A *Candida albicans* Strain Expressing Mammalian Interleukin-17A Results in Early Control of Fungal Growth during Disseminated Infection

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Candida albicans is normally a commensal fungus of the human mucosae and skin, but it causes life-threatening systemic infections in hospital settings in the face of predisposing conditions, such as indwelling catheters, abdominal surgery, or antibiotic use. Immunity to *C. albicans* involves various immune parameters, but the cytokine interleukin-17A (IL-17A) (also known as IL-17) has emerged as a centrally important mediator of immune defense against both mucosal and systemic candidiasis. Conversely, IL-17A has been suggested to enhance the virulence of *C. albicans*, indicating that it may exert detrimental effects on pathogenesis. In this study, we hypothesized that a *C. albicans* strain expressing IL-17A would exhibit reduced virulence *in vivo*. To that end, we created a *Candida*-optimized expression cassette encoding murine IL-17A, which was transformed into the DAY286 strain of *C. albicans*. *Candida*-derived IL-17A was indistinguishable from murine IL-17A in terms of biological activity and detection in standard enzyme-linked immunosorbent assays (ELISAs). Expression of IL-17A did not negatively impact the growth of these strains *in vitro*. Moreover, the IL-17A-expressing *C. albicans* strains showed significantly reduced pathogenicity in a systemic model of *Candida* infection, mainly evident during the early stages of disease. Collectively, these findings suggest that IL-17A mitigates the virulence of *C. albicans*.

Candida albicans is a commensal fungus of human skin and mucosal surfaces. Although normally nonpathogenic, *C. albicans* can cause disease in settings of immunodeficiency, abdominal surgery or otherwise disrupted barriers, antibiotic use, or other conditions (1, 2). By far the most serious form of candidiasis is a systemic infection characterized by fungal invasion into target organs, particularly the liver, kidney, and brain (3). Disseminated candidiasis is the 4th most common cause of hospital-acquired infections and has a high mortality rate (averaging 40%) (4).

In recent years, accumulating data have implicated the cytokine interleukin-17A (IL-17A) (also known as IL-17) in immunity to *Candida* and other fungi (5, 6). IL-17A- and IL-17 receptor-deficient mice are highly susceptible to candidemia (7–9). Similarly, humans with defects in the IL-17 pathway are especially sensitive to *Candida* infections; the defects arise from mutations in genes controlling IL-17 signaling (*ACT1*, *IL17RA*, *IL17RC*, and *IL17F*) or genes that drive Th17 cell differentiation (*DECTIN1*, *CARD9*, *STAT3*, *STAT1*, *IL12B*, and *IL12RB*) or arise in individuals with naturally occurring anti-IL-17 antibodies (occurring in certain thymomas and *AIRE* deficiency, also known as autoimmune polyglandular syndrome type 1 [APS-1] or autoimmune polyendocrinopathy candidiasis ectodermal dystrophy [APECED]) (10, 11).

Although *Candida* infections can be treated pharmacologically, currently available antifungal medications are limited by significant drug-drug interactions, the emergence of drug resistance, and toxicity (12, 13). To date, there are no vaccines available for any fungal species, although an experimental vaccine to treat vaginal candidiasis is in development (14, 15). Experimental vaccines for *Candida* and other pathogenic fungi have been shown to require intact Th17 responses (16, 17). In contrast, it has been

suggested that IL-17A may act directly on *C. albicans* to increase its virulence in a gastric model of candidiasis (18). Therefore, understanding the correlates of immunity and particularly the impact of IL-17 activity in the context of *Candida* infections is important in designing new drugs or preventive strategies to treat candidiasis. Accordingly, our goal in this study was to determine whether ectopic expression of IL-17A by *Candida* was protective or pathogenic in the context of disease. To that end, we created a yeast strain expressing murine IL-17A and demonstrated that there is an improved host response to this strain during a mouse model of disseminated candidiasis.

