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IL-23 and IL-17A, but Not IL-12 and IL-22, Are Required for Optimal Skin Host Defense against *Candida albicans*

Shinji Kagami,[*,1] **Heather L. Rizzo,**[*,1] **Stephen E. Kurtz,**[†] **Lloyd S. Miller,**[‡] **and Andrew Blauvelt**[*,†,§]

IL-23 and Th17 cells play important roles in host defense against systemic infections with extracellular bacteria and fungi, although their roles in immunity against localized skin infections are less well defined. Here, the contributions of IL-23 and Th17 cytokines in host defense against cutaneous *Candida albicans* infection were evaluated. Mice deficient in IL-23 or IL-17A demonstrated delayed healing and decreased IL-17A production after skin infection with *C. albicans* compared with wild-type mice or mice deficient in IL-12 or IL-22. Histologic examination revealed epidermal hyperplasia overlying infected dermis four days postinoculation in wild-type mice. In IL-23–deficient mice, fungal burden was greater in skin, neither *IL-17A* nor *IL-22* mRNAs were expressed postinfection, and these mice demonstrated only minimal epidermal hyperplasia. Exogenous recombinant IL-17A injected at the site of skin infection promoted more rapid healing of candidiasis in both wild-type mice and mice deficient in IL-23 and IL-12. Taken together, these results demonstrate that IL-23 and IL-17A, but not IL-12 and IL-22, are required for optimal host defense against cutaneous candidiasis. In addition, recombinant IL-17A may serve as a potential therapy to enhance healing in individuals with chronic cutaneous candidiasis. *The Journal of Immunology*, 2010, 185: 5453–5462.

C utaneous infections with the ubiquitous yeast *Candida albicans* are common throughout the world (1). Although many infections occur in warm, moist areas of the body, such as in the inguinal and inframammary folds, in otherwise healthy individuals, cutaneous candidiasis is also an important marker of innate or acquired immune dysfunction. For example, individuals with diabetes mellitus, HIV disease, chronic systemic corticosteroid usage, or chemotherapy-induced neutropenia are predisposed to cutaneous candidiasis (2–5). This diverse range of clinical settings associated with susceptibility to candidiasis fits with what is currently known about immune responses directed against *C. albicans*, as host defense against this pathogen involves complex coordination of both innate and acquired immune responses. Pattern recognition of organisms via TLRs (e.g., TLR2 and TLR4) and C-type lectin receptors (e.g., dectin-1, dectin-2, and mannose receptor), Th1 and Th17 number and function, and neutrophil number and function have all be reported to be critical pieces in controlling infection by *C. albicans* (6–10).

Regarding T cell studies, Th1 cells and IFN-γ production are believed to be critical in host defense against *C. albicans* (11–15). IL-12 production, priming of Th1 responses, and IFN-γ production were critical for optimal control of *C. albicans* infection (14, 15). In IFN-γR–deficient mice, Th2 responses were enhanced, which led to increased susceptibility to infection (15). These studies suggest that Th1 responses promote resistance to *C. albicans* infection, whereas Th2 cytokines are associated with increased susceptibility to infection.

Th17 cells play a role in host defense against *C. albicans* as well (16, 17). Th17 cells produce cytokines, principally IL-17A and IL-22, which mediate protection from infection at mucocutaneous surfaces (18, 19). Specifically, IL-17A stimulates neutrophil accumulation and function, whereas IL-22 stimulates proliferation and antimicrobial peptide production by epithelial cells, key defense strategies designed to combat infection. Notably, individuals with Job's syndrome (also known as hyper-IgE syndrome) have been found to have mutations in *STAT3* that lead to a deficiency in Th17 cells, rendering them susceptible to chronic mucocutaneous infections with *C. albicans* (20–22). Furthermore, humans with mutations in *dectin-1* or a protein downstream of dectin-1 signaling, CARD9, have defective IL-23 production, subsequent decreases in IL-17A production by Th17 cells, and increased susceptibility to chronic mucocutaneous candidiasis (23, 24). Collectively, these data suggest that both IL-12/Th1 responses and IL-23/Th17 responses contribute to host defense against *C. albicans*. It is unclear, however, which response predominates in combating cutaneous candidiasis.

To define further the specific roles of IL-12 and IL-23 as well as cytokines produced by Th17 cells (e.g., IL-17A and IL-22) in host defense against *C. albicans*, a cutaneous infection model in mice was established. This model involved the inoculation of *C. albicans* into the dorsal back skin of various strains of mice that are genetically deficient in key components of the IL-12/Th1 and IL-23/Th17 inflammatory pathways, and the ensuing healing rates, fungal burden, leukocyte infiltration, epidermal hyperplasia, and cytokine production were compared.

Materials and Methods

Mice

All breeding and experiments were undertaken with review and approval from the Portland Veterans Affairs Medical Center Institutional Animal Care and Use Committee. C57BL/6 wild-type (WT) mice, *IL-12p35*^{−/−} mice, and *IL-12/23p40*^{−/−} mice were purchased from Jackson Lab-

oratories (Bar Harbor, ME). Through material transfer agreements, *IL-17A*$^{-/-}$ mice were obtained from Dr. Yoichiro Iwakura (Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Japan) (25), and *IL-22*$^{-/-}$ mice and *IL-23p19*$^{-/-}$ mice were obtained from Dr. Wenjun Ouyang and Dr. Frederic de Sauvage (Genentech, South San Francisco, CA) (26, 27). All knockout mice had been bred for many years on a C57BL/6 background.

C. albicans

C. albicans (strain SC5314) was obtained from Dr. Janet Staab (The Johns Hopkins University, Baltimore, MD) and maintained in 1%yeast extract, 2% peptone, 2% dextrose (YPD). To generate pseudohyphae, an isolate of strain SC5314 was grown in YPD at 30˚C with shaking for 16 h. Cells were adjusted to 5×10^6 cells/ml in YPD with 10% FBS and incubated at 37˚C for 2 h. Microscopic examination confirmed that >95% of the cells converted to pseudohyphae.

Clinical course of C. albicans-injected mice

C. albicans pseudohyphae (5×10^6 cells) were injected into the deep dermis and superficial fat of the shaved dorsal region of recipient mice. In some experiments, mice were injected with a mixture of *C. albicans* pseudohyphae and 1 μg recombinant murine IL-17A (R&D Systems, Minneapolis, MN). Mice were assessed for presence of nodule, ulceration, erythema, and crust three times weekly thereafter by an evaluator blinded to the genotype of the mice. Disease was scored positively if any one of the four clinical features was present. In three separate experiments, at least 12 mice for each genetic background were studied for the clinical course experiments. In the experiments using recombinant murine IL-17A, a total of seven mice in each treatment group were studied in three separate experiments.

Immunohistochemistry

A dose of 5×10^4 *C. albicans* pseudohyphae was injected to improve recovery of intact skin tissue from infected skin. Four days postinfection, 4-mm punch biopsies were performed in the center of the *C. albicans* nodules. Sections from formalin-fixed, paraffin-embedded biopsies were stained. Blocking solutions, primary Abs, and secondary Abs are shown in Supplemental Table 1. For immunohistochemistry, sections were deparaffinized and hydrated by washing sections in xylene followed by a graded alcohol series. The sections were incubated in 10 mM citric acid (pH 6) at 95˚C for 30 min to unmask Ags, and endogenous peroxidase activity was quenched by treating sections with 3% hydrogen peroxide for 5 min at room temperature. Sections were blocked for 60 min at room temperature, followed by incubation with primary Abs overnight at 4˚C. Samples were washed and incubated at room temperature for 60 min with secondary Abs, followed by a 30-min incubation with streptavidin-conjugated HRP (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA), developed using diaminobenzidine substrate kit for peroxidase (Vector Laboratories), and counterstained with hematoxylin (Vector Laboratories). To quantify epidermal hyperplasia, defined as area/length, randomly chosen fields for each section were measured using Image Pro software (version 4.5; Media Cybernetics, Bethesda, MD). To quantify Gr-1$^+$ cells, an observer blinded to the genotype of the mice graded Gr-1–stained specimens from 0 (few) to 3 (many). At least three mice per group were stained, and photographs were taken from three representative ×40 magnification fields per section. Average numbers of positive cells per picture were counted for quantification.

