gut bacterial regulation, sIgA targets the uniquely fungal phenomenon of hyphal formation. Our findings indicate that antifungal sIgA produced in the gut can play a role in regulating intestinal fungal commensalism by coating fungal morphotypes linked to virulence, thereby providing a protective mechanism that might be dysregulated in patients with Crohn’s disease.
and host changes. Recent studies also demonstrate that sIgA in the healthy gut and oral mucosa bind to specific fungal species\(^{29,30}\). During homeostasis, commensal fungi, similar to commensal bacteria, interact with the host in mutualistic ways\(^{1,29-31}\), raising a question of how fungal commensalism in the gastrointestinal tract is maintained. We hypothesized that exposure to morphotype-dependent fungal factors and their interactions with sIgA in the gut regulate fungal commensal states.

**Results**

A subset of gut mycobiota is coated by sIgA induced predominantly by *Candida albicans*. To visualize fungal cell in the intestines, murine faeces from C57BL/6J mice housed in our facility\(^{11}\) were stained with DNA-binding SYBR-Green (SYBR) to distinguish live microbes from Sybr\(^\text{gb}\) debris and microbial components found in sterilized food and cage bedding (Extended Data Fig. 1a). Co-staining with chitin-binding Calcofluor White (CFW) dyes distinguished the CFW\(^\text{gb}\) fungal populations from the Sybr\(^\text{gb}\) microbial populations as a whole (Extended Data Fig. 1b)\(^{11}\). The material was then stained with fluorescently labelled anti-IgA, anti-IgM and anti-IgG antibodies to visualize the intestinal fungi-bound antibody repertoire. Consistent with luminal sIgA abundance and previous assessments of intestinal bacteria-bound antibody repertoires\(^{1,3,11,35}\), this approach revealed a large fraction

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**Fig. 1** | Under homeostatic conditions, the majority of the mouse and human gut mycobiota is coated by sIgA primarily induced by *C. albicans*. a,b, Representative flow cytometry histogram displaying IgA-, IgG- and IgM-bound fungi (a), quantified as percentage of total fungi (within the Sybr\(^\text{gb}\)* fraction) of faeces collected from SPF WT WCM-CE mice (b), assessed by flow cytometry. Grey curves in a represent corresponding Ig-isotype control staining. Data are representative of three independent experiments. n = 5. c,d, Representative flow cytometry histogram displaying IgA-bound fungi (c), quantified as percentage of total fungi (d), assessed in WCM-CE SPF mouse faeces from Rag\(1^-\) and B cell-deficient μMT\(^{-/-}\) mice in comparison with WT mice, assessed by flow cytometry. The filled-in grey curves in c represent IgA-isotype control staining. Data represent two independent experiments. Kruskal–Wallis test followed by Dunn’s multiple comparisons test. WT, μMT, c represent IgA-isotype control staining. Data represent two independent experiments; one-way analysis of variance, followed by Sidak’s test, \(n = 4, 8\). e,f, Representative histogram of IgA- and IgG-bound fungi (e), quantified as percentage of total fungi (f), assessed in faeces collected from healthy human individuals, assessed by flow cytometry. Grey curves in e represent IgA-isotype control staining. Two-sided Mann–Whitney test. n = 8. g, Total free sIgA levels in the faeces of GF mice monoclonized with common commensal and dietary fungal species. Data are representative of two independent experiments. Kruskal–Wallis test followed by Dunn’s multiple comparisons test. GF, n = 12; *C. albicans*, n = 15; *S. cerevisiae*, n = 4; *S. fibuligera*, n = 4; *A. amstelodami*, n = 4; *W. sebi*, n = 5. h, GF mice were orally gavaged with *C. albicans* (+Ca) and sIgA binding to intestinal *C. albicans* was observed by flow cytometry in faeces collected at days 2, 4, 8 and 14 post-colonization. Analysis of IgA binding representative of two independent experiments; one-way analysis of variance, followed by Sidak’s test, \(n = 6\). i, GF mice were orally gavaged with PBS (GF) or colonized for two weeks with *C. albicans* (+Ca). PP of all mice were harvested at day 14 for flow cytometry analysis of frequency of B220+IgA\(^+\) among live CD45+CD4\(^-\) cells. Pooled from two independent experiments; two-sided Mann–Whitney test. GF, n = 9; +Ca, n = 14. j-m, Representative plots (j) and flow cytometry analysis of frequency (k) of B220+IgA\(^+\) in PP B cells, as well as representative plots (l) and flow cytometry analysis of frequency (m) of GC-B cells. In the PP of mycobiota-free ASF mice orally gavaged with PBS (ASF) or colonized for two weeks with *C. albicans* (+Ca). Pooled from two independent experiments; two-sided Mann–Whitney test. ASF, n = 8; +Ca, n = 10. Dots, faecal samples/ gut-associated lymphoid cells of individual mice (a-d,g-i-m) or healthy humans (e,f); error bars, s.e.m.
of intestinal fungi bound by sIgA (Fig. 1a,b). Neither sIgG nor sIgM fungal binding was observed in the gut (Fig. 1a,b), reflecting a lack of IgG and IgM transcytosis in adult mammalian intestine-3 and distinguishing IgA as the primary immunoglobulin contributing to intestinal homeostasis during the steady-state. The presence of antifungal sIgA was dependent on the presence of mature functional B cells because these antibodies were absent in the lumen of both B cell-deficient μMT−/− and B/T cell-deficient Rag1−/− mice (Fig. 1c,d). Notably, the lack of an antifungal sIgA response in μMT−/− mice negates the contribution of unconventional IgA induction pathways through peritoneal B cells1,2,6. Similar results were obtained on assessment of the faecal mycobiota of healthy human subjects (Fig. 1e,f), suggesting that substantial antifungal sIgA binding of gut mycobiota occurs in both mice and humans.

Although sIgA can be induced by both food antigens and the gut microbiota, and total IgA levels increase continuously with age in both GF and colonized mice6,8, monoclonization experiments have consistently demonstrated that a fraction of sIgA is both microbiota-induced and microbiota-reactive1,6,20,25. Having previously demonstrated that the gut microbiota contributes to the host circulating IgA antibody pool26, we investigated whether intestinal fungi contribute to the antifungal sIgA repertoire observed under steady-state. Fungal monoclonization experiments were performed in GF mice using several fungal species found in the human or the murine gut27. Surprisingly, among those, C. albicans was the species that induced a robust luminal sIgA response (Fig. 1g). sIgA binding to C. albicans cells was already detectible during early colonization and increased over time (Fig. 1h), consistent with the course of sIgA production induced by this fungus (Extended Data Fig. 1c). These results were corroborated on analysis of the Peyer’s patches (PP) of C. albicans-colonized mice in which we observed a notable increase in IgA frequency in the B cell compartment relative to non-colonized GF mice (Fig. 1i) and Extended Data Fig. 1d).

Bacterial composition can dramatically influence fungal colonization in the gut1,28-31. To assess the potential role of intestinal bacteria in this process, we next utilized altered Schaedler flora (ASF)-colonized mice that carry a defined set of bacteria but are mycobiota-free32,33. Consistent with our findings in GF mice, intestinal colonization with C. albicans correlated with increased frequencies of IgA+ B cells in the PP (Fig. 1j,k). Notably, within the germinal centre B cell (GC-B) subset, which consists of highly proliferative mature B cells that are undergoing affinity maturation and associated with high-affinity antibody responses, the same patterns of increased IgA class-switch recombination (CSR) were observed (Fig. 1l,m). Together, these results suggest that C. albicans intestinal colonization induces strong IgA CSR responses in the PP independent of the presence of intestinal bacteria.

