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CX_3CR1 Is Dispensable for Control of Mucosal *Candida albicans* Infections in Mice and Humans

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***Candida albicans* is part of the normal commensal microbiota of mucosal surfaces in a large percentage of the human population. However, perturbations of the host's immune response or bacterial microbiota have been shown to predispose individuals to the development of opportunistic *Candida* infections. It was recently discovered that a defect in the chemokine receptor CX_3CR1 increases susceptibility of mice and humans to systemic candidiasis. However, whether CX_3CR1 confers protection against mucosal *C. albicans* infection has not been investigated. Using two different mouse models, we found that Cx3cr1 is dispensable for the induction of interleukin 17A (IL-17A), IL-22, and IL-23 in the tongue after infection, as well as for the clearance of mucosal candidiasis from the tongue or lower gastrointestinal (GI) tract colonization. Furthermore, the dysfunctional human CX_3CR1 allele *CX_3CR1-M280* was not associated with development of recurrent vulvovaginal candidiasis (RVVC) in women. Taken together, these data indicate that CX_3CR1 is not essential for protection of the host against mucosal candidiasis, underscoring the dependence on different mammalian immune factors for control of mucosal versus systemic *Candida* infections.**

Candida albicans is a normal constituent of the human mucosal microbial ecology. However, inherited and acquired immunodeficiency syndromes and iatrogenic factors, such as catheter and antibiotic use, result in perturbations in the local mucosal immune environment and predispose patients to development of opportunistic mucosal *Candida* infections and systemic candidiasis due to translocation of yeast from mucosal surfaces into the systemic circulation (1, 2). The most common forms of mucosal candidiasis are oropharyngeal candidiasis (OPC) and vulvovaginal candidiasis (VVC), while infections of the skin and nails occur less often (3). Although human mucosal candidiasis is not life threatening, it has a substantial global disease burden. For example, the majority of HIV-infected patients develop oral mucosal candidiasis (4), and approximately 75% of healthy reproductive-age women develop at least one episode of VVC during their lifetime. Furthermore, about 50% of these women develop at least one episode of recurrent infection, and 5 to 10% of them experience recurrent VVC (RVVC), defined as ≥3 episodes of infection per year (5). The substantial incidence and morbidity of mucosal candidiasis, the significant cost associated with it (i.e., the estimated annual cost of VVC exceeds $2 billion in the United States alone) (6), and the emerging resistance of *Candida* spp. to available antifungal agents that limits therapeutic options (7) highlight the importance of a better understanding of the cellular and molecular immune factors that mediate effective anti-*Candida* host defense at the mucosa and systemically, with an aim to develop immune-based strategies for risk stratification, prognostication, and/or treatment of affected patients.

The chemokine receptor CX_3CR1 binds specifically to its sole ligand, CX_3CL1 (fractalkine), and is expressed by hematopoietic (i.e., mononuclear phagocytes and subsets of NK and T cells) and nonhematopoietic (i.e., epithelial and endothelial) cells, in which it mediates cell adhesion, proliferation, differentiation, recruitment, survival, and effector functions (8, 9). We have previously shown that Cx3cr1 is critical for host survival and control of proliferation of *C. albicans* in the kidneys of mice, by promoting resident kidney macrophage survival, accumulation in tissue, and contact with and killing of *C. albicans in vivo*. In agreement with the mouse data, the mutant human *CX_3CR1-M280* allele was shown to be an independent risk factor for the development of candidemia and disseminated candidiasis in two different cohorts of patients from the United States and Europe (10). However, whether this receptor plays a role in mucosal host defense against *Candida* is unknown.

In recent years, it has become evident that interleukin 23 (IL-23)-dependent IL-17 and IL-22 signaling is critical for protection against mucosal candidiasis in mice and humans (11–17). Of interest, Cx3cr1 has previously been shown to promote IL-23-dependent IL-22 production and mucosal immune responses in the context of bacterial gastrointestinal (GI) tract infection and intestinal inflammation (18, 19). Similarly, it has also been found that Cx3cr1 modulates IL-17 responses, both at the mucosal level in

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the setting of intestinal inflammation (20) and systemically in models of experimental autoimmune encephalomyelitis and collagen-induced arthritis (21, 22). Therefore, the aim of this study was to determine whether a deficiency in CX_3CR1 impairs the production of IL-17A, IL-22, and IL-23, while enhancing susceptibility of mice or humans to mucosal *Candida* infections. Interestingly, Cx3cr1-deficient mice did not have impaired expression of IL-17A, IL-22, or IL-23 in the context of mucosal candidiasis. Also, unlike the case with systemic candidiasis, Cx3cr1-deficient mice did not exhibit an impaired ability to control *C. albicans* infection in the oral cavity or yeast colonization of the lower GI tract. Furthermore, the dysfunctional human *CX_3CR1-M280* allele was not associated with development of RVVC in a cohort of 113 affected patients. Therefore, CX_3CR1 protects the host specifically during systemic but not mucosal *C. albicans* challenge.