Fungal burden assay

Mice were injected with 5×10^6 pseudohyphae as described above, and 4-mm punch biopsies were taken at the injection site 4 d postinfection. The biopsies were weighed and then gently homogenized using a Dounce homogenizer. Homogenates were diluted, and 100-μl aliquots were plated onto YPD growth medium to permit *Candida* colony growth. Plates were incubated at room temperature for 2 d, and the numbers of CFU per gram of tissue were calculated. Experiments were repeated at least three times.

RNA isolation and quantitative real-time PCR

Infected skin was collected, placed into TRIzol (Invitrogen, Carlsbad, CA), and homogenized with a mechanical rotor for 1 min. Total RNA was isolated and purified using PureLink RNA Mini Kit (Invitrogen) with the protocol for using TRIzol with the PureLink RNA Mini Kit as recommended by the manufacturer. cDNA was prepared from 1 μg total RNA by reverse transcription using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Reactions were diluted three times with water for all quantitative real-time PCR (qPCR) experiments.

qPCR was performed on the MyiQ system (Bio-Rad) using TaqMan Gene Expression Master Mix with the following TaqMan primers and fluorescent probes from Applied Biosystems: *Gapdh* (catalog no. Mm9999991-5_g1), *Il23a* (catalog no. Mm00518984_m1), *Il12b* (catalog no. Mm0128-8990_m1), *Il12a* (catalog no. Mm01218136_m1), *Il17a* (catalog no. Mm01268754_m1), *Il22* (catalog no. Mm01226722_g1), *Ifnγ* (catalog no. Mm00801778_m1), *Il17f* (catalog no. Mm00521423_m1), *Il21* (catalog no. Mm00517640_m1), and *Tnfα* (catalog no. Mm00443258_m1). Expression of each transcript was calculated relative to *Gapdh* expression.

Protein isolation and ELISA

Infected skin was collected and protein was extracted using the Qproteome Mammalian Protein kit (Qiagen, Germantown, MD). Briefly, skin was placed in lysis buffer containing benzonuclease and a protease inhibitor solution and homogenized for 1 min with a mechanical rotor. Homogenates were then centrifuged, and the clear supernatants were collected. Mouse IL-17A and IL-22 ELISA kits (R&D Systems) and mouse IL-21 ELISA kit (eBioscience, San Diego, CA) were used according to the manufacturer's instructions.

Statistical analyses

The chi-square goodness of fit test was used to evaluate normality for all parameters. The *F* test and Bartlett test were used to evaluate equality of variance between two or more groups, respectively. Student *t* test, Welch's *t* test, or Mann–Whitney *U* test was used for comparison between WT and knockout mice. For testing equality of population means among three or more groups, Kruskal–Wallis test and Scheffe's *F* test were used. Kaplan–Meier method and log rank test were used for the analysis of clinical course. The *p* values ≤ 0.05 were considered significant.

Results

### IL-23–deficient mice have impaired clearance of C. albicans skin infection

After inoculation of *C. albicans* within skin, WT mice developed visible skin nodules that took a median of 13 d to heal (Fig. 1*A*). By contrast, IL-23–deficient mice (*IL-23p19*$^{-/-}$ mice) and mice deficient in both IL-12 and IL-23 (*IL-12/23p40*$^{-/-}$ mice) developed skin lesions that took much longer to heal with a median of >15 d ($p < 0.025$ versus WT mice for both knockout strains) (Fig. 1*A*). Furthermore, 20 d postinfection, all WT mice had resolved the infection versus only 50% of the *IL-12/23p40*$^{-/-}$ mice and 62% of the *IL-23p19*$^{-/-}$ mice. IL-12–deficient (*IL-12p35*$^{-/-}$) mice healed *Candida* lesions in a time span similar to that of WT mice ($p < 0.19$; Fig. 1*A*). *IL-12/23p40*$^{-/-}$ mice also demonstrated higher fungal burdens in skin compared with that in WT mice ($p < 0.001$; Fig. 1*B*), which was consistent with the clinical course (Fig. 1*A*). The average CFU/gram of tissue obtained from *IL-23p19*$^{-/-}$ mice was higher than that of WT mice, although this was not statistically significant ($p < 0.066$; Fig. 1*B*).

Histologic examinations of WT mouse skin 4 d postinfection showed a large collection of fungal organisms deep within the dermis and superficial fat (Fig. 2*A*) surrounded by numerous Gr-1$^+$ neutrophils (Fig. 2*A*). The number of CD3$^+$ cells was decreased in *IL-23p19*$^{-/-}$ mice and *IL-12p35*$^{-/-}$ mice ($p < 0.01$ and $p < 0.05$ versus WT mice, respectively; Fig. 2*A*, *2B*). There were less F4/80$^+$ cells in *IL-23p19*$^{-/-}$ mice than in WT mice ($p < 0.05$; Fig. 2*A*, *2B*). Fewer neutrophils infiltrated infection sites of *IL-12/23p40*$^{-/-}$ mice compared with that in WT mice ($p < 0.05$; Fig. 2*A*, *2B*).

C. albicans induces epidermal hyperplasia overlying infected dermis that is dependent on IL-23

Notably, histologic analyses of infected skin of WT mice also revealed prominent epidermal hyperplasia overlying the nidus of infection in the deep dermis compared with nonlesional skin of WT mice (mean ± SD of thickness = 47.7 ± 8.4 μm and 19.0 ± 3.0 μm, respectively, $p < 0.001$; Fig. 3*A*, *3B*). In mice deficient in IL-23 (*IL-23p19*$^{-/-}$ mice) and mice deficient in both IL-12 and
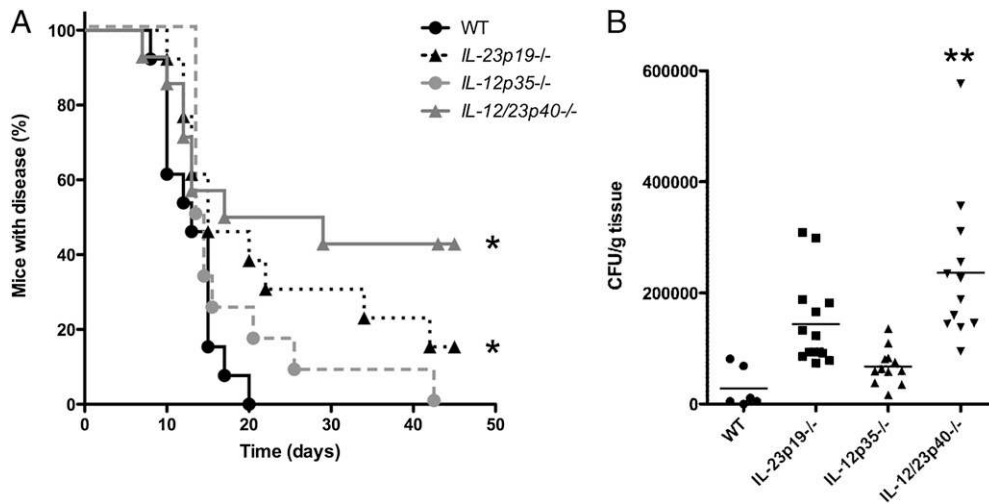
FIGURE 1. Optimal resolution of cutaneous *C. albicans* infection is dependent upon IL-23. *A*, Kaplan–Meier curves demonstrating the percentage of *C. albicans*-infected mice with disease, as defined by the presence of nodule, ulcer, erythema, or crust at the skin site of inoculation. This was assessed by an investigator (blinded to the genotype of the mice) three times weekly postinfection. In three separate experiments, at least 12 mice for each genetic background were evaluated. $*p < 0.025$ versus WT. *B*, Quantification of *C. albicans* fungal burden in skin 4 d after inoculation. Infected tissue was isolated, weighed, minced, and cultured on agar plates to determine CFU/gram skin tissue. $**p < 0.025$ versus WT.