**Antifungal sIgA antibodies direct fungal commensalism by preferentially targeting hyphal morphotypes.** In contrast to bacteria, fungal cells express specific morphotypes in the gut34 (yeast and hyphae) that differ in both cell size and function. An analysis of fungal cells size and granularity in sIgA+ and sIgA− fractions (based on forward and side scatter) revealed enrichment of larger and more granular fungi in the sorted sIgA-bound fraction relative to unbound fungal cells or fungi in unsorted material overall (Fig. 2a–c and Extended Data Fig. 2a–e), suggesting preferential binding of sIgA to specific fungal morphologies. C. albicans is the most abundant and common dimorphic fungus in the human gastrointestinal tract, is present in patients with inflammatory bowel disease (IBD)35 and was recently demonstrated to be capable of producing pseudohyphae on intestinal colonization11. Because C. albicans made up the majority of fungi found in both sIgA+ and sIgA− fractions in the assessed human faecal samples36, but larger and more granular fungal cells were preferably bound to sIgA (Fig. 2a–c), we hypothesized that sIgA preferentially binds fungal hyphal. Thus, we used human faecal slurry as a source of sIgA to stain C. albicans. To observe morphological differences in C. albicans antifungal sIgA binding in vitro or in vivo, we generated a double-reporter C. albicans strain (Ca-dREP) in which the enolase 1 (ENO1) promoter was used to constitutively express relatively high amounts of green fluorescent protein (GFP) resulting in green fungal cells (Extended Data Fig. 3, GFP), whereas red fluorescent protein (RFP) was expressed under the hyphal wall protein 1 gene (HWP1) promoter resulting in strong RFP expression specifically in hyphae during filamentation (Extended Data Fig. 3, RFP). Using human faecal slurry as a source of sIgA, we first investigated sIgA binding on incubation with in vitro cultures of this double-reporter mutant. Analysis by

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Fig. 2 | sIgA antibodies preferentially bind fungal hyphae and influence C. albicans morphotypes in the gut. **a,b,** Representative plots of forward scatter (a, left) and side scatter (a, right), analysis (b) of all CFW+Sybr® stain fungi (All), and the IgA+/IgA− fungal fractions, collected from healthy human faecal samples. Mixed effects analysis with Geisser–Greenhouse correction, n = 6. **c,** SPF WT, JAX mice were fed with 1 × 108 C. albicans and faeces collected four days later. Average forward scatter (left) and side scatter (right) values of all CFW+Sybr® stain fungi (All), as well as the IgA+ and IgA− CFW+Sybr® fractions are shown. Mixed effects analysis with Geisser–Greenhouse correction, n = 8. **d–f,** Immunofluorescence microscopy of Ca-dREP incubated with human faecal supernatant as a source of sIgA, stained with 4,6-diamidino-2-phenylindole and anti-human IgA-APC (d) or an APC isotype control (e). GFP–RFP+ yeast, GFP–RFP− hyphal and IgA+ events for each were counted for calculation of IgA-coating frequency by morphotype (f). Composite images of x20 magnification and enlarged dotted regions of each are shown in the left and right panels, respectively. Single channels are shown in Extended Data Fig. 3 (upper rows). Scale bars, 50 μm (x20 magnification panel) and 20 μm (enlarged panel). Data are representative of three independent experiments. Two-tailed Wilcoxon matched pairs signed rank test. **g,h,** C. albicans were cultured for 18 h in hyphae-inductive media and stained with faecal supernatant from C. albicans-colonized IgA-sufficient WT or IgA-deficient Rag1−/− mice, followed by sIgA staining. Representative plots (g) and analysis (h) of IgA binding from two independent experiments. Grey curves in g represent IgG-isotype control staining. Multiple two-tailed t tests. WT, n = 4; Rag1−/−, n = 4. **i,j,** SPF WT (i) and Rag1−/− (j) mice were treated with cefoperazone and infection with a 1:1 mix of 1 × 108 C. albicans strain CAF2-1–RFP (WT, black) and sIgG+ (pink) C. albicans, after which the numbers of colony-forming units (cf.u.) for each were tracked for 20 days. For each mouse genotype, competition indices (CI) were generated for each experiment, calculated as CI = log10(recov. cf.u./original inoculum cf.u.). Multiple two-tailed t tests. WT, n = 4; Rag1−/−, n = 4. Data are representative of two independent experiments. **k,l,** Representative flow cytometry plots (k) and analysis (l) of RFP+ hyphal in WT, μMT−/− and Rag1−/− faeces of mice four days after cefoperazone-aided colonization with 1 × 108 C. albicans strain CAF2-1–RFP. Plots gated on Ca-dREP. Data are representative of three independent experiments. Kruskal–Wallis test followed by Dunn’s multiple comparisons test. WT, n = 10; μMT−/−, n = 5; Rag1−/−, n = 8. **m–o,** Quantification of IgA-coating and Ca-dREP morphotypes in SPF WT JAX mice faeces 4 and 14 days after cefoperazone-aided colonization. Plots in m and n represent colon contents gated on CFW+ events, with GFP–RFP+ yeast and GFP–RFP− hyphal Ca-dREP populations easily distinguished. Plot o represents the hyphal (left plot, red box) and yeast (right plot, green box) fractions of Ca-dREP shown in m. Connected dots in o represent ≥ 1% of IgA− fungal cells within the hyphal (red) and yeast (green) Ca-dREP gates in a single faecal sample collected at day 4 or 14, respectively. Two-tailed Wilcoxon matched pairs signed rank test. n = 7. **p,q,** sIgA from healthy human faeces was applied (5 μg well−1) at 0 and 6 h to a two-partner system comprising of Ca-dREP and Caco-2 cells on Ca-dREP infection. The frequency of hyphal (p) and yeast (q) morphologies was measured by flow cytometry using the gating strategy described in m. Each dot represents a well. Multiple unpaired two-tailed t tests followed by Sidak’s test. Error bars, s.e.m.
fluorescent microscopy revealed preferential sIgA hyphae coating, whereas yeast cells were targeted substantially less (Fig. 2d–f and Extended Data Fig. 3a,b, upper rows). The same staining approach using faeces from \( \text{C. albicans} \)-colonized IgA-sufficient or IgA-deficient \( \text{Rag1}^{-/-} \) mice revealed similar preferential coating of \( \text{C. albicans} \) hyphae by murine sIgA (Fig. 2g,h). As expected, this
Consistent with previous reports, co-colonization of specific pathogen-free (SPF) WT mice with C. albicans resulted in decreased fitness of the hyphae-sufficient strain (Fig. 2i). Notably, we observed the opposite result in Rag1−/− mice (Fig. 2i), suggesting that adaptive immunity-dependent features might play a role in regulating C. albicans commensalism in the gut.
In light of these results and the observed morphological dependency of sIgA binding in vitro, we sought to investigate whether this phenomenon occurs in vivo and whether sIgA plays any role in *C. albicans* morphogenesis and commensal states in the gut. sIgA-sufficient (wild-type; WT) and sIgA-deficient (*Δrgr1* and *μMT−/−*) mice were colonized with Ca-dREP for 4 and 14 days, after which colon contents were assessed by flow cytometry for frequency of RFP+ hyphae. Notably, in both *Δrgr1* and *μMT−/−* mice, a higher frequency of hyphae was detected within the colonized Ca-dREP population relative to similarly colonized WT control mice (Fig. 2k,l). Furthermore, sIgA-binding assessment of the WT mice colonized with Ca-dREP recapitulated the sIgA-binding results observed in vitro (Fig. 2g,h), with substantially higher frequencies of sIgA binding observed among hyphal Ca-dREP relative to yeast morphology at both the early and later stages of colonization with an increase at the later time point (Fig. 2m–o), consistent with the course of free sIgA production (Extended Data Fig. 1c) and binding (Fig. 1h) to *C. albicans*. To assess whether a similar phenomenon takes place in humans, we isolated sIgA from healthy human faeces that was applied to the two-partner system of Ca-dREP and intestinal epithelial cell line Caco-2 (used as an inducer of hyphal morphogenesis53). Consistently, sIgA treatment limited the frequency of *C. albicans* hyphal morphologies in this assay while a complimentary increase in yeast morphotype frequencies was observed (Fig. 2p,q). Together, these data suggest that the absence of gut antifungal sIgA antibodies results in increased hyphal morphogenesis of *C. albicans*, implicating a role for sIgA in maintaining *C. albicans* commensalism.