MATERIALS AND METHODS

Mice. Age- and sex-matched *Cx3cr1*$^{-/-}$ mice and C57BL/6 controls were obtained from Taconic Farms. The *Cx3cr1*$^{-/-}$ mice were developed and backcrossed to the C57BL/6 background as described previously (23). All mice were used between 7 and 14 weeks of age and were housed under specific-pathogen-free conditions. All experiments were conducted in accordance with guidelines set forth by the *Guide for the Care and Use of Laboratory Animals* (24) under a protocol approved by the Animal Care and Use Committee of the NIAID in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility.

Fungal strains. *C. albicans* strain SC5314, recovered from a patient with systemic candidiasis, was used for the majority of the experiments performed. In some experiments of oropharyngeal candidiasis (OPC), other strains of *C. albicans* were used. The oropharyngeal clinical strain 529L has been described before (25). Strains Y42 and Y72 are clinical isolates recovered from patients at the NIH with chronic mucocutaneous candidiasis due to autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), while they had active oral *C. albicans* infection. The organisms were grown in yeast extract, peptone, and dextrose (YPD) media that contained penicillin and streptomycin (Mediatech, Inc.) in a shaking incubator set at 30°C.

Mouse model of OPC. To induce OPC in mice, sublingual infections were performed as previously described (26). Briefly, mice were sedated with ketamine and xylazine, and a cotton swab (Puritan Medical Products) saturated in medium containing 1×10^7 *C. albicans* organisms/ml was placed securely under the tongue for 90 min, using subsequent doses of anesthesia to keep animals sedated for the entirety of the infection. No corticosteroid or other immunomodulatory treatment was administered to mice prior to oral *C. albicans* infection.

Mouse model of GI *Candida* colonization. The GI tracts of mice were colonized with *C. albicans* as previously described (27). Briefly, in order to eradicate the endogenous microbial flora in the mouse gut, streptomycin (2 mg/ml; Research Products International Corp.), penicillin G (1,500 U/ml; Research Products International Corp.), and fluconazole (0.25 mg/ml; Roxanne Laboratories) were added to the drinking water for 3 days, after which the drinking water was replaced with water containing streptomycin and penicillin G at the same concentration for 2 more days. Following antibiotic-antifungal treatment, the mice were given water containing 1×10^7 *C. albicans* organisms/ml, streptomycin (2 mg/ml), and penicillin G (1,500 U/ml) for the next 5 days. After 5 days of *C. albicans* administration, stools were collected from the mice, weighed, homogenized in 1 ml of phosphate-buffered saline (PBS), and quantitatively cultured to determine the fungal load. Following stool harvesting, mice were observed for 4 weeks for signs of development of systemic candidiasis caused by GI tract translocation.

Determination of gene expression via real-time qPCR. *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice were sublingually infected with *C. albicans* and euthanized 1 day postinfection (p.i.). Tongues were harvested, and mRNA was extracted using TRIzol (Invitrogen) and the RNeasy kit (Qiagen), according to the manufacturer's instructions. To convert mRNA to cDNA, the qScript cDNA Supermix kit (Quanta BioSciences) was used. Quantitative PCR (qPCR) was then performed with TaqMan detection (PerfeCTa qPCR FastMix ROX; Quanta BioSciences), using the 7500 real-time PCR system (Applied Biosystems) and predesigned primer and probe mixes for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Applied Biosystems or Integrated DNA Technologies), IL-17A (Integrated DNA Technologies), IL-22, or IL-23p19 (Invitrogen). All qPCR assays were performed in duplicate, and results were normalized to GAPDH transcript levels using the threshold cycle (C_T) method.