IL-23 (*IL-12/23p40$^{-/-}$* mice), epidermal hyperplasia was much less prominent compared with WT epidermis (26.1 ± 4.5 μm and 23.1 ± 4.9 μm, respectively, $p < 0.001$ versus WT mice for both strains; Fig. 3*A*, 3*B*). In contrast, infected skin of IL-12–deficient mice (*IL-12p35$^{-/-}$* mice) developed prominent epidermal hyperplasia that was similar to the epidermal hyperplasia observed in WT mice (Fig. 3*A*, 3*B*).

C. albicans *induces rapid upregulation of IL-12 and IL-23 expression within infected skin*

To determine whether IL-12 and IL-23 expression was induced in skin after *C. albicans* infection, RNA was isolated from WT skin at various time points postinfection, and *IL-23p19*, *IL-12p35*, and *IL-12/23p40* mRNAs were measured by qPCR. Prior to infection, mRNA levels for *IL-23p19*, *IL-12p35*, and *IL-12/23p40* were absent or minimal (data not shown). In contrast, mRNA expression for all three subunits peaked at 24 h in WT mice after skin inoculation with *C. albicans* (data not shown), and thus all data presented are from this time point (Supplemental Fig. 1*A*). *IL-23p19* mRNA levels were induced to high levels in WT mice, *IL-12p35$^{-/-}$* mice, and *IL-12/23p40$^{-/-}$* mice. *IL-12p35* mRNA levels were induced to high levels in WT mice, *IL-23p19$^{-/-}$* mice, and *IL-12/23p40$^{-/-}$* mice. As an expected control, *IL-23p19*, *IL-12p35*, and *IL-12/23p40* mRNA levels were absent in *IL-23p19$^{-/-}$* mice, *IL-23p35$^{-/-}$* mice, and *IL-12/23p40$^{-/-}$* mice, respectively. Thus, the presence of IL-12 in *IL-23p19$^{-/-}$* mice could not compensate for the lack of IL-23 in these mice, suggesting that IL-23 is a more critical determinant than IL-12 to the increased healing times observed in *IL-23p19$^{-/-}$* mice. Confirmation of mRNA results was performed via protein analysis using immunohistochemistry. There were more IL-23p19$^+$ cells in WT mice and *IL-12p35$^{-/-}$* mice compared with that in *IL-23p19$^{-/-}$* mice and *IL-12/23p40$^{-/-}$* mice (Supplemental Fig. 1*B*, 1*C*). The majority of IL-23p19$^+$ cells were also positive for F4/80, indicating that macrophages were the primary producers of IL-23 in this model (Supplemental Fig. 1*D*).

IL-17A *and* IL-22 *expression in* C. albicans-*infected skin are dependent on IL-23*

To investigate the downstream effects of activation of the IL-12/Th1 and IL-23/Th17 inflammatory pathways postinfection, mRNA expression levels of *IL-17A*, *IL-17F*, *IL-22*, *IL-21*, *IFN-γ*, and *TNF-α*

were analyzed. In the absence of infection, mRNA amounts for all of six genes were absent or minimal in WT mice (data not shown). In contrast, mRNA levels of all gene products reached maximal levels by 48 h postinfection in WT mice (data not shown), and thus all data presented are from this time point (Fig. 4). *IL-17A*, *IL-17F*, and *IL-22* mRNA were not induced in *IL-23p19$^{-/-}$* mice or in *IL-12/23p40$^{-/-}$* mice after *C. albicans* cutaneous infection (Fig. 4). *IL-12p35$^{-/-}$* mice exhibited similar *IL-17A* and *IL-22* mRNA expression as WT mice (Fig. 4). There were no differences in *IL-21* mRNA expression among WT and knockout mice (Fig. 4). *IFN-γ* mRNA expression was markedly decreased in *IL-12p35$^{-/-}$* mice and in *IL-12/23p40$^{-/-}$* mice after *C. albicans* cutaneous infection ($p < 0.001$ and $p < 0.01$ versus WT mice, respectively; Fig. 4). Of note, *IFN-γ* mRNA was increased in IL-23–deficient mice compared with that in WT mice ($p < 0.01$; Fig. 4). In *IL-12/23p40$^{-/-}$* mice, *TNF-α* mRNA expression was lower compared with that in WT mice ($p < 0.05$; Fig. 4). Taken together, these data indicated that *IL-17A*, *IL-17F*, and *IL-22* mRNA production was completely dependent upon IL-23 and that *IFN-γ* mRNA production was largely dependent upon IL-12.

Confirmation of mRNA results was performed via protein analyses using ELISA 48 h after *C. albicans* inoculation and immunohistochemistry 4 d after *C. albicans* inoculation. Both IL-17A and IL-22 protein levels as measured by ELISA were nearly absent in infected skin lesions of *IL-23p19$^{-/-}$* and *IL-12/23p40$^{-/-}$* mice ($p < 0.025$ versus WT mice for both cytokines and both strains of mice; Supplemental Fig. 2). Neither IL-17A$^+$ nor IL-22$^+$ cells were visualized in *IL-23p19$^{-/-}$* mice and *IL-12/23p40$^{-/-}$* mice (Fig. 5*A*, 5*B*), corroborating the mRNA and ELISA data. In contrast, both IL-17A$^+$ and IL-22$^+$ cells were readily observed in the dermis of infected WT and *IL-12p35$^{-/-}$* mice (Fig. 5*A*, 5*B*). Of note, protein levels of IL-21, which is also expressed by Th17 cells, were equal among WT mice and all of the knockout mice, suggesting that IL-21 production was not dependent on either IL-23 or IL-12 (Supplemental Fig. 2). It should also be mentioned that IFN-γ protein levels as measured by ELISA among all of the samples were below the level of detection (data not shown). Next, we stained serial sections to detect double-positive cells. IL-17A was mainly produced by CD3$^+$ cells; some Gr-1$^+$ cells also pro-
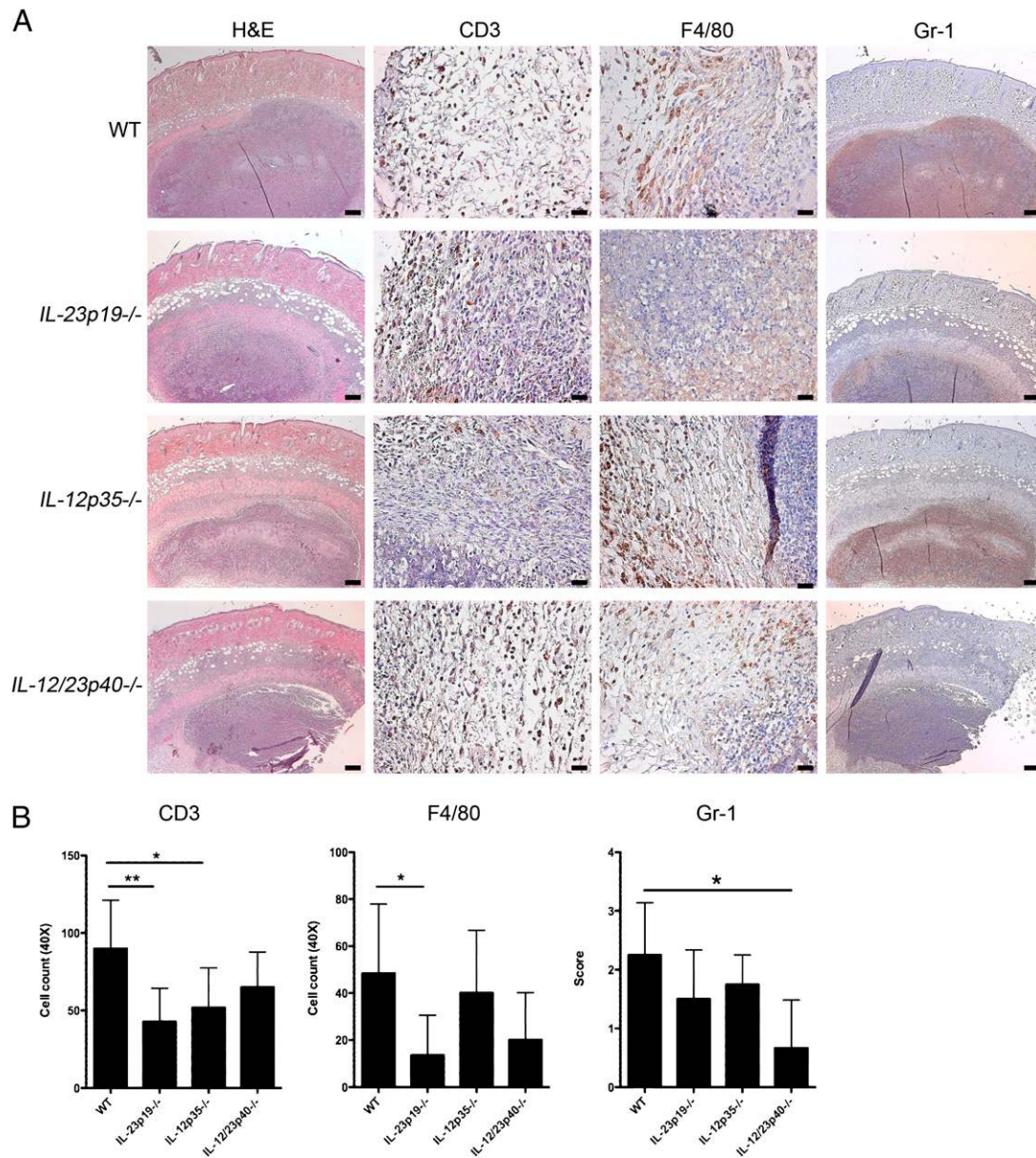
FIGURE 2. Increased numbers of infiltrating cells in WT mouse skin compared with knockout mouse skin. *A*, H&E, CD3 (T cells), F4/80 (macrophages), and Gr-1 (neutrophils) staining of skin 4 d postinfection with *C. albicans* showing a large collection of fungal elements in the deep dermis and superficial fat. H&E and Gr-1 staining, original magnification ×10; scale bars, 0.2 mm. CD3 and F4/80 staining, original magnification ×40; scale bars, 0.025 mm. *B*, Quantification of CD3$^+$ and F4/80$^+$ cells and grading of Gr-1 staining in each of the four groups of mice shown in *A*. Data are expressed as mean ± SD. Bar graphs were analyzed with Kruskal–Wallis test and Scheffe's *F* test.*$p < 0.05$; **$p < 0.01$ versus WT.