MATERIALS AND METHODS

Plasmids, *Candida* transformation, and culture. An expression cassette was constructed containing the first 1,000 bp of the *Candida* *TDH3* pro-

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motor, the secreted *Candida* SAP5 signal sequence (SS), and the open reading frame (ORF) of murine *Il17a*. The *TDH3* promoter (driving *Candida albicans* glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) was chosen due to its high constitutive expression in yeast. The sequence of mouse *Il17a* was optimized for expression in *C. albicans* by GeneArt (Life Technologies, Grand Island, NY) with its native SS removed. The murine *Il17a* SS was identified by comparison to the known human *IL17A* SS (19) and prediction software from SignalP 4.0 server (20). To assemble the cassette, fragments were recombined into histidine-negative *Saccharomyces cerevisiae* by homologous recombination (gap repair) in the plasmid pSG1 (histidine positive) and grown on selective media. The resulting plasmid was purified from *S. cerevisiae*, transformed into *Escherichia coli*, and verified by sequencing. The linearized cassette was recombined into the *C. albicans* strain DAY286 genome by lithium acetate transformation. Positive colonies were identified by growth on selective media and colony PCR. The *Candida* IL-17A primers were 5'-GCTATGACCATGATTA CGCC and 3'-TATAATGTTTATCTAACAAAGATGTTGTGT. Strains were grown in yeast extract-peptone-dextrose (YPD) at 30°C or stored at -80°C in 15% glycerol.

Filamentation assay. Strains were cultured overnight at 30°C and diluted into fresh RPMI 1640 at 37°C at an optical density at 600 nm (OD_{600}) of 0.2 for 90 min. Samples were fixed in 4% formaldehyde in phosphate-buffered saline (PBS), stained with Calcofluor (200 μ g/ml), and visualized with a Zeiss Axio Observer Z.1 fluorescence microscope and a 20 $\times$ objective with 1.6 optivar. Seven Z-stack images were assembled per image. To obtain a quantitative assessment of filamentation, filament length was measured in a blind manner on enlarged images and presented as a filament index with arbitrary units.

Cell culture, cytokine stimulations and histology. ST2 stromal cells were cultured with Eagle minimum essential medium alpha modification (α -MEM) (Sigma, St. Louis, MO) with 10% fetal bovine serum (FBS), L-glutamine, and antibiotics (Invitrogen, Carlsbad, CA). Recombinant IL-17 and tumor necrosis factor alpha (TNF- α) were from Peprotech (Rocky Hill, NJ) and used at 10 ng/ml and 1 ng/ml, respectively. The cells were stimulated with supernatant from IL-17A-secreting *Candida* or the parent DAY286 strain diluted 1:10 in α -MEM with 10% FBS with or without TNF- α for 6 h at 37°C. Additional samples were incubated with anti-IL-17A monoclonal antibodies (MAbs) (10 μ g/ml) or isotype controls (R&D Systems, Minneapolis, MN) starting 1 h prior to cytokine stimulation and continuing throughout the incubation period. Supernatants were analyzed for IL-6 and chemokine (C-X-C motif) ligand 5 (CXCL5) by enzyme-linked immunosorbent assays (ELISAs). IL-6 ELISA kits were from eBioscience (San Diego, CA), and CXCL5 ELISA kits were from R&D Systems. For histology, kidney samples were stained with hematoxylin and eosin stain (H&E) or periodic acid-Schiff stain (PAS) by the University at Buffalo Histology Core (Buffalo, NY). The slides were analyzed in a blind manner, and images were captured on an EVOS FL microscope system (Life Technologies). RNA from kidney was prepared with Qiagen RNeasy kits with an on-column DNase digestion step, and cDNA was prepared with SuperScript III reverse transcriptase (Life Technologies). PCR primers for detection of *Candida* in kidney tissue were as follows: *TDH3* (5'-ATCCCACAAGGACTG GAGA and 3'-ATCCCACAAGGACTGGAGA), *Ca-IL-17A* (5'-TTT GCTTTATTAATTGATGCT and 3'-ATGAACTTTAGCACCCAATG). Samples were visualized on a 2.5% agarose gel and imaged on a Protein Simple instrument (Wallingford, CT).