**Hyphal morphotypes drive the robust *C. albicans*-induced sIgA responses.** We next engineered hyphal-competent (WT) and hyphal-deficient (yeast-locked; *egf1Δ/cph1Δ/Δ* strain60) strains of *C. albicans* to express RFP or GFP, respectively, both to further explore preferential sIgA binding to hyphae in vivo and to assess differences in sIgA repertoires induced in the presence or absence of the hyphal morphology. WT mice were colonized with a 1:1 mixture of hyphal-competent (WT) and hyphal-deficient (yeast-locked; *egf1Δ/cph1Δ/Δ* strain60) strains of *C. albicans* that could be later detected in faecal material (Fig. 3a). Consistently, hyphal-competent WT *C. albicans* strain was bound by sIgA with higher frequency when compared with the co-colonized yeast-locked strain (Fig. 3b,c).

To explore the mechanisms behind sIgA induction, we next colonized mice with hyphal-sufficient WT or yeast-locked *egf1Δ/cph1Δ/Δ* *C. albicans* strains (Extended Data Fig. 3c). Interestingly, hyphal-sufficient *C. albicans* induced substantially more IgA+ CSR in the B cells of the PPs than the *egf1Δ/cph1Δ/Δ* strain, despite greater expansion of the later in the murine gut (Fig. 3d–f). Since polyreactivity is a common trait identified in recent characterizations of gut sIgA repertoires35, hyphae-induced IgA CSR could either be inducing antibodies that bind cellular structures common to fungi and present in all *C. albicans* morphotypes or exhibit specific reactivity to hyphae-associated virulence factors. We therefore colonized ‘mycobacteria-naive’ ASF mice with *C. albicans* and assessed sIgA reactivity against cell lysates derived from either yeast-locked or hyphal-sufficient *C. albicans*. This assessment revealed a preference of sIgA to lysates of hyphal *C. albicans* as compared to those of the hyphal deficient strain (Fig. 3g). Notably, no morphotype-specific preference was observed in *egf1Δ/cph1Δ/Δ* colonized mice or fungi-naive ASF controls (Fig. 3g). Altogether these data suggest that hyphal morphotypes of *C. albicans* are potent inducers of antifungal sIgA and that such antibodies sway fungal commensalism by preferentially targeting hyphal morphotypes.

sIgA responses to *C. albicans* hyphal morphotypes are mediated through innate immune interaction with intestinal phagocytes. Several intestinal populations of phagocytes play important roles in microbiota regulation, barrier protection, priming of antigen-specific responses to microbial antigens and regulation of sIgA responses to intestinal microbes37,45,47. Recent findings suggest that a population of mononuclear phagocytes that express high levels of the fractalkine receptor CX3CR1 (CX3CR1* mononuclear phagocytes (MNP)s))34,45 is both involved in IgA responses to bacteria62 and central to fungal sensing and priming of antifungal T cell responses in the intestine54,55, and plays a role in the protection of intestinal epithelial cells from fungal toxins56. A loss-of-function mutation in CX3CR1, leads to loss of systemic antifungal IgG responses in humans and mice42,43. By contrast, CD11c+/CD11b+/CD103+ dendritic cells (DCs) that depend developmentally on the transcription factor IRF457,58, have long been known to play an important role in the PPs and control of IgA responses to bacterial and food antigens in the small intestine49,50.

The PPs in the small intestine play a key role in the induction of sIgA responses specific to penetrant commensals and invasive pathogens59, whereas lamina propria and adjacent isolated lymphoid follicle-produced sIgA target both invasive and non-invasive bacteria as well as food antigens52,53. A recent study demonstrated that a subset of CX3CR1* MNP56 are required for the development of tertiary lymphoid structures in the colon serve as a site for a Salmonella-specific IgA response52. Because intestinal colonization with *C. albicans* that led to sIgA production (Fig. 3h), induced an increase of IgA+ B cells in the PPs and in the lamina propria (Fig. 3i–k and Extended Data Fig. 4a,b), we next explored whether either of these two populations of gut-resident phagocytes have any role in the induction of antifungal sIgA in PPs and in the lamina propria. We generated ΔCX3CR1 mice (C3xcr1−/− × CD11c+/−) to selectively deplete intestinal CX3CR1+ MNP56. To selectively target CD11c+/CD11b+/CD103+ DCs (cDC2), we crossed flox-inducible Irf4fl/fl allele mice with transgenic Cd11c+/+ mice (ΔIrf4). Intestinal colonization of ΔIrf4 mice and their respective littermates with hyphae-sufficient *C. albicans* revealed a reliance of anti-*C. albicans* sIgA production and *C. albicans*-dependent induction of IgA+ B cells in the PPs on cDC2 as described previously for bacteria36,56 (Fig. 4a–d). Targeting of CX3CR1* MNP56, by contrast, affected *C. albicans*-dependent induction of IgA+ B cells in the lamina propria without affecting these cells in the PPs (Fig. 4e–h). Notably, depletion of either phagocyte population led to a decrease in luminal anti-*Candida* sIgA (Fig. 4d,h) that coincided with an increase in granular fungal morphologies in the gut (Fig. 4l–1 and Extended Data Figs. 2 and 4c). Altogether the data suggest that cDC2 and CX3CR1* MNP56 might be interdependent by regulating different pathways of antifungal sIgA induction: one affecting IgA+ B cells in the PPs and the other affecting IgA+ plasmablasts in the lamina propria.

**C. albicans**-induced sIgA targets hyphae-associated virulence factors. As one of the most successful fungal opportunists, *C. albicans* has evolved to survive in the gut or invade host tissues: a process associated with yeast-to-hyphal transition7,63. Among several key virulence factors, hyphae-produced proteins such as secreted aspartyl proteinases (specifically Sap6 in the gut31), agglutinin-like protein 3 precursor (Alix36) and Ece1-derived cytolytic toxin candidalysin (Ece1-III)63 have been implicated in tissue adhesion and damage associated with fungal pathogenesis. Thus, we next explored whether hyphae-reactive sIgA induced by *C. albicans* intestinal colonization (Fig. 5a) was driven by reactivity to these virulence factors. Although sIgA exhibited only a minimal binding to cell wall mannann (Fig. 5b), these antibodies were distinguished by distinct increase in reactivity to candidalysin and to a lesser extent to Sap6 (Fig. 5c,d). Furthermore, anti-bacterial flagellin sIgA titres in mice were not affected by *C. albicans* colonization (Fig. 5e), suggesting the fungal reactivity of *C. albicans* induced sIgA antibodies.
Fig. 4 | *C. albicans* sIgA responses are mediated through interaction with DC2 and CX3CR1+ MNPs.  