duced IL-17A (Fig. 5*C*). IL-22 was primarily produced by CD3$^+$ cells (Fig. 5*C*). Some IL-17A$^+$ cells were also IL-22$^+$ (Fig. 5*C*).

IL-17A–deficient mice but not IL-22–deficient mice have impaired clearance of C. albicans *skin infection*

Because IL-23–deficient mice had defective host defense against cutaneous *C. albicans* infection and had impaired production of IL-17A and IL-22, the specific roles of IL-17A versus IL-22 in combating cutaneous candidiasis were investigated further using *IL-17A$^{-/-}$* and *IL-22$^{-/-}$* mice. *IL-17A$^{-/-}$* mice developed skin lesions that took longer to heal than those in WT mice with a median of >15 d ($p < 0.025$ versus WT mice; Fig. 6*A*). In contrast, *IL-22$^{-/-}$* mice healed *Candida* lesions in a similar time span as that of WT mice (Fig. 6*A*). *IL-17A$^{-/-}$* mice, but not *IL-22$^{-/-}$* mice, demonstrated increased fungal burdens in skin compared with that in WT mice ($p < 0.001$; Fig. 6*B*), which is con-

sistent with clinical course data. Four days postinfection, lesional mouse skin from *IL-17A$^{-/-}$* and *IL-22$^{-/-}$* exhibited fewer CD3$^+$ cells than skin from WT mice ($p < 0.01$ and $p < 0.05$, respectively; Fig. 7*A*, 7*B*). However, both strains of knockout mice showed similar levels of Gr-1$^+$ and F4/80$^+$ cells compared with those in WT mice (Fig. 7*A*, 7*B*).

C. albicans *induces epidermal hyperplasia overlying infected dermis in mice deficient in either IL-17A or IL-22*

Infected skin of *IL-17A$^{-/-}$* and *IL-22$^{-/-}$* mice showed prominent epidermal hyperplasia that was similar to WT mice (52.0 ± 10.9 μm and 43.8 ± 10.2 μm, respectively, versus 47.7 ± 8.4 μm in WT mice; Supplemental Fig. 3*A*, 3*B*). Thus, epidermal hyperplasia induced during cutaneous *C. albicans* infections was not dependent upon IL-17 or IL-22. Furthermore, these results suggest that the presence of prominent epidermal hyperplasia as seen in

FIGURE 3. *C. albicans* infection in skin induces epidermal hyperplasia that is dependent upon IL-23. *A*, H&E staining of mouse skin 4 d postinfection with *C. albicans*. Original magnification ×20; scale bars, 0.05 mm. *B*, Quantification of epidermal hyperplasia in each of the five groups of mice in *A*. Data are expressed as mean epidermal thickness (μm) ± SD. Data were analyzed using Kruskal–Wallis test and Scheffe's *F* test. *$p < 0.001$ versus WT.
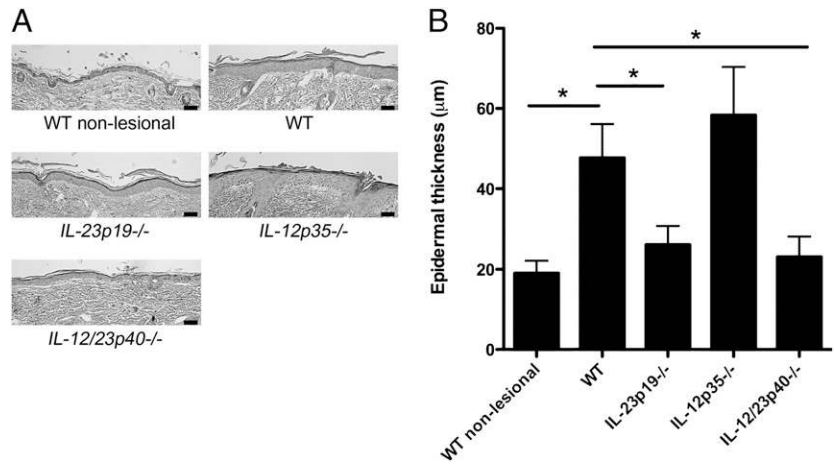
$IL\text{-}17A^{-/-}$ mice and $IL\text{-}22^{-/-}$ mice did not play a major role in contributing to the delayed *Candida* healing times as observed in $IL\text{-}23p19^{-/-}$ and $IL\text{-}12/23p40^{-/-}$ mice (Fig. 1*A*).