Determination of cytokine and chemokine protein concentrations. *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice were sublingually infected with *C. albicans* SC5314 as described above, and tongues were harvested at day 1 p.i. Tongues were homogenized with an Omni tissue homogenizer (Omni International) in PBS with 0.5% Tween 20 (Sigma-Aldrich) and a protease inhibitor cocktail (Roche Applied Science). The homogenate was then centrifuged at 16,168 × *g* for 10 min at 4°C, and supernatants were filtered through an 0.22-μm filter (EMD Millipore) and frozen at 80°C until use. Cytokine and chemokine protein concentrations were determined using a multiplex bead array assay. Antibodies and cytokine standards were purchased from R&D Systems or Peprotech as antibody pairs. Individual Luminex bead sets were coupled to capture antibodies for each cytokine or chemokine measured as previously described (28). As recommended by the manufacturer, biotinylated antibodies were used at twice the recommended concentration for enzyme-linked immunosorbent assay (ELISA), and all procedures were performed in PBS with 1% normal mouse serum (Gibco BRL), 1% normal goat serum (Gibco BRL), and 20 mM Tris-HCl (pH 7.4). A total of 1,200 beads were used for each cytokine or chemokine per sample. The plates were read on a Luminex MAGPIX platform, and at least 50 beads were collected for each set of beads per sample. The median fluorescence intensity for each bead was determined for analysis with Milliplex software using a five-parameter regression algorithm.

Quantification of CFU. In order to assess fungal load, the tongues or stool of mice were harvested, weighed, and homogenized with the Omni tissue homogenizer. The homogenate was then serially diluted or plated undiluted on YPD agar plates containing penicillin and streptomycin. After incubation at 37°C for 24 to 48 h, fungal colonies were counted, and the data were expressed as CFU/gram of tissue. To lower the limit of fungal burden detection in tongues during infection with SC5314, the entire tissue homogenate was plated onto three plates. If no colonies were counted from the entire tongue homogenate, then a value of 0 was assigned.

Histology. Tongues were harvested at days 3 or 5 after sublingual infection, fixed in 10% formalin, and embedded in paraffin. Longitudinal sections of the tongue were prepared for hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) staining (Histoserve, Inc.). Sections were then viewed under a microscope, and representative pictures were taken.

Association of *CX_3CR1* genotype with susceptibility to RVVC. One hundred thirteen microbiologically validated RVVC patients with at least 3 episodes of VVC per year were recruited at Radboud University Medical Centre (Nijmegen, The Netherlands) and Wayne State University School of Medicine (Detroit, MI). Patient inclusion took place between 2010 and 2011. Patients gave written informed consent, and the study was approved by the institutional review boards of both medical centers. One hundred sixty-seven healthy, asymptomatic controls were also recruited and gave written informed consent. Both the RVVC and control groups had a West European genetic ancestry. For both cohorts, venous blood from EDTA tube was collected and genomic DNA was isolated using the Qiagen isolation kit by following the manufacturer's standard protocol.