**a**–**h**, PP B cells and cLP plasmablasts were assessed for IgA+ CSR after two weeks of cefoperazone-aided colonization with WT *C. albicans in Cd11c-cre<sup>−/−</sup>×IRF4<sup>fl/fl</sup> (ΔIRF4; a–c) or Cd11c-cre<sup>−/−</sup>×C3cr1<sup>DTR</sup> (ΔCX3CR1; e–g) SPF mice relative to Cd11c-cre<sup>−/−</sup> littermates (Litt.). Representative plots and analyses of PP B220<sup>+</sup>IgA<sup>+</sup> B cells (a, e), Fas<sup>−</sup>GL7<sup>+</sup> GC-B and IgA<sup>+</sup> GC-B cells (b, f). Representative plots and analyses of B220<sup>+</sup> IgA<sup>+</sup> plasmablasts in the cLP are shown in c and g. ELISA-based characterization of luminal anti-*C. albicans* sIgA in small intestines of ΔIRF4 (d), ΔCX3CR1 (h) mice and their respective littermates after two weeks of intestinal colonization by WT *C. albicans*.  

**i**–**l**, Representative plot of side and forward scatter (i–k) and respective analysis (l) of CFW<sup>+</sup>Sybr<sup>+</sup> *C. albicans* (gated as shown in Extended Data Fig. 4c) in luminal content of ΔIRF4 (i), ΔCX3CR1 (j, k) mice and their respective littermates. Mixed effects analysis with Geisser–Greenhouse correction. Data represent two or three independent experiments. Two-tailed Mann–Whitney test. ΔIRF4, n = 12 (one sample was excluded from the ΔIRF4 experimental group because of failed fluorescent staining); Litt., n = 7; ΔCX3CR1, n = 8; Litt., n = 12. Error bars, s.e.m.
Increase in granular hyphal morphologies and dysregulated antifungal sIgA responses are observed in CD. Given these findings and because we found that the hyphal programme is involved in the induction of antifungal sIgA, we next explored whether intestinal inflammation affects antifungal sIgA in the human intestinal mucosa where these antibodies are produced. Thus, we focused these investigations on CD as a disease in which serum circulating antibodies against fungal mannan (anti-*Saccharomyces cerevisiae* antibodies; ASCA) are elevated and used as a marker of disease severity. We examined mucosal intestinal washings and serum from healthy controls and patients with CD. As expected, circulating ASCA IgA and IgG antibodies were increased in patients with CD compared with controls, although a differential increase in these antibodies did not reach statistical significance in mucosal washings of patients with CD in our cohort (Fig. 5f,g). By contrast, sIgA antibodies reactive to *Candida* hyphae-produced proteins such as Sap6 and candidalysin were affected in patients with CD from this cohort (Fig. 5h,i). Consistent with dysregulation of antibody responses, flow cytometry-based analysis revealed an increase in granular fungal morphologies in CD mucosal washings (Fig. 5j). These data suggest a dysregulated antibody response against factors produced by hyphal morphotypes and an increase in hyphal morphologies in the mucosa might be occurring during CD.

Taken together, the data indicate that although human sIgA with reactivity to both yeast and hyphal morphotypes exists in the human gut, sIgA with preferential binding to hyphae might arise in response to specific hyphal-associated virulence factors (Extended Data Fig. 5). Furthermore, CD might alter this process by affecting the targeting of hyphae-associated virulence factors by sIgA.
Discussion

In addition to the intestinal mucus layer and antimicrobial peptides, sIgA antibodies have long been considered an important player in intestinal homeostasis by providing a ‘first line’ of defence against invasive pathogens, toxins or other harmful food and metabolic bacterial products. Here, we describe that a fraction of sIgA present in the intestinal lumen is directed towards fungi. It has previously been established that sIgA displays broad cross-bacterial reactivity with occasional preferential coating of distinct bacterial species. However, we discovered that a phenomenon specific to the fungal kingdom, namely the ability to produce hyphae, was the main target of sIgA antifungal antibodies. We found that among several gut fungal species, C. albicans and its hyphal morphotype were key targets and the most potent inducers of antifungal sIgA, suggesting that selected fungal species and specific morphotypes are involved in its induction (Extended Data Fig. 5). Because multiple fungal species are capable of hyphae or pseudohyphae formation, future research should determine whether this is due to an inability of specific fungi to produce hyphae in the intestinal environment, a requirement of specific virulence factors and metabolites, or a combination of all of the above.

Because hyphal formation is a primary mechanism used by dimorphic fungi to invade and translocate between environments within their hosts, targeting of these structures might have important functional consequences for fungal commensalism, fungal pathogenesis and immune responses to these processes by the host. Indeed, we show here that the absence of sIgA potentiates hyphal growth in the murine intestines. Taken together, our findings and recent findings by others suggest that the ability to thrive in the gut, a switch between specific fungal morphotypes (and a switch between commensal versus pathobiont states) and the associated production of virulence factors might all be involved in the capacity of C. albicans to induce sIgA, and that antifungal sIgA has a role in the control of fungal commensalism in the gut.

Although internal transcribed spacer sequencing methodologies are successfully used to explore the mycobacteriota composition in different IBD cohorts, changes in specific fungal genera are observed inconsistently across cohorts. Our findings suggest that an assessment of additional parameters (Fig. 5g–j) might provide a better understanding of the role of the gut mycobacteriota in IBD.

Despite an inability to efficiently track yeast-to-hyphae transition and IgA binding (facilitated by the use of Ca-DRP in mice), in humans, as well as the limitation of using an individual cohort, we found that sIgA antibodies that bind to hyphae-associated factors are affected in patients with CD (Extended Data Fig. 5) where Candida species expansion has been reported. Although studies in multiple cohorts demonstrate that systemic ASCA develops consistently in patients with CD,26–28 our findings suggest a potentially differential role for systemic and secretory antibodies in this patient population that warrants further investigation, which may lead to a better understanding of mycobacteriota involvement in the pathogenesis of human inflammatory diseases.

Methods

Contact for reagents, data, resource sharing and code availability statement.

All data needed to evaluate the conclusions in the manuscript are available within the main text or supplementary materials. Further information and requests for resources and reagents should be directed to and will be fulfilled by the corresponding author. No custom code was used.

Mice. Seven to eight-week-old WT C57BL/6J, Igf1r−/−/Igf1r−/−, Rag1−/− mice (Rag−/−), Tg(Igακερε−1)Reiz (Cd11c−−/−), Cx3cr1−/−/Cx3cr1−/− mice were purchased from the Jackson Laboratories (Supplementary Table 1). Igf8Δ or Ccex1−/− mice were bred with hemizygous Cd11c−−/− mice for selective depletion of Cd11c+ DC11b+ CD103+ DCs (ΔIFb4 mice) or Cd11c+ CD11b+ CX3CR1+ MNP after administration of diphtheria toxin in the latter case. Depletion was achieved in Cd11c−−/−Cx3cr1−/−, but not Cd11c−−/−Cd3e1−/−Ccxr1−/−, which were injected on three consecutive days with 100 ng of diphtheria toxin intraperitoneally followed by maintenance injection every other day for the length of the experiments. Depleted mice were designated ΔCcxr1−/− mice. Mice bred for several generations with the indicated genotypes and females. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications.24,25 Mice were housed under SPF conditions at Weil Cornell Medicine unless otherwise described.

GF C57BL/6J male mice were maintained in sterile vinyl isolators at Weil Cornell Medicine’s gnotobiotic mouse facility. ASF mice were generated by colonizing GF mice with the defined ASF community78 and allowing five generations of breeding before use.

The investigators were not blinded to the conditions of the experiments during data collection and analysis.