IL-22 is expressed in $IL\text{-}17A^{-/-}$ mice and IL-17A is expressed in $IL\text{-}22^{-/-}$ mice after cutaneous C. albicans *infection*

Induction of *IL-23p19, IL-12/23p40,* and *IL-12p35* mRNA levels in response to cutaneous *C. albicans* infection was similar in WT, $IL\text{-}17A^{-/-}$, and $IL\text{-}22^{-/-}$ mice (data not shown). *IL-17A* mRNA was induced similarly in $IL\text{-}22^{-/-}$ mice and WT mice (Fig. 8*A*). *IL-22* mRNA expression, however, was significantly decreased in $IL\text{-}17A^{-/-}$ mice compared with that in WT mice ($p < 0.001$). As an expected control, IL-17A expression was absent in $IL\text{-}17A^{-/-}$ mice, and IL-22 expression was absent in $IL\text{-}22^{-/-}$ mice ($p < 0.001$). These data suggest that IL-17A contributes to regulation of *IL-22* mRNA expression, but not vice versa. *TNF-α* mRNA
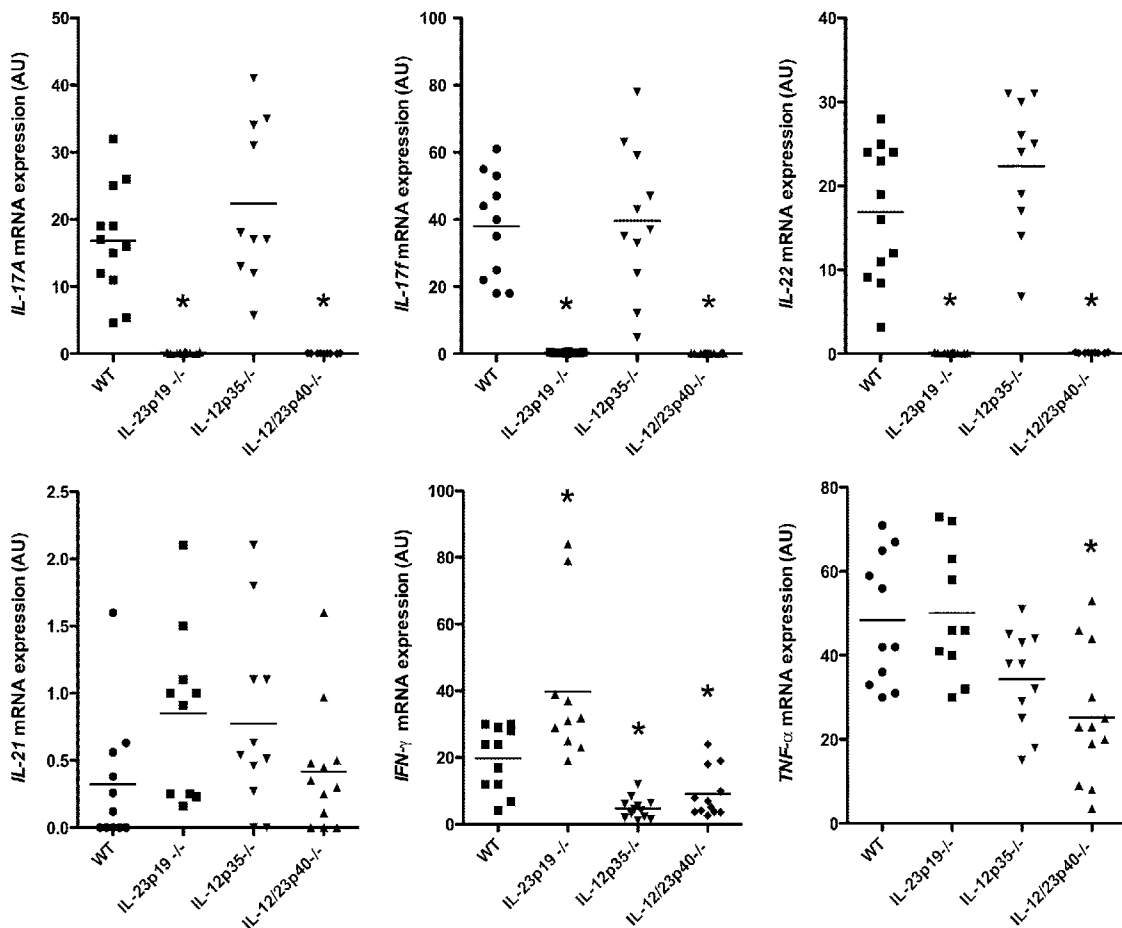
FIGURE 4. Impaired production of *IL-17A, IL-17F,* and *IL-22* in IL-23–deficient mice. *IL-17A, IL-17F, IL-22, IL-21, IFN-γ,* and *TNF-α* mRNA expression 48 h after *C. albicans* skin infection in WT and the various knockout mice as measured by qPCR. Each symbol indicates the amount of mRNA transcript as measured by arbitrary units (AU) of a single specimen, and horizontal bars denote the mean of scatterplots. Data were analyzed using Kruskal–Wallis test and Scheffe's *F* test. *IL-17A*: *$p < 0.001$ versus WT. *IL-17F*: *$p < 0.001$ versus WT. *IL-22*: *$p < 0.001$ versus WT. *IFN-γ*: *$p < 0.01$ for $IL\text{-}23p19^{-/-}$ and $IL\text{-}12p35^{-/-}$ versus WT; *$p < 0.01$ for $IL\text{-}12/23p40^{-/-}$ versus WT. *TNF-α*: *$p < 0.01$ versus WT.
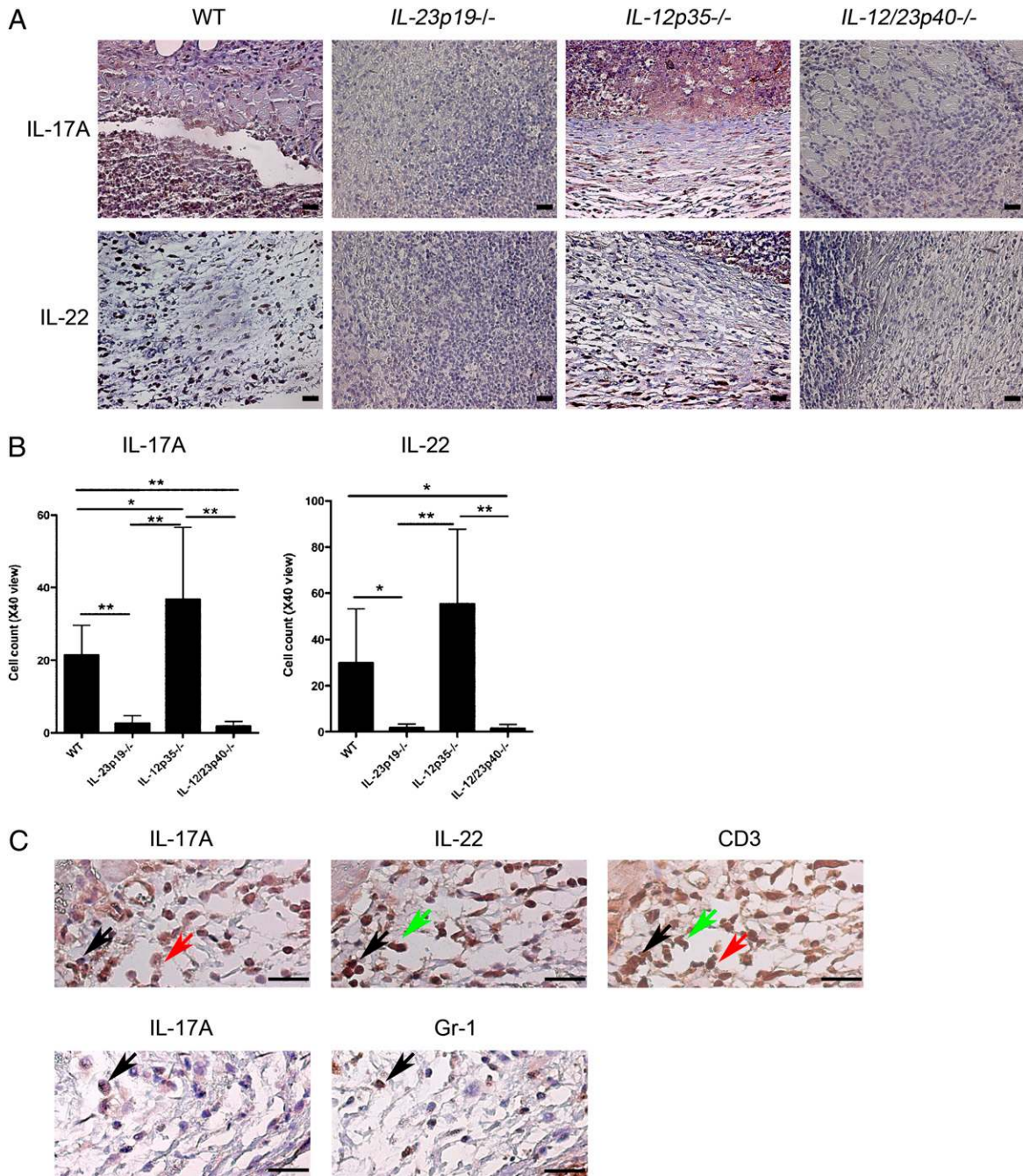
FIGURE 5. Impaired protein production of IL-17A and IL-22 in IL-23–deficient mice. *A,* Anti–IL-17A Ab-stained or anti–IL-22 Ab-stained sections of skin 4 d postinfection with *C. albicans*. Original magnification ×40; scale bars, 0.025 mm. *B,* Quantification of IL-17A$^+$ and IL-22$^+$ cells in each of the four groups of mice shown in *A*. Bar graphs were analyzed using Kruskal–Wallis test and Scheffe's *F* test. IL-17A: $*p < 0.05$; $**p < 0.01$ versus WT. IL-22: $*p * 0.05$; $**p < 0.001$ versus WT. *C,* IL-17A, IL-22, and CD3, or IL-17A and Gr-1 staining of serial sections of WT mouse skin 4 d postinfection with *C. albicans*. Original magnification ×100; scale bars, 0.025 mm. Black arrows indicate cells positive for IL-17A, IL-22, and CD3 (*top panels*) and cells positive for IL-17A and Gr-1 (*bottom panels*). Green arrows indicate cells positive for IL-22 and CD3, but negative for IL-17A. Red arrows indicate cells positive for IL-17A and CD3, but negative for IL-22.