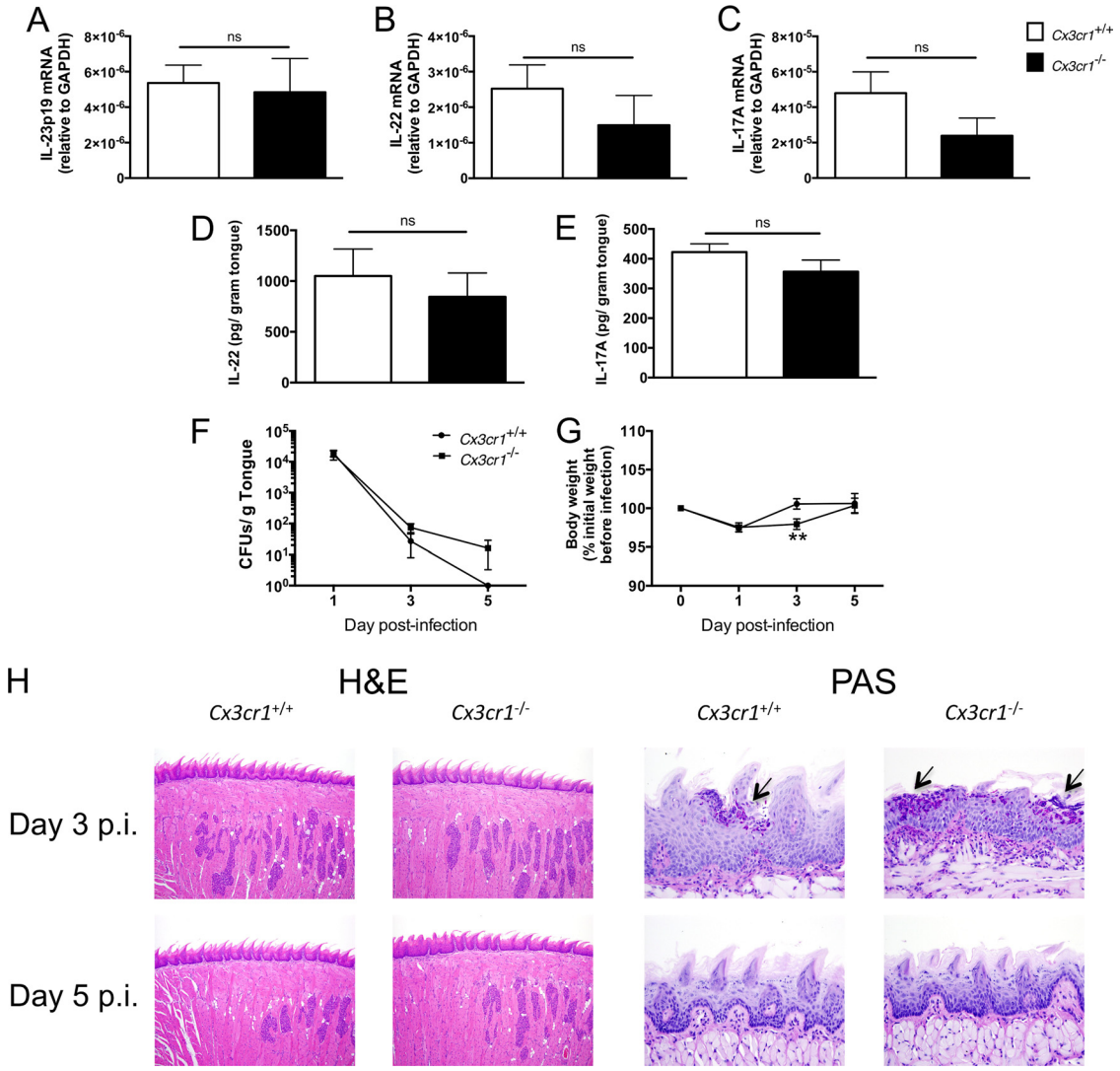

FIG 1 Cx3cr1 is not required for the induction of IL-23p19, IL-22, or IL-17A in the tongue or for control of oral *C. albicans* infection. *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice were infected sublingually with *Candida albicans* strain SC5314. One day p.i., the mRNA levels of IL-23p19 (A), IL-22 (B), and IL-17A (C), relative to GAPDH, were determined from tongue homogenates. Protein concentrations of IL-22 (D) and IL-17A (E) in tongue homogenates at day 1 p.i. were also determined. (F) Tongue fungal burden after infection. (G) Percent weight change p.i. relative to mouse weight before infection. (H) Representative H&E and PAS images of infected tongues (magnifications, ×100 and ×400, respectively). Arrows indicate areas of neutrophilic abscesses containing degraded fungal elements. Data in panels A to E were analyzed using Mann-Whitney tests or unpaired *t* tests, where appropriate, while data in panel G were analyzed using multiple *t* tests by the Holm-Sidak method. The data in panels A to E are combined from two independent experiments ($n = 6$ mice/group). The data in panel G are combined from 2 to 4 independent experiments ($n = 8$ to 16 mice/group). The data in panel H are representative images from 2 independent experiments. **, data are significantly different at a *P* value of <0.01. ns, not significant.

***CX$_3$CR1-M280* genotyping.** To determine whether individuals carried the *CX$_3$CR1-M280* mutant allele, a PCR was performed on DNA that had been isolated from whole blood as previously described (10), using the following primers (15 pmol): 5′-AGAATCATCCAGACGCTGTTTTCC-3′ and 5′-CACAGGACAGCCAGGCATTTCC-3′. The PCR also included the following components: 2.0 mM $MgCl_2$, a 175 μM concentration of each deoxynucleoside triphosphate (dNTP), 1.5 U of *Taq* polymerase, and 1× buffer (Life Technologies). To amplify the product, the following protocol was used: a single cycle of 95°C for 3 min, followed by 35 cycles of 95°C, 69°C, and 72°C at 30 s each and a single cycle of 72°C for 10 min. The resulting product is 311 bp in length. In order to type the alleles at codon 280, the resulting PCR product was digested with 1.5 U of Bst4Cl (New England BioLabs) via overnight incubation at 65°C. The method of digestion results in two fragments (107 bp and 204 bp in length) when a C is present at nucleotide 839 of the open reading frame, while an uncut product remains when this C is mutated to a T. Therefore, individuals with the "wild-type" (WT) allele will have two bands on the gel, those heterozygous for the M280 mutation will have three bands, and those homozygous for the M280 mutation will have only one band. Genotyping was determined by electrophoresis using a 2% agarose gel containing Gelstar (FMC Bioproducts).