Fungal strains and lysates preparation. C. albicans SC5314 (ATCC MYA-2876), S. cerevisiae (ATCC MIA-796), Aspergillus amstelodami (ATCC 46362) and Wallenius sebi (ATCC 82964) were obtained from the American Type Culture Collection (Supplementary Table 1). Saccharomyces fibuligera was isolated from murine faeces material (Supplementary Table 1). Ca-DRP, a C. albicans strain that emits green fluorescence in the yeast phase and red fluorescence in the hyphal phase was constructed as follows. The promoter of WHI1, a hyphal-specific gene in C. albicans, was ablated from C. albicans 5178 and replaced with the SC5314 genome79 using primers pWH1-pu (AGCTTTCAATATTTGATATTTACGATGTC) and pWH1-plo (ACGTCTCAGATGCTAAGCAAACAAAAGCGAGTGAC) that incorporate their 5′ end restriction sites for XbaI and XhoI, respectively (underlined in the primers sequence). The 2,084 bp amplified fragment was purified and after digestion with the indicated endonucleases, was cloned in a SpeI- and SalI-digested pAS15 plasmid, pASM0–RFP, generating pASM10–RFP. pASM0–RFP is a C. albicans integrative plasmid expressing a C. albicans optimized RFP80 under control of the TET7 promoter; pASM10–RFP plasmid was digested with the restriction enzymes KpnI and SacII and used to transform strain SN100 (his3Δ/Δ his3Δ URA3/ara3:Ura3::mdm634 IRG1/iro1:Ura3::mdm634)71 using electroporation, freezing the AH1 genomic region. The 2,198 bp selected on yeast extract peptone dextrose plates supplemented with 200 µg ml−1 nourseothricin sulfate. The proper integration of the construction was checked using primers upstream of the WHI1 promoter and within the inserted construction, as well as by examination of red fluorescence in cells triggered by filamentation. This intermediate was named ERG1390. We then used the YY1 promoter to generate a yeast-phase specific reporter. We first amplified the YY1 promoter from the SC5314 strain using primers pYY1-pu (ACACTGATCCCATCTATTATTTCCTTGGAAAAGT) and pYY1-plo (ACGCTGTCAGATTATTACATTCCTGGATGATAAATTAAT) that incorporate their 5′ end restriction sites for XbaI and XhoI, respectively (underlined in the primers sequence). The 2,198 bp amplified fragment was digested with BamHI and SacII and cloned in a BamHI- and SalI-digested pAS15 plasmid, pDH0M–GFP85, an integrative plasmid vector that uses HIS1 as genetic marker allowing integration of any promoter upstream of GFP. The resulting plasmid, pDH111–GFP was digested with KpnI and SacII and used to transform intermediate ERG1390, directing the promoter to the ARDA genome through the standard yeast protocol as indicated above and minimal (His-) medium plates giving rise to the C. albicans double-reporter Ca-DRP.

WT C. albicans reporter strain CAF2–RFP that constitutively expresses RFP was generated by replacement of the 5′ ADH1 Xbal-SacII region of pmY10–GFP with a 1,630 bp region derived from pmN166–GFP that comprises the C. albicans TDH3 promoter. Yeast-locked reporter cph1Δ/Δ cph1Δ/Δ GFP strain was constructed inserting a fragment carrying the GFP under the TET7 promoter derived from KpnI and Ksp restriction enzyme digest of plasmid pmY10–GFP to recombination to the ADHI region on a URA+ derivative strain of HLC69 (cph1Δ/Δ cph1Δ/Δ ura3Δ/Δ ura3Δ/Δ)72 which was constructed by integration of pmY10–GFP in the LEU2 genomic region. M. grisea cec1Δ/Δ and parental strains were generated as described previously.

All fungi were cultured under aerobic conditions overnight in Sabouraud Dextrose Broth (EMD Chemicals) at 37°C. In experiments in which lyses from a yeast-locked strain were used, egf1Δ/Δpkh1ΔΔ33 and WT C. albicans strains were cultured hypha-inducing conditions in liquid media containing 0.2% N-acetylgalactosamine-supplemented media86. For fungal lysate preparation, fungal cultures were fixed in 4% paraformaldehyde for 60 min at 4°C. Fungal suspensions were washed three times by pelleting at 900g for 2 min, aspirating the supernatant and resuspending the pellet in molecular-grade water. Fungi were washed three times with three freeze-thaw cycles and resuspended in a total volume of 75 µl in a 75°C incubator. Finally, fungal suspensions were sonicated for eight cycles of 15 on 30 s off. Fungal debris was centrifuged at 4,000 r.p.m. for 2 min, after which supernatant was used for enzyme-link immunosorbant assay (ELISA) plate coating.

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Faecal and mucosal washing samples flow cytometry-based analysis. Age and sex information for human samples assessed in this study can be found in Supplementary Table 2. For assessments of the sIgA-bound fractions of murine faecal and human mucosal or mucosal lavage samples by flow cytometry, samples were collected from 7–8-week-old mice or healthy human individuals, homogenized in sterile PBS at 25 mg ml−1 and filtered through a 70-μm strainer-capped tube (Falcon, 5 ml). C57/B6 WT mice were in our facility (WCM-CE) or were used for the assessment of sIgA binding to native mycobacteria, whereas C57BL/6 J WT mice raised in the Jackson Laboratory (JAX) were used in Ca-dREP colonization studies. Size separation was achieved by centrifugation at 900g for 10 min. The resulting large (fungal) and small (bacterial) fractions were used in applications such as flow cytometry analysis and/or sorting and/or DNA isolation. After samples were blocked for 30 min with staining buffer (2% BSA, 0.05% sodium azide), mouse samples were stained with 1:50 mAb against P. aeruginosa (ThermoFisher Scientific) and goat anti-human IgA (Miltenyi) secondary antibodies instead of PE-labelled, with the same clones at the same concentration. Stained faeces were then fixed in 4% formalin, followed by staining with 1:1,000 SYBR Green I (Invitrogen)/1:500 Calciofluor-white (Sigma).

Antifungal ELISA assay. For assessment of free sIgA titre in human mucosal lavage samples or mouse faecal pellets, samples were diluted, homogenized and centrifuged at 8,000g for 10 min. The resulting supernatant was weighted, resuspended to 100 μg material ml−1 with sterile PBS containing protease inhibitor (Sigma) and used in our assays. Total levels and antifungal titres of sIgA in human and mouse faeces or mucosal washings were measured by ELISA: 96-well high-binding polystyrene plates (Corning) were coated with 50 μl per well. Total free sIgA was assessed with anti-mouse IgG–IgA–IgM (Sigma) or anti-human Ig (Southern) diluted 1:2,000 in coating buffer (50 mM carbonate–bicarbonate buffer, pH 9.5). Fungal lysates prepared as described above were diluted 1:50 in coating buffer. S. cerevisiae mannann (Sigma), bacterial bagellin from S. typhimurium (InvivoGen), EceI-1 (Candida) and Sap6 (MyBioSource) were coated at 2 μg ml−1 in buffer, respectively. For total IgA analyses in mouse and human faeces or mucosal washings, sIgA-containing supernatant was diluted 250× and 4,000× in 0.5% BSA, and 50 μl of suspension was applied per well. After total IgA measurements, antigen-specific IgA was similarly plated with 50 μl after normalizing total IgA concentrations between all samples. Total IgA titres were measured against serial dilution standards of mouse (Bethyl Laboratories) and human (Southern) unconjugated IgA antibodies. After overnight incubation at 4 °C, plates were washed and incubated for 1 h at room temperature with 1,000× goat anti-human or anti-mouse IgA–horseradish peroxidase (HRP) or IgG–HRP antibodies (Southern). Plates were washed, followed by development with TMB HRP substrate (BD Biosciences) and quenched with 0.36 M H2SO4. Plates were washed, followed by development with TMB HRP substrate (BD Biosciences) and quenched with 0.36 M H2SO4. Plates were washed, followed by development with TMB HRP substrate (BD Biosciences) and quenched with 0.36 M H2SO4.