expression was decreased in both *IL-17A$^{-/-}$* mice and *IL-22$^{-/-}$* mice ($p < 0.01$). In contrast with the differential mRNA induction of other cytokines, *IL-17F, IFN-γ,* and *IL-21* mRNA expression was similar among *IL-17A$^{-/-}$*, *IL-22$^{-/-}$*, and WT mice (Fig. 8). To confirm the mRNA results, protein analyses using ELISA and immunohistochemistry were performed. The ELISA data demonstrated similar findings to the mRNA data where IL-17A protein levels were induced similarly in *IL-22$^{-/-}$* mice and WT mice (Supplemental Fig. 4*A*). However, in contrast with the mRNA data, IL-22 protein levels were not significantly different between

IL-17A$^{-/-}$ mice and WT mice. As expected, protein levels of IL-17A and IL-22 were at or below the level of detection in *IL-17A$^{-/-}$* mice and *IL-22$^{-/-}$* mice, respectively. IL-21 protein levels were similar in *IL-17A$^{-/-}$*, *IL-22$^{-/-}$*, and WT mice (Supplemental Fig. 4*A*). It should also be noted that IFN-γ protein levels as measured by ELISA among all of the samples were below the level of detection (data not shown). Finally, to confirm the mRNA and ELISA data, IL-22$^+$ cells were readily observed in the dermis of infected *IL-17A$^{-/-}$* mice, and IL-17A$^+$ cells were readily observed in the dermis of infected *IL-22$^{-/-}$* mice (Supplemental Fig. 4*B*, 4*C*).
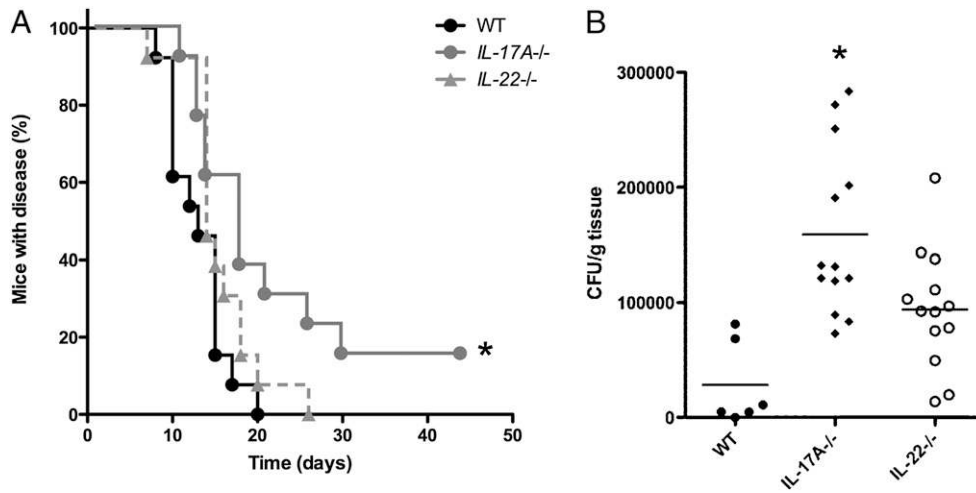
FIGURE 6. Optimal resolution of cutaneous *C. albicans* infection is dependent upon IL-17A but not IL-22. *A*, Kaplan–Meier curves demonstrating the percentage of *C. albicans*-infected mice with disease, as defined by the presence of nodule, ulcer, erythema, or crust at the skin site of inoculation. This was assessed by an investigator (blinded to the genotype of the mice) three times weekly postinfection. $*p < 0.025$ versus WT. *B*, Quantification of *C. albicans* fungal burden in skin 4 d after inoculation. Infected tissue was isolated, weighed, minced, and cultured on agar plates to determine CFU/gram skin tissue. $*p < 0.001$ versus WT.

Recombinant murine IL-17A delivered at the site of skin infection promotes more rapid healing of candidiasis in IL-12/23p40$^{-/-}$ mice

To determine whether exogenous IL-17A could promote healing in mice deficient in both IL-12 and IL-23, we injected a mixture