Statistics. To determine whether values reached statistical significance for the mouse experiments, multiple *t* tests using the Holm-Sidak method, unpaired *t* tests, or Mann-Whitney tests were performed, where appropriate. For the comparison of the frequency of the *CX$_3$CR1* genotypes from human samples, a χ^2 test was performed. All statistical analyses were performed using Prism 6 software (GraphPad), and data are presented as

TABLE 1 Production of inflammatory mediators after oral *C. albicans* infection does not depend on Cx3cr1

Cytokine or chemokine	Concn (pg/g of tongue) in[a]:		*P* value
	Cx3cr1$^{+/+}$ mice	*Cx3cr1*$^{-/-}$ mice	
IL-1β	13,183 ± 2,279	9,106 ± 1,765	0.1797
IL-2	446.4 ± 66.23	417.5 ± 49.95	0.7352
IL-4	2,680 ± 138.9	2,711 ± 174.6	0.8918
IL-6	22,695 ± 2,667	23,947 ± 3,888	0.7960
IL-10	2,511 ± 239.8	2,151 ± 222.7	0.2975
IL-12p70	33,945 ± 8,534	19,851 ± 6,782	0.2251
IL-15	2,089 ± 193.5	1,909 ± 183.7	0.5163
IL-17a	422.2 ± 28.23	356.5 ± 39.25	0.2045
IL-22	1,050 ± 266.5	844.1 ± 235.5	0.7879
Ccl2	46,214 ± 5,721	37,332 ± 11,366	0.5011
Ccl3	1,042 ± 219.9	666 ± 216.8	0.2509
Ccl4	983.9 ± 278.9	615.1 ± 234.1	0.2381
Ccl5	240.4 ± 24.92	328.3 ± 62.87	0.2381
Ccl7	5,375 ± 754	4,826 ± 1,518	0.7528
Ccl20	7,477 ± 2,206	6,995 ± 3,357	0.5714
Cxcl1	5,325 ± 725.3	4,719 ± 1,497	0.7231
Cxcl2	11,298 ± 1,327	7,420 ± 2,454	0.1947
Cxcl12	35,654 ± 3,609	33,012 ± 2,223	0.5470

[a] Concentrations were determined at day 1 postinfection. Data are mean values ± SEMs and are combined from two independent experiments with 6 mice/group.

the means ± standard errors of the means (SEMs). In all cases, a *P* value of less than 0.05 was considered significant.

RESULTS

Cx3cr1 is not required for the induction of IL-23, IL-22, or IL-17A or control of oral *C. albicans* infection in mice. To determine the importance of Cx3cr1 in *C. albicans* clearance from the oral mucosa, a mouse model of OPC was used as previously described (26). To determine whether a deficiency in Cx3cr1 impacts the IL-23/IL-22/IL-17 axis during mucosal fungal infection, real-time quantitative PCR was performed on tongue homogenates from *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice. Consistent with previous reports, uninfected mice had very little, if any, IL-17A mRNA expression in their tongues (17, 29). Similarly, steady-state IL-22 mRNA levels were low or undetectable, whereas modest constitutive IL-23p19 expression was equally detected in both *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice (data not shown). Importantly, upon infection, IL-17A and IL-22 were highly induced, and IL-23p19 was also significantly induced (~3-fold), though to a lesser extent than IL-17A and IL-22, compared with findings for tongues from uninfected *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice. However, there was no difference in the relative mRNA levels of IL-23p19 (Fig. 1A), IL-22 (Fig. 1B), or IL-17A (Fig. 1C) at day 1 p.i. between the two groups, highlighting the Cx3cr1-independent regulation of IL-23/IL-22/IL-17A signaling in the tongue during *C. albicans* infection. Consonant with the mRNA data, there was no difference between *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice in protein concentrations of IL-22 or IL-17A in the tongue at day 1 p.i. (Fig. 1D and E and Table 1). In agreement with the lack of difference in IL-23p19, IL-22, and IL-17A induction, which is indispensable for mucosal anti-*Candida* host defense (11–17), we found no statistically significant difference between *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice in their ability to clear *C. albicans* from the tongue at days 1, 3, and 5 p.i. (Fig. 1F). Importantly, both groups of mice controlled the infection rapidly, as very few CFU could be recovered from the tongue at either day 3 or 5 p.i., despite a similarly high fungal load observed at day 1 p.i. In agreement with the fungal burden data, *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice developed similar degrees of weight loss at day 1 after infection, a surrogate marker of disease severity (Fig. 1G). The recoveries of weight loss by day 5 p.i. were also similar between *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice, despite a slight delay in weight gain seen in Cx3cr1-deficient mice (Fig. 1G). At the histological level, H&E staining did not reveal any differences between *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice in the overall architectural integrity of the tongue epithelium or in the inflammatory cell infiltrate of the tongue (Fig. 1H, left). Furthermore, PAS staining showed that both *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice formed neutrophilic abscesses in the tongue at day 3 p.i., which contained degraded hyphal elements, while no *C. albicans* abscesses were found at day 5 p.i. (Fig. 1H, right), confirming the microbiological data showing that the vast majority of *C. albicans* had been cleared by day 5 p.i. (Fig. 1F). Finally, to further understand the role of Cx3cr1 in the production of inflammatory mediators in the tongue after infection, protein concentrations for 9 cytokines and 9 chemokines were determined in tongue homogenates at day 1 p.i. with *C. albicans*. There were no significant differences in the protein concentrations of these cytokines and chemokines (Table 1), highlighting the lack of dependence on Cx3cr1 for the generation of an inflammatory response in the tongue during *C. albicans* infection. These results collectively indicate that Cx3cr1 is not essential for the control of oral *C. albicans* infection.