Cell processing, flow cytometry, fungal cell sorting, imaging and antibodies. Cell suspensions, prepared as described above, were blocked with CD16/CD32 (Mouse BD Fc Block, 2.4G2, BD Biosciences). For B cell compartment analysis, suspensions were stained with antibodies (Table 1) against CD45 (30-F11, BD Biosciences). Dead cells were excluded using eBioscience Anti-Dead Cells Fixable viability dye eFluor 780 (ThermoFisher Scientific). For at least two independent experiments, suspensions were stained with antibodies against CD3 (145-2C11, BD Biosciences), CD4 (RM4-5, BD Biosciences), CD8 (53-6.7, BD Biosciences), CD11b (M1/70, eBioscience), Cx3cr1 KO (183011F11, BD Biosciences) and CD103 (2E7, ThermoFisher Scientific). Dead cells were excluded using eBioscience Fixable viability dye eFluor 506 (ThermoFisher Scientific). Flow cytometry was performed using an LSRFortessa (BD Biosciences) and data were analysed with Flowjo v10 software (TreeStar). Images were acquired using an inverted Nikon Eclipse Ti microscope (Nikon).

In vivo models of intestinal fungal colonization. For monocolonization of GF mice, FS and ASF mice, 7–8-week-old mice were orally gavaged with 1×1010 fungal cells cultured from pure isolates grown overnight at 37 °C. Faecal sIgA assessment was conducted at the indicated time points and tissue harvesting for B cell compartment and staining was conducted after two weeks. For colonization of SPF mice, oral gavage of 7–8-week-old mice occurred two days after administering 0.4 g kg−1 cepazoline ad libitum and continued throughout the entirety of the experiment.

Isolation of PPs and cLP. Four to six PPs were excised from the ileum of each mouse. Colonos were isolated, opened longitudinally, washed of faecal contents and then cut into two pieces. The obtained tissues were placed in HBSS medium (ThermoFisher Scientific), supplemented with 2 mM EDTA, and were shaken for 10 min at 37 °C, followed washing and mincing in a medium consisting of RPMI 1640 (ThermoFisher Scientific), 5% fetal bovine serum, 0.5 mg ml−1 collagenase type VIII (Sigma), 5 U ml−1 DNase (Roche Diagnostics), 100 IU ml−1 penicillin and 100 μg ml−1 streptomycin (ThermoFisher Scientific), and filtered through a nylon mesh (70 μm). Processed tissues were once again filtered through mesh, washed twice in PBS and resuspended in cold PBS supplemented with 2% BSA for antibody staining.

In vitro antifungal IgA binding assay. For assessment of free sIgA binding to C. albicans, human mucosal lavage samples, mouse faecal samples or mouse faecal pellets were used as a source of sIgA. Samples were diluted, homogenized and centrifuged at 8,000g for 10 min. The resulting supernatant was separated from solid pelleted material, sterilized by passage through a 0.2 μm filter and resuspended to 100 μg material ml−1 with sterile PBS containing protease inhibitor (Sigma). Silica beads were used for staining of Ca-dREP grown previously under hypoxia-inducing conditions and incubated with staining buffer (2% BSA/0.05% sodium azide) for 30 min. Samples were then washed and stained with anti-human or anti-mouse IgA respectively, followed by fixation in 4% formalin (for microscopy evaluation) or in 1% paraformaldehyde/0.05% sodium azide, containing buffer (for flow cytometry).

In vitro two-partner assay for hyphal morphogenesis assessment in the presence of human sIgA. sIgA from human faecal samples prepared as described above was isolated using Peptide M Agarose (InvivoGen) loaded into G-Trap FPLC columns (G-Biosciences). Pooled and purified IgA was then desalted through buffer exchange to PBS using Amicon Ultra-15 Centrifugal filter tubes (Millipore) for dilution. Caco-2 cells (ATCC) were seeded into 24-well tissue culture–treated plates at a concentration of 0.2×103 ml−1 in DMEM (10% foetal bovine serum, 1% GlutaMAX solution ( Gibco), 1% penicillin/streptomycin solution (Corning) for 3 days at 37 °C. Each well was co-incubated with live Ca-dREP at multiplicity of infection 1 in DMEM (serum free, 1% penicillin/streptomycin solution) and supplemented with 5 μg sIgA per well at 0 and 6 h. The assay was terminated at 24 h (used as the last time point). Samples were collected at the indicated time points and fungal morphology was assessed by flow cytometry using the in vitro antifungal IgA-binding assay as described above.

Quantification and statistical analysis. All flow cytometry data were collected using FACSdiva Software (v.9.0). Statistics were computed using GraphPad Prism v.8 (GraphPad Software). Statistical details of experiments are reported in the figure legends. The P value(s) reported in the figure legends are the likelihood(s) of observing the effect size(s) if the null hypothesis of zero difference is true. All statistical tests represented are two-sided analyses. Apart from two mice from the experiment in Fig. 3b–k (referred in figure legend), no mice were excluded from our analyses. Normal distribution was not assumed in the statistical analyses shown in this study, and exact P values of these analyses are shown separately on each graph. P<0.05 is considered statistically significant.

Ethics. All mouse experiments were performed after approval by the Institutional Animal Care and Use Committee of Well Cornell Medicine. Human faecal samples or mucosal washings from healthy and CD-affected de-identified individuals were obtained with informed consent following institutional review board-approved protocols at Well Cornell Medicine.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Source data are provided with this paper. The data that support the findings of this study are available from the corresponding author on request.

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Author contributions

I.D. and I.D.I. conceived and designed the experiments. I.D., M.M., D.G.S., X.V.L., I.L., T.K., W.D.F., W.Y.L., E.R. and M.B.-D. performed the experiments. J.P., R.S.L. and P.C.W., generated key research materials and contributed to interpretation of the experiments. J.D. and I.D.I. generated figures and legends from analysed data. I.D.I acquired funding for the project. I.D. and I.D.I. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Identification of gut fungi from fecal material by flow cytometry and anti-C. albicans sIgA dynamics. a. Microbes in fecal material from SPF WT WCM-CE mice were distinguished as a Sybrhi population that is absent in GF mouse feces. b. Fungi (SybrhiCFW−) were enriched from bacteria (SybrhiCFW+) through size separation by 900g centrifugation and calcofluor white (CFW) staining of the resulting pellet. c. C. albicans cultured for 18 hours in hyphae-inductive media was stained with fecal supernatant from C. albicans-colonized GF mice (N=6) collected at 0, 2-, 4-, 8- and 14-days post colonization, followed by sIgA staining. Analysis of IgA binding representative of two independent experiments, one-way ANOVA, followed by Sidak’s test. d. Representative flow cytometry plots of frequency of B220+IgA− among Live CD45−CD4+ cells in the PP of germ-free (GF) mice orally gavaged with PBS (GF) or colonized for two weeks with C. albicans (+Ca). Data in (c) represents mean ± SEM.
Extended Data Fig. 2 | CFW−Sybr+ FSC−SSC− C. albicans population in feces represents hyphal/ pseudohyphal fungal morphologies that are preferentially bound by slgA. a, CFW−Sybr+ fungal population from feces of SPF mice colonized with CAF2-RFP C. albicans was sorted into FSC−SSC− and FSC−SSC+ fractions. Constitutive expression of RFP in this strain allows for high visibility and resistance to signal quenching upon prolonged light exposure during flow cytometry and microscopy on the same material. b, Immunofluorescence microscopy of sorted material from (a). Composite images at 20X magnification of FSC−SSC− and FSC−SSC+ shown in left and right panels, respectively. Scale bar represents 25μm. Data representative of two independent experiments. c, CFW−Sybr+ fungal population from feces of SPF mice colonized with CAF2-RFP was sorted into IgA+ and IgA− populations. Gray histograms represent IgA− isotype control staining used to distinguish sorted populations. d−e, Area (d) and perimeter length (e) of CAF2-RFP were compared between IgA+ and IgA− sorted populations. Data represents two independent experiments, mean ± SEM. Two-sided Mann-Whitney test. N = 5.
Extended Data Fig. 3 | Assessment of Ca-dREP C. albicans double reporter strain upon IgA staining and hyphae forming deficiency of efg1Δ/Δ cph1Δ/Δ C. albicans strain. a−b, Immunofluorescence microscopy of Ca-dREP incubated with human fecal supernatant as a source of sIgA and stained with DAPI and anti-human IgA-APC (a) or an APC isotype control (b). Single channel staining of 2 samples shown. Left to right: DAPI, constitutive ENO1-GFP expression, hyphae-specific HWP1-RFP expression, and anti-human IgA-APC (a) or APC isotype control (b). Top rows in a and b correspond to composite images in Fig. 2d,e, representing three independent experiments. Scale bar represents 50μm. c, Hyphae-competent (WT), but not hyphae-deficient (yeast-locked; efg1Δ/Δ cph1Δ/Δ) strains of C. albicans forms hyphae upon hyphae-inducing stimuli in vitro. Scale bar represents 25μm.
Extended Data Fig. 4 | Flow cytometry gating strategy in PPs, LP and in feces. a–b, Cell gating strategy for assessment of IgA+ GC B cell in PPs (a) and IgA+ plasmablasts in lamina propria (b). c, gating strategy of *C. albicans* cells in feces pre- and post-*C. albicans* (*C.a*) colonization.
Extended Data Fig. 5 | Graphical abstract for the model of antifungal IgA induction by and regulation of intestinal fungal commensalism. (Credit: Created with BioRender.com).
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection All flow cytometry data were collected by FACS Diva Software (version 9.0).