of *Candida* pseudohyphae and recombinant murine IL-17A into mouse skin on day 0. In a series of three independent experiments performed with a total of seven mice in each treatment group, 14% of WT mice were healed 12 d postinfection, whereas no *IL-12/23p40$^{-/-}$* mice were healed at this time point (Fig. 9*A*). In con-
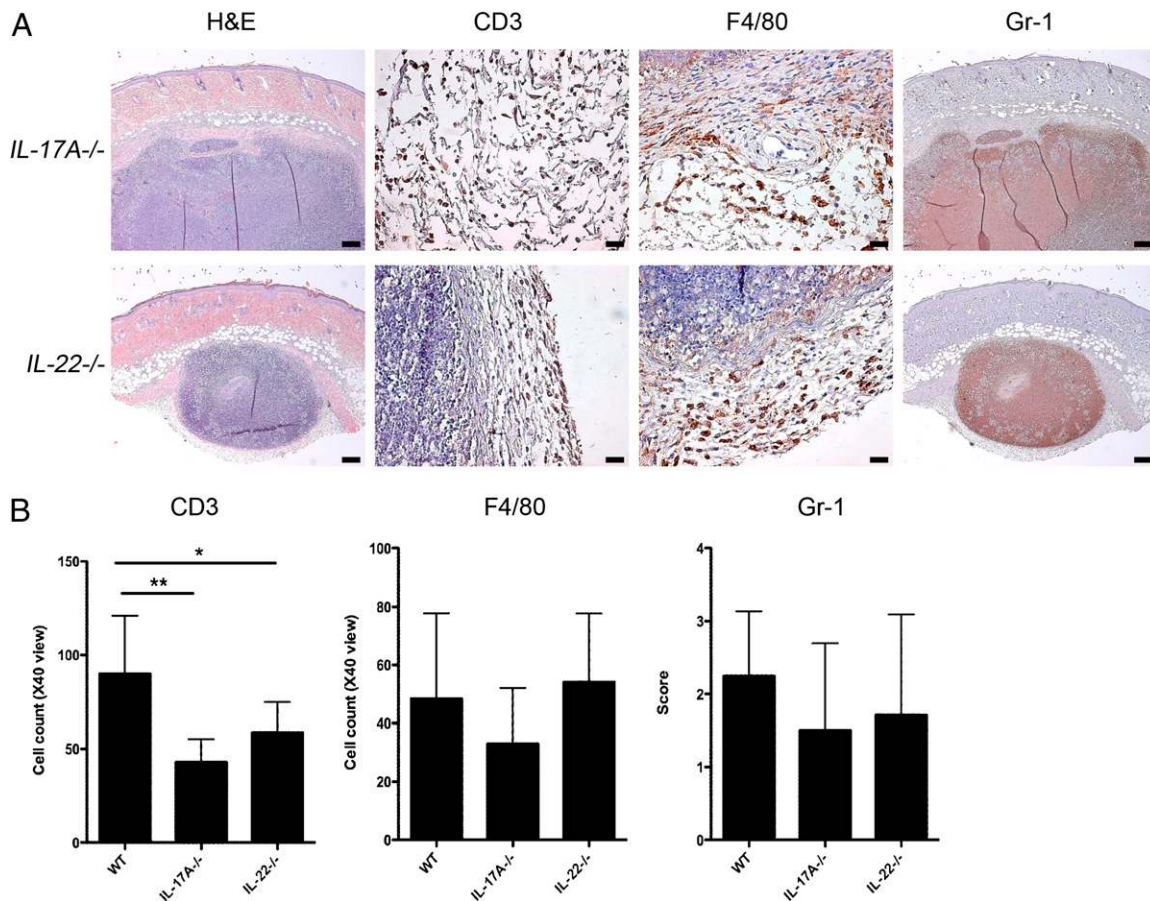
FIGURE 7. Higher numbers of CD3$^+$ T cells in WT mouse skin compared with that in *IL-17A$^{-/-}$* and *IL-22$^{-/-}$* mice. *A*, H&E, CD3, F4/80, and Gr-1 staining of skin 4 d postinfection with *C. albicans* showing a large collection of fungal elements in the deep dermis and superficial fat. H&E and Gr-1 staining, original magnification ×10; scale bars, 0.2 mm. CD3 and F4/80 staining, original magnification ×40; scale bars, 0.025 mm. *B*, Quantification of CD3$^+$ and F4/80$^+$ cells and grading of Gr-1 staining in each of the four groups of mice shown in *A*. Data are expressed as mean ± SD. Bar graphs were analyzed with Kruskal–Wallis test and Scheffe's *F* test. $*p < 0.025$; $**p < 0.001$ versus WT.
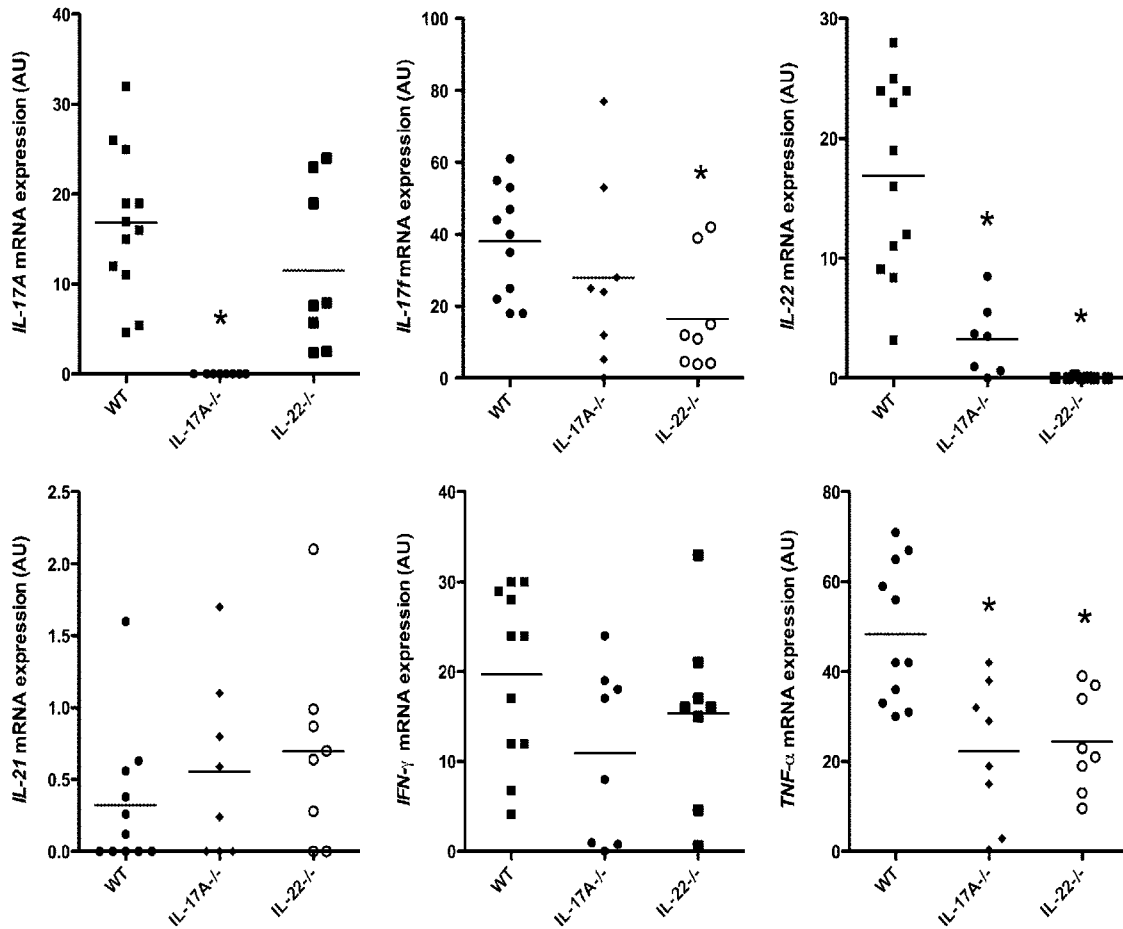
FIGURE 8. *IL-22* expression is decreased in *IL-17A*$^{-/-}$ mice, whereas *IL-17A* expression is normal in *IL-22*$^{-/-}$ mice after cutaneous *C. albicans* infection. *IL-17A, IL-17F, IL-22, IL-21, IFN-γ,* and *TNF-α* mRNA expression at 48 h after *C. albicans* skin infection in WT and the various knockout mice as measured by qPCR. Each symbol indicates the amount of mRNA transcript (as measured in AU) of a single specimen, and horizontal bars denote the mean of scatterplots. Data were analyzed using Kruskal–Wallis test and Scheffe's *F* test. *IL-17A* and *IL-22*: $*p < 0.001$ versus WT. *IL-17F* and *TNF-α*: $*p < 0.01$ versus WT. *IL-21* and *IFN-γ*: no differences versus WT.

trast, 43% of WT mice infected in the presence of exogenous IL-17A were healed and 29% of *IL-12/23p40*$^{-/-}$ mice infected in

FIGURE 9. Administration of exogenous recombinant IL-17A at the onset of infection promotes more rapid healing of cutaneous candidiasis in both WT and *IL-12/23p40*$^{-/-}$ mice. *A,* The percentage of mice healed 12 d postinfection. *B,* The percentage of mice healed 19 d postinfection. Data shown were generated after completing three independent experiments using a total of seven mice in each group.

the presence of exogenous IL-17A were healed on day 12 (Fig. 9*A*). Similar trends were observed 19 d postinfection (Fig. 9*B*). WT mice infected in the presence of exogenous IL-17A healed more quickly (86%) compared with WT mice infected without exogenous IL-17 (57%), and *IL-12/23p40*$^{-/-}$ mice infected in the presence of exogenous IL-17A healed more quickly (57%) compared with *IL-12/23p40*$^{-/-}$ mice infected without exogenous IL-17 (29%) (Fig. 9*B*).