Mice and infections. C57BL/6 female mice from The Jackson Laboratory (Bar Harbor, ME) were used at 6 to 10 weeks of age. IL-17RA^{-/-} (IL-17RA stands for interleukin 17A receptor) mice (C57BL/6 background) were provided by Amgen and bred in-house (21). Mice were housed under a 12-h light/dark cycle and specific-pathogen-free (SPF) conditions and provided with autoclaved food and water *ad libitum*. Mice were injected in the tail vein with the indicated *Candida* strains at 2×10^5 yeast cells or sterile saline vehicle control as described previously (22). For injections, mice were briefly held in a commercial restraining apparatus

(Braintree Scientific, Braintree, MA). Mice were monitored visually and weighed at least once daily. Mice were humanely sacrificed by CO₂ inhalation at the termination of each experiment or if animals exhibited >20% weight loss or showed signs of pain or distress as delineated by the approved animal protocol. The University of Pittsburgh Institutional Animal Care and Use Committee (IACUC) approved all animal protocols used in this study (Animal welfare assurance number A3187-01). All efforts were made to minimize suffering in accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* (23). Tissues were harvested in a Miltenyi GentleMACS dissociator and plated in serial dilutions on YPD-Amp (YPD with ampicillin) agar plates.

RESULTS

To create a strain of *C. albicans* that secretes a form of IL-17A that can be recognized *in vivo*, we synthesized a recombinant cassette composed of a constitutive *Candida albicans* *TDH3* promoter (which regulates the *C. albicans* *GAPDH* gene) driving the murine *Il17a* gene (Fig. 1A). The native signal sequence (SS) of *Il17a* was replaced by the SS of the fungal secreted aspartyl proteinase 5 (SAP5). The IL-17A sequence was optimized for expression in *C. albicans* (Fig. 1B). These cassettes were recombined into the pSG1 plasmid with a histidine selective marker (24). The plasmid was transformed into *C. albicans* strain DAY286 (25), positive colonies were identified by growth on selective media, and colony PCR was performed to verify insertion of the *Il17a* gene (data not shown).

To determine whether the recombinant *C. albicans* strains expressed IL-17A protein, two independently derived strains of strain DAY286 expressing IL-17A (DAY286-IL-17A) were cultured for 16 h at 30°C, and conditioned supernatants were serially diluted and analyzed by ELISAs. As shown, both strains exhibited substantial production of IL-17A (with a mean production of 74,829 pg/ml), whereas no IL-17A or cross-reactive contaminants were detected in the supernatant from the parent DAY286 strain (Fig. 1C). Therefore, *C. albicans* can be engineered to secrete mammalian IL-17A.

Although IL-17A has been reported to negatively impact the virulence of *C. albicans* by direct action on the fungus (18), the growth curve of IL-17A-expressing *C. albicans* DAY286 was indistinguishable from the parent strain, indicating that ectopic expression of IL-17A is not detrimental to *C. albicans* growth *in vitro* (Fig. 2A). Moreover, expression of IL-17A in the positive clones was stable over at least 2 months of culture (data not shown). In characterizing this strain, we noticed that one of the IL-17A-producing strains exhibited enhanced sensitivity to caspofungin (C. A. Woolford, unpublished data), perhaps suggesting alterations in the cell wall. Accordingly, we measured the filamentation capacity of the parent and IL-17A-expressing strains. *Candida* strains were cultured in RPMI 1640 for 90 min, stained with Calcofluor, and visualized as a Z-stack of seven compiled images (Fig. 2B). Filamentation length was measured in a blind manner on representative enlarged images. Unexpectedly, there was a difference in the average germ tube length between parental and IL-17A-expressing strains, with the DAY286-IL-17A germ tubes ~25% shorter than the parent strain germ tubes (Fig. 2C). To determine whether IL-17 signaling acting directly on the fungus caused these differences, yeast strains were cultured in 100 ng/ml recombinant murine IL-17A during filamentation. However, there was no difference in either the parent or the IL-17A-expressing strain upon addition of IL-17, indicating that exogenous ex-