Data analysis Flow cytometry data analyzed by FlowJo V10. Statistical analysis analyzed by R and Graphpad Prism V8.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are available from the corresponding author upon request. No custom code was used.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
No statistical methods were used to predetermine sample size. Instead, they were chosen based on the current standards in the field, as well as the availability of GF, ASF, or SPF mice of the same age and sex which ensured sufficient power for downstream statistical analyses. Four or more mice per group were used in each experiment.

**Data exclusions**
Based on quality control, two mice were excused from data analysis. No other data were excluded for analysis.

**Replication**
All experiments were performed successfully at least independently twice or three biologically independent for all results in the manuscript.

**Randomization**
Age and sex matched groups of mice were randomly allocated to the experimental groups. For human antibody titers measured by ELISA, samples were randomized within healthy and CD groups and aside alongside each other.

**Blinding**
The investigators were blinded during sample collection, but not blinded during the experiments and analysis, since properly conducting these experiments required identifying the group to which samples belonged. Additionally, none of the experiments described required an investigator’s judgement

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology and archaeology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |
| ☑   | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChiP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

### Antibodies

**Antibodies used**

- Rat IgG1 kappa Isotype Control (eBRG1), PE, Thermo Fisher Scientific (eBioscienceTM), Cat # 12-4301-82; IgA Monoclonal Antibody (MA-6E1), PE, Thermo Fisher Scientific (eBioscienceTM), Cat # 12-4204-82; Rat IgG1 kappa Isotype Control (eBGR1), APC, Thermo Fisher Scientific (eBioscienceTM), Cat # 17-4301-82; IgA Monoclonal Antibody (MA-6E1), APC, Thermo Fisher Scientific (eBioscienceTM), Cat # 17-4204-82; Alexa Fluor® 647 ChromPure Goat IgG, whole molecule, Jackson ImmunoResearch, Cat # 005-600-003; Alexa Fluor® 647 AffinPure Goat Anti-Mouse IgG, Fc fragment specific, Jackson ImmunoResearch, 115-605-071; Rat IgG2a kappa Isotype Control (eBR2a), PE-Cy5, Thermo Fisher Scientific (eBioscienceTM), Cat # 25-4321-82; IgM Monoclonal Antibody (Il/41), PE-Cy5, Thermo Fisher Scientific (eBioscienceTM), Cat # 25-5790-82; Isotype Control Antibody, mouse IgG1 (IS5-21F5), PE, Miltenyi Biotec, Cat # 130-113-200; IgA Antibody, anti-human (IS11-8E10), PE, Miltenyi Biotec, Cat # 130-113-476; Isotype Control Antibody, mouse IgG1 (Isotype Control Antibody, mouse IgG1 (IS5-21F5), PE, Miltenyi Biotec, Cat # 130-113-200; IgA Antibody, anti-human (IS11-8E10), PE, Miltenyi Biotec, Cat # 130-113-476; Isotype Control Antibody, mouse IgG1 (IS5-21F5), PE, Miltenyi Biotec, Cat # 130-113-200; IgA Antibody, anti-human (IS11-8E10), PE, Miltenyi Biotec, Cat # 130-113-476; Isotype Control Antibody, mouse IgG1 (IS5-21F5), PE, Miltenyi Biotec, Cat # 130-113-200; IgA Antibody, anti-human (IS11-8E10), PE, Miltenyi Biotec, Cat # 130-113-476; Goat IgG-Alexa Fluor® 647, Southern Biotech, Cat # 65-0866-14; Brilliant Violet 605TM Anti-mouse I-A/I-E a (M5/114.15.2), Biolegend, Cat # 107639; Anti-mouse CD11b monoclonal antibody (M1/70), PE, Cyntellect, Thermo Fisher Scientific (eBioscienceTM), Cat # 25-0112-82; Anti-mouse CX3CR1 antibody (SA011F11), BV421, Biolegend, Cat # 149023; Anti-
Validation

All antibodies used in this study are commercially available. All antibodies have been validated by the manufacturer:

- Rat IgG1 kappa Isotype Control (eBioG1, PE, Cat # 12-4301-82) “This PE rat IgG1 isotype control has been tested by flow cytometric analysis of mouse sphenocyte suspensions.”

- IgA Monoclonal Antibody (mAb-6E1, PE, Cat # 12-4304-82) “This mAb-6E1 antibody has been tested by flow cytometric analysis as a second step to detect mouse IgA primary antibodies.”

- Rat IgG1 kappa Isotype Control (eBioG1, APC, Cat # 17-4301-82) “This rat IgG1 isotype control has been used by flow cytometric analysis of mouse sphenocyte suspension.”

- IgA Monoclonal Antibody (mAb-6E1, APC, Cat # 17-4204-82) “This mAb-6E1 antibody has been tested by flow cytometric analysis of mouse sphenocytes stained with a mouse IgA primary antibody.”

- Alexa Fluor® 647 ChromoPure Goat IgG (whole molecule, Jackson Immunoresearch, Cat # 006-605-003) “Based on immunoelectrophoresis at an antigen concentration of 20 mg/ml, the pattern of precipitation against anti-goat whole serum is the same as that against anti-goat IgG, Fc fragment specific.”

- Alexa Fluor® 647 AffiniPure Goat Anti-Mouse IgG (Fcy fragment specific, Jackson Immunoresearch, Cat # 115-605-071) “Based on immunoelectrophoresis and/or ELISA, the antibody reacts with the Fc portion of mouse IgG heavy chain but not with the Fab portion of mouse immunoglobulins. No antibody was detected against mouse IgM or non-immunoglobulin serum proteins. The antibody has been tested by ELISA and/or solid-phase adsorbed to ensure minimal cross-reaction with human, bovine, and horse serum proteins, but it may cross-react with immunoglobulins from other species.”

- Rat IgG2a kappa Isotype Control (eBR2a, PE-Cyanine7, Cat # 25-4211-82) “This eBR2a antibody has been tested by flow cytometric analysis of mouse sphenocytes.”

- IgM Monoclonal Antibody (IV/41, PE-Cyanine7, Cat # 25-5750-82) “This IV/41 antibody has been tested by flow cytometric analysis of mouse spleen cells.”