Discussion

The observations reported here demonstrate that host defense against a cutaneous *C. albicans* challenge is dependent on IL-23 and IL-17A but not on IL-12 and IL-22. Importantly, all of the mice that demonstrated delayed healing of cutaneous candidiasis, that is *IL-23p19*$^{-/-}$, *IL-12/23p40*$^{-/-}$, and *IL-17A*$^{-/-}$ mice (Figs. 1*A*, 5*A*), shared a deficiency of IL-17A (Figs. 4, 7) and increased fungal burdens in skin (Fig. 1*B, 1C* and Fig. 5*B, 5C*). In contrast, mice deficient in *IL-12p35*, which had levels of IL-17A that were similar to WT mice, had no impairment in healing time. Furthermore, of the Th17 cytokines, IL-17A was shown to be a much more critical determinant for host defense than IL-22, as IL-22–deficient mice did not exhibit delayed healing or increased fungal burdens (Fig. 5). Also of note, *IL-22*$^{-/-}$ mice expressed relatively normal levels of IL-17A, which likely accounted for their ability to clear *C. albicans* from skin effectively (Fig. 7). Importantly, administration of exogenous recombinant murine IL-17A directly at the

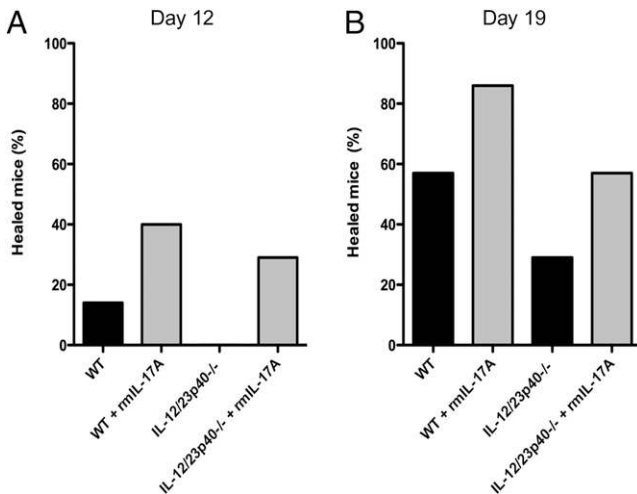sites of skin infection promoted more rapid healing of candidiasis in both WT and $IL-12/23p40^{-/-}$ mice (Fig. 9). Taken together, these results demonstrate the importance of IL-17A, whose production was dependent upon IL-23, but not IL-12 or IL-22, as a key mediator for an effective immune response against *C. albicans* within skin.

These findings have direct implications for human disease because they help provide an explanation for the increased susceptibility to *C. albicans* in patients who suffer from Job's syndrome, chronic mucocutaneous candidiasis, and HIV/AIDS (2, 23, 24, 28). It has recently been described that defective immune responses in Job's syndrome result from a defect in the ability to make Th17 cells due to mutations in *STAT3*, a transcription factor essential for Th17 cell differentiation (20, 21, 29). In addition, individuals with chronic mucocutaneous candidiasis have T cells that produce low amounts of Th17 cytokines (18, 23, 24). Patients with HIV/AIDS have also been demonstrated to have defects in Th17 responses (30). Thus, these current findings demonstrating that IL-17A is essential for host defense against cutaneous *C. albicans* infections provide a mechanism by which Th17 dysregulation leads to increased susceptibility to *C. albicans* infections among individuals with these disparate diseases.

We showed here that both CD3$^+$ T cells and Gr-1$^+$ neutrophils produced IL-17A (Fig. 5), but we cannot rule out the possibility that NK cells and $\gamma\delta$ T cells also produced this cytokine. A recent study has demonstrated that IL-17A, which mediated neutrophil recruitment against cutaneous *Staphylococcus aureus* infection in mice, is produced by resident $\gamma\delta$ T cells located in mouse epidermis (31). Thus, IL-17A–producing $\gamma\delta$ T cells may also play a role in cutaneous *C. albicans* infection. Regardless of the source, IL-17A signaling through the IL-17RA receptor activates both the NF-κB and MAPK intracellular pathways within a variety of cell types (32). IL-17A regulates the production of G-CSF, which in turn promotes the proliferation of promyelocytes and maturation of neutrophils. This homeostatic mechanism plays an important role in normal physiology and in host defense against bacterial infections (33). The results shown here do not support an additional major role for IL-17F (Fig. 8), which is closely related to IL-17A and is also produced by Th17 cells (34). A previous report demonstrated that IL-17F levels were elevated in $IL-17A^{-/-}$ mice (35). Thus, IL-17F homodimers, which have similar affinity for the IL-17R, do not appear to compensate for the loss of IL-17A and associated impaired healing of cutaneous candidiasis.

Results shown here using a cutaneous *C. albicans* infection model are supported by other mouse studies of *C. albicans* infection in other tissues. IL-17AR–deficient mice had reduced survival rates after systemic *Candida* challenge (36). In that study, IL-17AR–deficient mice had increased *C. albicans* burden in the kidneys and impaired neutrophil influx, which resulted in reduced survival compared with WT mice. In addition, another study using a mouse model of oral candidiasis demonstrated that mice deficient in Th17 cells developed more severe disease than Th1 cell-deficient mice (17). Similar to the current study, this study also found that IL-17A was a more critical determinant for host defense against oral *C. albicans* infection than IL-22, as IL-22–deficient mice were only mildly susceptible to oral disease (17).

It should be mentioned that when epidermal hyperplasia was measured above the *C. albicans* dermal abscess, IL-23–deficient mice had significantly decreased epidermal thickening compared with that in WT mice or IL-12–deficient mice. These findings indicate that epidermal hyperplasia was mediated by IL-23 and not by IL-12. We identified F4/80$^+$ macrophages as a source of IL-23 (Supplemental Fig. 1); we cannot rule out production by cutaneous dendritic cells. We also observed that *C. albicans*

infection readily induced epidermal hyperplasia in $IL-22^{-/-}$ mice (Fig. 6), suggesting that epidermal hyperplasia observed in response to *C. albicans* cutaneous infection is not entirely dependent upon IL-22. This is a somewhat surprising finding given that injections of recombinant IL-23 into murine skin induced epidermal hyperplasia in WT mice but failed to do so in mice deficient in IL-22 (26). Thus, IL-23–mediated epidermal hyperplasia induced by a cutaneous pathogen is likely more complex than epidermal hyperplasia induced by recombinant IL-23, involving cytokines other than IL-22. A recent study revealed that injection of IL-21 into mouse skin could also induce epidermal thickening (37). Therefore, IL-21 production was studied here, but no significant differences in IL-21 production were detected among WT and various knockout mice after *C. albicans* infection, suggesting that IL-21 was not the key mediator for epidermal hyperplasia in this model (Figs. 4B, 7B). Other studies have demonstrated that IL-19, IL-20, and IL-24 can induce epidermal hyperplasia directly (38). IL-23 injection into mouse skin induced $IL-19$ and $IL-24$ but not $IL-20$ mRNA (39). Thus, it could be that multiple inflammatory cytokines downstream of IL-23, such as IL-19 and IL-24, contribute to epidermal hyperplasia induced by cutaneous *C. albicans* infection.

From a clinical point of view, the findings shown here suggest that mechanisms that enhance IL-23 and/or IL-17A responses may help prevent or treat *C. albicans* cutaneous infections in humans. This may be particularly important in the development of future immunomodulatory therapy and vaccine development against *C. albicans*, especially for those patients with altered Th17 responses that suffer from chronic candidiasis. Conversely, therapeutic blockade of IL-23/IL-17A–mediated immune responses may result in increased skin infections with *C. albicans*. For example, ustekinumab is an mAb directed against IL-12/23p40 and has recently been approved for use in individuals with moderate-to-severe psoriasis (40, 41). Similar drugs that target IL-23 alone or IL-17A are also in clinical development for psoriasis, rheumatoid arthritis, and other T cell-mediated inflammatory diseases. Our results suggest that patients treated with these drugs should be closely monitored for cutaneous infections with *C. albicans* and perhaps other pathogens that are controlled by IL-23/IL-17A–mediated immune responses. They also suggest that individuals with chronic candidiasis may benefit from exogenous recombinant IL-17A delivered directly into sites of skin infection.

Disclosures
The authors have no financial conflicts of interest.

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