FIG 1 Engineering an IL-17A-secreting strain of *Candida albicans*. (A) Diagram of the construct used to express IL-17A in *C. albicans*. The constitutive *TDH3* promoter encoding GAPDH of *C. albicans* was used to drive expression. The Sap5 signal sequence (Sap5SS) is derived from the yeast secreted aspartyl proteinase gene and is fused to a codon-optimized open reading frame encoding murine IL-17A without its native signal sequence. (B) Sequence of the *TDH3* promoter (red) and the SAP5SS-IL-17A fusion gene (SAP5SS sequence shown in blue, and IL-17A sequence shown in purple) are shown. The intervening pSG1-derived sequence is shown in black. (C) Expression of murine IL-17A was detected by ELISA in conditioned media from two *C. albicans* clones (clones 1 and 2) compared to the parent *C. albicans* DAY286 strain. Two clones of strain DAY286 expressing IL-17A (DAY286-IL-17A) were tested. The experiment was repeated three times.

posure to this cytokine does not alter the filamentation capacity of the fungus (Fig. 2B and C).

To determine whether the IL-17A produced by these *Candida* strains was biologically active, we used a mammalian cell culture assay to detect signaling by yeast-derived IL-17A. In mammals, IL-17A is produced dominantly by lymphocytes, but its main biological activity is on nonhematopoietic cells, particularly fibroblasts and epithelial cells. Even in these cell types, IL-17A-mediated signaling by itself is fairly modest. However, IL-17A exhibits potent signaling synergy in cooperation with TNF- α to induce expression of downstream cytokines such as IL-6 (26, 27). Therefore, we evaluated the activity of *Candida*-derived IL-17 in the presence or absence of suboptimal concentrations of TNF- α using a mouse bone marrow stromal cell line, ST2, which responds to IL-17 by expressing characteristic gene products such as IL-6 and the chemokine CXCL5 (28). First, we confirmed that recombinant murine IL-17 (rIL-17A) (10 ng/ml) and TNF- α (1 ng/ml) induced IL-6 and CXCL5 in ST2 cells. As expected, activation of these genes was blocked by a neutralizing antibody (Ab) against IL-17A (Fig. 3A and B). We also verified that there was no inductive effect of *Candida* conditioned medium from the parent DAY286 strain on ST2 cells. However, conditioned medium from DAY286-IL-17A cells (diluted 1:10 to a concentration of ~6 to 8 ng/ml of IL-17A) induced marked secretion of both IL-6 (Fig. 3A) and CXCL5 (Fig. 3B) into the ST2 cell culture supernatant. There was no detectable difference in production of CXCL5 in response to rIL-17A compared to *Candida*-derived IL-17A under these con-

ditions, although IL-6 production was reduced ~2-fold with the *Candida*-derived IL-17A. We do not know the basis for these differences, though they were reproducible (A. R. Huppler, unpublished observations). Importantly, neutralizing Abs against IL-17A, but not isotype control Abs, blocked the activity of the DAY286-IL-17A culture supernatants, demonstrating specific activity of the *Candida*-derived IL-17A. Thus, the IL-17A produced by *C. albicans* was functionally comparable to mouse IL-17A.

Because immunity to systemic candidiasis requires intact IL-17A signaling (7, 8), we hypothesized that IL-17A-secreting *Candida* strains would be less pathogenic than the parental wild-type (WT) strain. To test this hypothesis, WT C57BL/6 mice (12 or 13 mice in each group) were infected intravenously with saline (mock infected [Sham]) or with 2×10^5 CFU of strain DAY286 or DAY286-IL-17A, a dose that is commonly used in this model. Expression of *Candida* IL-17A in infected kidney was verified by quantitative PCR (qPCR) and sequencing (see Fig. S1 in the supplemental material; also data not shown). After 4 days, the mice were sacrificed, and kidney fungal burdens were assessed by plating serial dilutions of tissue homogenate on YPD agar followed by colony enumeration in triplicate. As expected, there was no *C. albicans* detected in the mock-infected kidneys. The geometric mean of the kidney fungal load in the DAY286-infected mice was 72,202 CFU/g, whereas in the DAY286-IL-17A-infected mice, the mean load was 4,895 CFU/g, representing an ~15-fold reduction in the fungal burden (Fig. 4A). These differences were reproducible and statistically significant ($P = 0.006$).