To verify the strain independence of our findings and to extend our analysis using *C. albicans* strains recovered from patients with mucosal candidiasis, three clinical oropharyngeal isolates from patients with OPC were used to sublingually infect *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice. *C. albicans* strain 529L has been shown to colonize the oral mucosa of mice for extended periods (25). Using this isolate, no differences were observed between *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice in the tongue fungal load at day 1, 3, or 5 p.i. (Fig. 2A). Interestingly, compared to infection with strain SC5314, there was less induction of IL-23p19, IL-22, and IL-17A at day 1 p.i. than for uninfected tongues (data not shown). However, consistent with our findings with SC5314 (Fig. 1A to C), there was no dependence on Cx3cr1 for induction of these cytokines in the tongues of mice after 529L infection (data not shown). Moreover, in agreement with our findings with strains SC5314 and 529L, *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice also exhibited equivalent fungal burdens in the tongue following infection with both Y42 (Fig. 2B) and Y72 (Fig. 2C) strains, which were recovered from patients with APECED and OPC. These data collectively indicate that Cx3cr1 is dispensable for fungal clearance from the tongue regardless of the strain of *C. albicans* used.

Cx3cr1 is dispensable for the control of *C. albicans* colonization from the lower GI tract. To further explore the importance of Cx3cr1 in the clearance of *C. albicans* from mucosal surfaces, a mouse model of GI tract colonization was utilized as previously described (see Fig. 3A for experimental setup) (27). In agreement with the results obtained with the OPC model of infection, there was no difference in the amount of *C. albicans* recovered from stools of *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice (Fig. 3B). In addition, neither *Cx3cr1*$^{+/+}$ nor *Cx3cr1*$^{-/-}$ mice developed systemic candidiasis due to translocation of *C. albicans* from the GI tract into the systemic circulation (data not shown). These data indicate that Cx3cr1 is not necessary for the local control of *Candida* colonization and translocation in the GI tract.

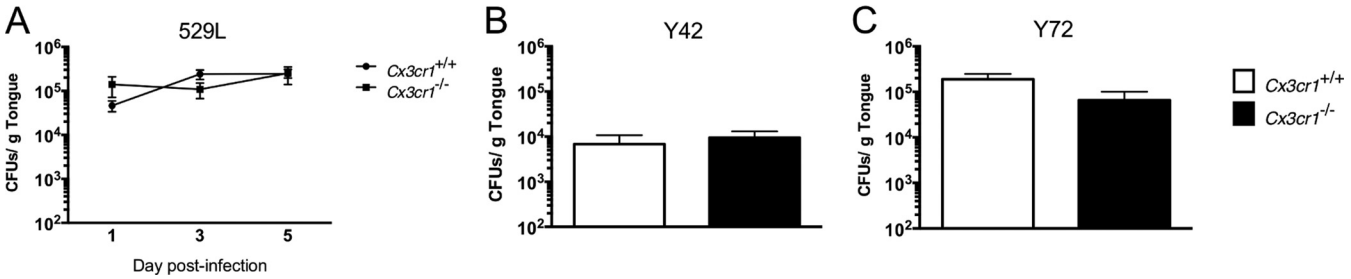

FIG 2 Cx3cr1 is not necessary for the control of *C. albicans* oral infection caused by clinical oropharyngeal isolates. *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice were infected sublingually with *C. albicans* strains 529L, Y42, and Y72. Tongue fungal burden was determined on days 1, 3, and 5 p.i. for strain 529L (A) or day 5 p.i. for strains Y42 (B) and Y72 (C). These data were analyzed using Mann-Whitney tests. The data are combined from 2 independent experiments ($n = 4$ to 7 mice/group).