- Isotype Control Antibody, mouse IgG1 (ISE-21F5, PE, Cat # 130-113-200) “Human peripheral blood lymphocytes (PBLs) were stained with Mouse IgG1 isotype control antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer.”

- IgA Antibody, anti-human (IL11-B10D, PE, Cat # 130-113-476) “Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgA antibodies as well as with CD19 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer.”

- Isotype Control Antibody, mouse IgG1 (ISE-21F5, APC, Cat # 130-113-196) “Human peripheral blood lymphocytes (PBLs) were stained with Mouse IgG1 isotype control antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer.”

- IgA Antibody, anti-human (IL11-B10D, APC, Cat # 130-113-472) “Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgA antibodies as well as with CD19 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer.”

- Goat IgG-Alexa Fluor® 647 (Cat # 0109-31) “This document certifies that this product has met the quality control standards defined by SouthernBiotech and is guaranteed through the expiration date when stored unilisted as directed.”

- Goat Anti-Human IgG-Alexa Fluor® 647 (Cat # 2040-31) “This document certifies that this product has met the quality control standards defined by SouthernBiotech and is guaranteed through the expiration date when stored unilisted as directed.”

- PerCP/Cy5.5 anti-mouse/human CD45R/B220 (RA3-6B2, Cat # 103255) “This product lot has passed BioLegend’s QC testing and is certified for use.”

- Purified Rat Anti-Mouse CD16/32 (Mouse BD FC BlockTM, Clone 2.4G2, Cat # 563141) “This antibody is routinely tested by flow cytometric analysis.” On website, vendors show two color analysis of the expression of CD16/32 on mouse spleen cells and Fcγreceptor-mediated non-specific staining.

- ebioscienceTM Fixable Viability Dye eFluor® 655UV (Cat # 65-0866-18) “Normal human peripheral blood cells were heat-killed at 65°C for 1 min then mixed 1:1 with live peripheral blood cells and then stained with staining buffer (blue histogram) or Fixable Viability Dye eFluor® 455UV (purple histogram). Cells in the lymphocyte gate were used for analysis.”

- Brilliant Violet 650TM anti-mouse CD4 Antibody (30-F11, Cat # 103151) “G7BL/6 mouse splenocytes were stained with CD4 (clone 30-F11) Brilliant Violet 650™ (filled histogram) or rat IgG2b, x Brilliant Violet 650™ isotype control (open histogram). This product lot has passed BioLegend’s QC testing and is certified for use.”

- Brilliant Violet 605TM anti-mouse CD4 Antibody (RM-4-S, Cat # 100548) “G7BL/6 mouse splenocytes were stained with CD3 PE and CD4 (clone RM-4-S) Brilliant Violet 605™. This product lot has passed BioLegend’s QC testing and is certified for use.”

- Alexa Fluor® 700 anti-mouse CD13 Antibody (eBio1D3, Cat # 56-0193-81) “This eBio1D3 (1D3) antibody has been tested by flow cytometric analysis of mouse sphenocytes.”
Eukaryotic cell lines

Policy information about: cell lines

| Cell line source(s) | Colon cancer cell line Caco-2 was obtained from ATCC. |
|---------------------|------------------------------------------------------|
| Authentication      | Cell lines purchased from ATCC with authentication. |
| Mycoplasma contamination | No Caco-2 Mycoplasma contamination. |
| Commonly misidentified lines (See CLIC register) | None |

Animals and other organisms

Policy information about: studies involving animals, ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | 7-8-week-old wild-type SPF C57BL/6 mice (Jackson:000664), IgM1m1Gv1/1 μMT−/−, Rag2m1Mom (Rag1−/−, Tg[ltg5−cre]-1.1F6iz [Cd11c-cr], IfnγR1ΔΔ[H/R/R], and Cd3e1m [DTR]tm1/J [Cd3e1DTR]) mice were purchased from the Jackson Laboratories (Bar Harbor, ME). Germ free (GF) mice were similarly bred and maintained within sterile vinyl isolators at Weill Cornell Medical College Cnootobiologic Mouse Facility. Altered Schaedler flora (ASF) mice were generated from germ-free wild-type C57BL/6 mice upon inoculation with ASF community. As specified in the "Methods" section of the manuscript, all laboratory animal experimental groups included equal mixes of male and female mice between 8-16 weeks of age. All mice used in these experiments were housed with a 12-hr light/dark cycle per day at a temperature of 72±2°F, and 30-70% relative humidity. |
| Wild animals | No wild animals were used in this study. |
| Field-collected samples | No field-collected samples used in the study. |
Ethics oversight

All animal experiments were approved and are in accordance with the Institutional Animal Care and Use Committee guidelines at Weill Cornell Medicine.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Healthy mucosal washing and serum samples (matched): N = 9; 5 male, 4 female; 18-62 years old.
CD mucosal washings and serum samples (matched): N = 12; 7 male, 5 female; 23-84 years old.

Recruitment

Fecal and corresponding serum samples were obtained from de-identified individuals following informed consent and Institutional-Review-Board-approved protocols at Weill Cornell Medicine. CD Patients were recruited and enrolled from the EPIC electronic medical record system by physicians or study coordinators after obtaining informed consent and collecting disease history, surgical history, disease phenotype, extraintestinal manifestations of disease, medications, and other clinical data. If patient did not have current IBD diagnosis, their reasons for undergoing endoscopy or colonoscopy was noted. Healthy individuals were identified through their medical chart and health history. Recruitment was kept broad to limit selection bias, recruiting any adults undergoing colonoscopy regardless of visit reason, age, gender, or race.

Policy information about studies involving human research participants

Population characteristics

Healthy mucosal washing and serum samples (matched): N = 9; 5 male, 4 female; 18-62 years old.
CD mucosal washings and serum samples (matched): N = 12; 7 male, 5 female; 23-84 years old.

Recruitment

Fecal and corresponding serum samples were obtained from de-identified individuals from the JRI IBD Live Cell Bank Consortium and Institutional-Review-Board-approved protocols at Weill Cornell Medicine.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Peyer’s Patches (PP) and Lamina Propria (LP) cells were prepared as described in the "Isolation of Peyer’s patches (PP) and colonic lamina propria (cLP)" subsection of the “Methods”. "Four to six PP were excised from the ileum of each mouse. Colon were isolated, opened longitudinally, washed of fecal contents and then cut into pieces. Such obtained tissues were placed into Hank’s Balanced Salt Solution (HBSS) medium (Thermofisher), supplemented with 2 mM EDTA, and were shaken for 10 min at 37°C, followed washing and mincing in a medium consisting of RPMI 1640 (Thermofisher), 5% FBS, 0.5 mg/ml collagenase type VIII (Sigma), 5 U/ml DNase (Roche Diagnostics), 100 IU/ml penicillin and 100 μg/ml streptomycin (Thermofisher) and filtered through a nylon mesh (70μm). Processed tissues were once again filtered through mesh, washed twice in PBS, and resuspended in cold PBS supplemented with 2% BSA for antibody staining.

Instrument

Fortessa II (BD Biosciences) and inverted Nikon Eclipse Ti microscope (Nikon)

Software

Flow cytometry data were collected via FACSDiva (version 9.0, BD Biosciences) and analyzed by FlowJo V10 (TreeStar).

Cell population abundance

No cells were sorted in this study. Additionally, no cell population abundances were reported.

Gating strategy

All gating of cells was determined after FSC/SSC gating on lymphocytes population. FSA-A vs FSC-H and SSC-A vs SSC-W gates were used to gate singlets. Only CD45 positive viable cells (Fixable Viability Dye 506, eBioscience) were included for further analysis. All gating of fungal material was determined after CPW and SYBR Green gating as presented in Suppl. Fig 1 in extended data.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.