FIG 2 Characterization of IL-17A-secreting *C. albicans*. (A) The indicated *C. albicans* strains were cultured in YPD broth over 16 h, the optical density at 600 nm (O.D.600) of samples of the strains were determined (left), and the strains were plated for colony enumeration (right) at regular intervals. The experiment was performed once. (B and C) The indicated strains were cultured in RPMI 1640 for 90 min to induce filamentation with recombinant mouse IL-17A (100 ng/ml) or without recombinant mouse IL-17A (untreated), and germ tube length was assessed in enlarged images. All the images in panel B were taken at the same magnification. Experiments were performed twice. In panel C, the treatments are recombinant mouse IL-17A (17) or untreated (U). Values that are significantly different are indicated by a bar and two asterisks ($P < 0.01$). Values that are not significantly different (*ns*) are also indicated. Significance was assessed by analysis of variance (ANOVA) with a *post hoc* Tukey's test.

Consistent with a reduced fungal burden, there was substantially more weight loss in the DAY286-infected group than in the DAY286-IL-17A-infected group (Fig. 4A). Note that, although there is a difference in histidine biosynthesis between these strains, it has been shown that the *his1* mutation does not impair pathogenicity in this model (29).

Histological analysis of infected kidneys was consistent with the fungal load profiles. Specifically, in kidneys taken from DAY286-infected mice, H&E staining revealed distinct patches of inflammatory infiltrates in the tubular space, composed mainly of neutrophils and monocytes. The glomeruli surrounded by inflammatory cells appeared inflamed, with prominent signs of tubular atrophy. These areas corresponded to regions of dense *C. albicans* invasive hyphae, as visualized by PAS staining. In contrast, in DAY286-IL-17A-infected tissue, there were no obvious patches of infiltrating cells and markedly less invasive *C. albicans* (see Fig. S2 in the supplemental material). There was no evidence of tubular atrophy or inflamed glomeruli in these mice. Therefore, ectopic expression of IL-17A by *C. albicans* did not cause obvious renal inflammation and was associated with reduced inflammatory infiltrates and a reduction in hyphal tissue invasion. In support of this observation, we have found that overexpression of murine IL-17A in an adenoviral vector system also demonstrated no obvious inflammatory changes in the kidney (P. S. Biswas, unpublished observations).

In order to verify that the reduced fungal loads observed upon infection with the DAY286-IL-17A strain were in fact

mediated by host responses rather than an intrinsic effect of the yeast (such as the reduced filamentation seen in Fig. 2), IL-17RA^{-/-} mice were infected with both the parent and IL-17-expressing strains. As shown, there was no difference in the fungal load or weight loss upon infection with either strain (Fig. 4B). These data indicate that ectopic expression of IL-17A by *C. albicans* significantly reduces fungal loads during disseminated candidiasis in a manner that is dependent on host responses to IL-17 signaling.

To determine whether the effect of IL-17A was long-lasting, we infected mice and tracked weight loss and survival over a 15-day time course. Again, during the first 4 days of infection, the mice infected with strain DAY286-IL-17A showed less pronounced weight loss than mice infected with the parental DAY286 strain (Fig. 4C). However, starting at day 5 and continuing for the remainder of the experiment, there was no difference in weight loss between mice infected with either strain. There was also no difference in survival to disease endpoint (Fig. 4D) or fungal burden (Fig. 4E) in the few surviving animals ($n = 3$ or 4) at day 15. Therefore, the main activity of IL-17A appears to be in the early time points postinfection, with other factors coming into play at later stages of disease.