CX_3CR1 deficiency does not predispose women to develop RVVC. Finally, the impact of CX_3CR1 deficiency on control of mucosal candidiasis was studied in humans using a cohort of women who developed RVVC and control women without mucosal candidiasis. As described previously, the mutant *CX_3CR1-M280* allele leads to defective fractalkine binding and downstream CX_3CR1 signaling and is a risk factor for the development of systemic candidiasis (10). However, whether the *CX_3CR1-M280* allele is associated with development of human mucosal *Candida* infection has not been addressed. As shown in Fig. 4, the frequencies of women carrying the mutant *CX_3CR1-M280* allele versus the WT *CX_3CR1* genotype among control and RVVC individuals were similar, indicating that genetic variation at *CX_3CR1* is not a risk factor for development of persistent mucosal candidiasis in humans.

DISCUSSION

In the present study, we showed that the chemokine receptor Cx3cr1 is dispensable for host defense against *C. albicans* at the mucosa in mice and humans. Specifically, we show that in contrast to the Cx3cr1-dependent IL-23 and IL-22 induction during mucosal bacterial infection (18), *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice exhibited similar levels of expression of IL-23 and IL-22 from tongue homogenates during OPC. In addition, we found no Cx3cr1-dependent regulation of IL-17A during oral fungal infection, which is in contrast to what has been observed in the context of inflammatory colitis, collagen-induced arthritis, and experimental autoimmune encephalomyelitis (20–22). Furthermore, the ability to control *C. albicans* in the oral cavity and the lower GI tract was not impaired in *Cx3cr1*$^{-/-}$ mice, and Cx3cr1-deficient mice colonized with *C. albicans* in their GI tracts did not spontaneously develop GI tract-derived systemic candidiasis. In addition, the dysfunctional human *CX_3CR1-M280* mutation was not associated with development of RVVC in women. These findings contradict the previously reported crucial role of Cx3cr1 in protection against systemic candidiasis in mice and against candidemia in humans (10). Taken together, these data further highlight the divergent mammalian immune factors that are required for control of mucosal versus systemic *Candida* infections.

In accordance with this observation, patients with oral or vaginal candidiasis do not spontaneously develop systemic candidiasis as a result of their mucosal infection (30). Indeed, by studying perturbations in cellular and molecular immune factors in human populations, it has been found that there is a segregation of factors necessary for control of systemic versus mucosal candidiasis. Specifically, it would appear that the adaptive immune response is most important for the development of effective host defense against mucosal infection, as patients with HIV and idiopathic CD4 lymphocytopenia are prone to mucocutaneous candidiasis but not systemic candidiasis (4, 31). Furthermore, patients with either mutations in various components of the IL-17 pathway (i.e., *IL-17RA*, *IL-17F*, and *ACT1* [14, 32]) or with inherited immunodeficiencies that result in impaired IL-17 signaling (i.e., mutations in *STAT1*, *STAT3*, *STK4*, *DOCK8*, *AIRE*, and *DECTIN-1* [11]) are susceptible to mucosal but not systemic candidiasis. In

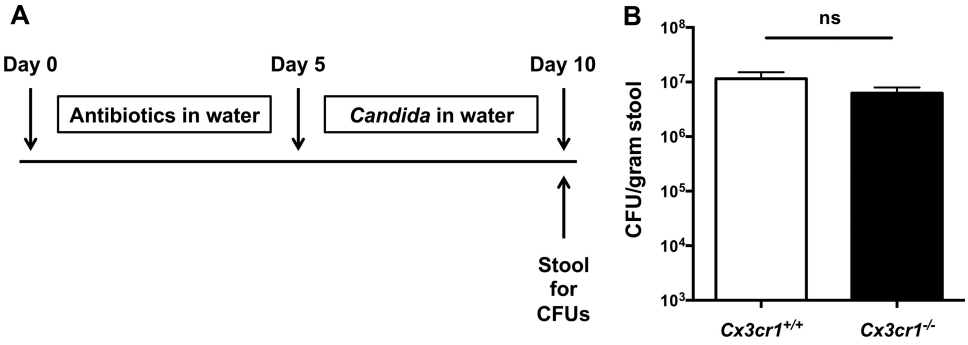

FIG 3 Cx3cr1 is dispensable for control of lower GI tract colonization by *C. albicans*. *Cx3cr1*$^{+/+}$ and *Cx3cr1*$^{-/-}$ mice were given antibiotics in their drinking water for 5 days prior to replacement with drinking water containing 1×10^7 *C. albicans* organisms/ml (strain SC5314) until day 10 after initiation of antibiotic treatment (A). At day 10, stools were collected from the mice, weighed, homogenized, and plated to determine fungal load (B). These data were analyzed using a Mann-Whitney test. The data are combined from 2 independent experiments ($n = 10$ mice/group).