DISCUSSION

It is now well established that the IL-17/Th17 pathway is an essential component of the immune response to pathogenic *Candida albicans* infections, not only in mouse models of disease but also in

FIG 3 IL-17A produced by *Candida* is biologically active. Mouse ST2 cells were treated (+) for 6 h with recombinant IL-17A (rIL-17A) (10 ng/ml), recombinant TNF- α (rTNF α) (1 ng/ml), or a 1:10 dilution of conditioned media from DAY286 or DAY286-IL-17A (clone 2) (6 to 8 ng/ml concentration) for 6 h. The indicated samples were also treated with 10 μ g/ml isotype control Ab or a neutralizing anti-murine IL-17A (anti-mIL-17A) Ab (white bars). (A and B) Supernatants were analyzed by ELISA for IL-6 (A) or CXCL5 (B). Experiments were performed three times. Statistical significance is indicated by bars and symbols as follows: *, $P < 0.05$ compared to the value for the unstimulated sample from the corresponding condition; ‡, $P < 0.05$ compared to the value for the isotype-treated sample from the corresponding condition; \$, $P < 0.05$ for the values for the indicated conditions; ns, not significant.

humans (10). However, this subject remains controversial, as IL-17A has been reported to be pathogenic in gastrointestinal *Candida* infections (30) and may exert a direct effect on the yeast to enhance its virulence (18). Conversely, IL-17 signaling is clearly protective in other forms of candidiasis (31–33). Moreover, the mechanisms by which IL-17 exerts its effects in the context of candidiasis are not fully understood. IL-17 is essential to control the neutrophil and antimicrobial peptide responses to candidiasis (31, 34–36). In addition, a recent study suggests that IL-17 may also be important for driving NK cell function in disseminated disease through a mechanism dependent on granulocyte-macrophage colony-stimulating factor (GM-CSF) (37).

In this study, we undertook to determine the effect of expressing IL-17A in *Candida albicans* during infection. We were not surprised to see that IL-17A expression reduced fungal burdens in a mouse model of disease, though it was unexpected to see that most of the beneficial effect is evident only during the first 4 days of infection (Fig. 4). This observation suggests that the most important impact of IL-17A occurs early, whereas at later stages other factors may come into play. It should be noted, however, that IL-17RA^{-/-} mice do not recover from candidiasis even after long-term infections (7, 38), though to our knowledge, IL-17A^{-/-} mice have not been tested in this regard. It is also possible that DAY286-IL-17A-infected mice fail to reach a threshold level of IL-17A because they are cleared rapidly during the first 4 days.

Since IL-17RA serves as an essential signaling receptor for several IL-17 family cytokines (IL-17F, IL-17A/F, IL-17C, and IL-25) (39), this finding may indicate a selective effect of IL-17A during early infection.

We unexpectedly observed that the IL-17A-expressing *Candida* strains exhibited altered filamentation *in vitro*, with the IL-17A-expressing strain consistently having ~25% shorter germ tubes than the parental strain did (Fig. 2C). The basis for this difference is unclear, though it was seen in both strains. It is possible that the additional energy expenditure required to express an additional secreted protein impacts the ability of the fungus to germinate. This finding raised the possibility that IL-17A might act directly on *C. albicans*, a phenomenon that was reported by Zelante and colleagues to affect *Candida* virulence in a gastrointestinal model of infection (18). However, when we treated the matched parental strain with IL-17A at 100 ng/ml (approximately the same concentration of cytokine produced by the recombinant strains), there was no detectable impact on germ tube growth (Fig. 2B and C). To rule out the possibility that the improved outcome in the IL-17A-producing strains was in some way related to this altered phenotype in a fungus-intrinsic manner, we evaluated candidiasis in IL-17RA^{-/-} mice, which cannot respond to IL-17 signaling. In contrast to WT controls, there was no change in fungal load in IL-17RA^{-/-} mice, indicating that the effect of *C. albicans*-produced IL-17A requires effective host responses, and

FIG 4 IL-17A-secreting *Candida albicans* exhibits reduced pathogenicity *in vivo* early during infection. (A) (Left) WT mice ($n = 12$) were injected via tail vein with saline (Sham) or with 2×10^5 CFU of the indicated *C. albicans* strains. After 4 days, the mice were sacrificed, and kidney burden (CFU/g of kidney) was assessed by plating. Each symbol represents the value for an individual mouse, and the short line represents the geometric mean value for the group of mice. The values for the DAY286-infected group and the DAY286-IL-17A-infected group are significantly different by analysis of variance (ANOVA) and Mann-Whitney correction. (Right) Weight loss at the indicated dates was assessed. The values for the DAY286-infected group that are significantly different from the values for the DAY286-IL-17A-infected group by ANOVA and Student's *t* test are indicated by an asterisk. (B) (Left) IL-17RA^{-/-} mice ($n = 3$ or 4) were injected via tail vein with saline (Sham) or with 2×10^5 CFU of the indicated *C. albicans* strains. After 4 days, mice were sacrificed, and kidney burden (CFU/g) was assessed by plating. (Right) Weight loss at the indicated dates was assessed. Values that are not significant (*ns*) are indicated. (C) WT mice ($n = 5$) were injected as described above for panel A, and weight loss was measured daily. The values for the DAY286-infected group that are significantly different from the values for DAY286-IL-17A-infected group by ANOVA and Student's *t* test are indicated by an asterisk. (D) Survival to endpoint (20% weight loss) of the indicated cohorts. (E) Fungal load at day 15 for the remaining surviving mice ($n = 3$ or 4). *ns*, not significant. Experiments were performed one to three times.

thus is mediated by IL-17 signaling in host immune system (Fig. 4B). These data indicate that the impact of IL-17A is extrinsic to the fungus and support our overall conclusions that the reduced fungal loads are due to the host response to the *Candida*-derived cytokine.

Ectopic expression of host immune genes has been previously used as an approach to influence the immune response to fungal organisms. For example, expression of S100A8/A9 (calprotectin) in *C. albicans* was shown to stimulate chemotaxis of neutrophils, though not to alter virulence *per se* (40, 41). Wormley et al. expressed murine gamma interferon (IFN- γ) in the fungal pathogen *Cryptococcus neoformans*, which conferred dramatic protection against secondary infection in a mouse model of disease (42). An intriguing translational potential application of *Candida* strains engineered to express immune genes is for use as a probiotic or prophylactic vaccine. *C. albicans* is a common commensal in humans, with a 30% colonization rate of mucosal surfaces, including skin, gastrointestinal (GI) tract, vaginal mucosa, and oral cavity. Mucosal application of the engineered strains could be used to induce protective immune responses against secondary exposure. Clearly, much further study in immunocompromised hosts would be required to evaluate the safety and efficacy of such an approach.

Antibodies to cytokines have revolutionized treatment for autoimmune disease. However, an inevitable risk with biologic therapies is infection (43). Antibodies to IL-17 and IL-17RA are currently being tested in advanced clinical trials to treat psoriasis (44–47). Some patients in these trials and also individuals with naturally occurring Abs against IL-17 (e.g., patients with autoimmune polyendocrinopathy syndrome 1 arising from *AIRE* deficiency) develop oropharyngeal candidiasis (OPC) or chronic mucocutaneous candidiasis (CMC) (48–50). CMC is also seen in rare individuals with mutations in genes that directly impact IL-17 signaling, including *IL17RA*, *ACT1*, or *IL17F* (51, 52). Although disseminated candidiasis is not typically seen in such patients, we recently found that patients taking tumor necrosis factor alpha (TNF- α) inhibitors to treat rheumatoid arthritis show elevated oral colonization with *C. albicans* and reduced IL-17-associated immune responses (53). Thus, it is plausible that patients taking anticytokine therapies, particularly those that target the Th17/IL-17 pathway, may be at a higher risk for developing systemic candidiasis in the face of predisposing factors, such as an indwelling catheter, abdominal surgery, or long-term antibiotic use.

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