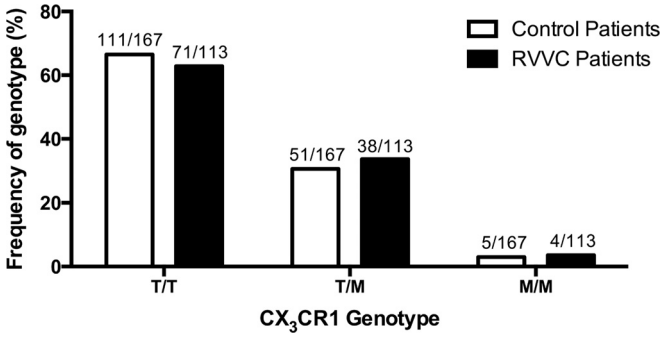

FIG 4 The dysfunctional CX_3CR1 allele *CX3CR1-M280* is not associated with development of recurrent vulvovaginal candidiasis. Patients with recurrent vulvovaginal candidiasis (RVVC) and healthy controls were genotyped for the presence of the *CX3CR1-M280* allele, and the frequencies of the genotype in the two cohorts were compared. The data are presented as such where T/T corresponds to individuals homozygous for the wild-type allele, T/M corresponds to heterozygous individuals, and M/M corresponds to individuals homozygous for the mutant allele. The data were analyzed using a χ^2 test, with no significant differences observed.

stark contrast, patients with inherited or acquired neutropenia, chronic granulomatous disease, and complete myeloperoxidase deficiency have an increased likelihood of developing systemic but not mucosal *Candida* infections (11, 33–35), indicating a stronger requirement for innate immune cell mechanisms for the protection against systemic infection. The only immune factor known to date to confer heightened susceptibility to both mucosal and systemic candidiasis on humans is CARD9, attesting to the central positioning of this adaptor protein downstream of several C-type lectin receptors (30, 36). However, the mechanisms by which CARD9 promotes effective mucosal and systemic anti-*Candida* effects in humans remain elusive.

In the mouse models of infection, there is evidence to suggest that a certain cellular or molecular immune factor may be protective for both systemic and mucosal *Candida* infections. For instance, neutrophils (29, 37) and cytokines such as IL-17, tumor necrosis factor alpha (TNF-α), and IL-6 (38–40) are indispensable for host defense against both systemic and mucosal mouse *Candida* infections. Nonetheless, the mechanisms involved in mucosal versus systemic anti-*Candida* immune protection by a given immune factor (e.g., IL-17) can be very distinct (12, 41). Our study indicates that Cx3cr1 does not mediate protective IL-23-dependent mucosal immune responses against *C. albicans* in mice and humans, despite its indispensable role in phagocyte-mediated protection during systemic candidiasis (10). Our data also underscore the divergent dependence on Cx3cr1 for induction of IL-22 and IL-17A-mediated mucosal immune responses during mucosal bacterial versus fungal infection (18).

In mice and humans, there are two distinct subsets of monocytes/macrophages based on expression of CX_3CR1 and CCR2. CX_3CR1 expression characterizes resident monocytes/macrophages with "patrolling" immune functions, whereas $CCR2^+$ cells are "inflammatory" and are recruited to tissues in high numbers after infection (42). Recent studies have highlighted the important role of $CCR2^+$ mononuclear phagocytes in mediating innate and adaptive antifungal immune responses during systemic and respiratory fungal disease, including systemic candidiasis (43–47). Therefore, future studies should examine the role of $CCR2^+$ inflammatory mononuclear phagocytes during mucosal *Candida* infection.

Although recent advances have been made in understanding immune responses against *Candida* and other fungal pathogens, there is still much to be learned. For example, the rate of mortality from systemic candidiasis still remains unacceptably high (~40%) despite antifungal use (48). Furthermore, mucosal *Candida* infections, including RVVC and chronic mucocutaneous candidiasis, lead to significant morbidity and hospital costs (49). In addition, because life-threatening systemic candidiasis originates from translocation of *Candida* from the mucosal surfaces into the bloodstream, a better understanding of the immune factors that control *Candida* at the mucosa will be important to devise preventive strategies against systemic infection. The fact that effective treatment approaches are still needed for candidiasis to improve patient outcomes underscores the importance of continued research in the area of mucosal and systemic fungal immunity.

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