

Unexpected kidney-restricted role for IL-17 receptor signaling in defense against systemic *Candida albicans* infection

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Kidney injury is a frequent outcome in patients with disseminated *Candida albicans* fungal infections. IL-17 receptor (IL-17R) signaling is critical for renal protection against disseminated candidiasis, but the identity and function of IL-17-responsive cells in mediating renal defense remains an active area of debate. Using BM chimeras, we found that IL-17R signaling is required only in nonhematopoietic cells for immunity to systemic *C. albicans* infection. Since renal tubular epithelial cells (RTEC) are highly responsive to IL-17 in vitro, we hypothesized that RTEC might be the dominant target of IL-17 activity in the infected kidney. We generated mice with a conditional deletion of IL-17 receptor A (*Il17ra*) in RTEC (*Il17ra*^{ΔRTEC}). Strikingly, *Il17ra*^{ΔRTEC} mice showed enhanced kidney damage and early mortality following systemic infection, very similar to *Il17ra*^{-/-} animals. Increased susceptibility to candidiasis in *Il17ra*^{ΔRTEC} mice was associated with diminished activation of the renal protective Kallikrein-kinin system (KKS), resulting in reduced apoptosis of kidney-resident cells during hyphal invasion. Moreover, protection was restored by treatment with bradykinin, the major end-product of KKS activation, which was mediated dominantly via bradykinin receptor b1. These data show that IL-17R signaling in RTEC is necessary and likely sufficient for IL-17-mediated renal defense against fatal systemic *C. albicans* infection.

Introduction

Candida albicans is a commensal fungus that causes severe disseminated infections (1, 2). Disseminated candidiasis is the third most common nosocomial infection in hospitalized patients, with a candidemia-attributable mortality rate ranging between 14.5%–49% (2–4). In most cases, autopsy studies revealed that kidneys are the major target organs following bloodstream *C. albicans* infection (5). Currently, there are no approved vaccines against any fungal pathogens. Moreover, antifungal drugs are administered too late in patients due to a lack of sensitive early diagnostic tools (6). Thus, the need for effective therapeutic strategies to counter this fatal infection is compelling and likely to increase with time. However, the development of new therapies is hindered by our lack of understanding of the host-pathogen interactions in driving protective antifungal immunity in the kidney.

Renal infections occur via hematogenous routes or from ascending spread from the bladder or urethra. The early innate response to invading *C. albicans* is mediated by a coordinated effort between the effectors of innate immunity and kidney-resident cells (5). This ensures successful clearance of fungal pathogens, followed by repair of damaged renal tissue. However, the cross-talk between the infiltrating immune cells and kidney-resident cells in shaping local anti-*Candida* immunity is a highly neglected area of inquiry.

In experimental models, IL-17A (IL-17) has emerged as a vital player of immunity against disseminated candidiasis (7–11). IL-17 signals through a receptor complex composed of IL-17RA and IL-17RC (12). However, our understanding of the downstream signaling events in IL-17 target cells in vivo is limited due to ubiquitous expression of the IL-17R and paucity of precise genetic tools to dissect IL-17 signaling in specific cell types. This has created major controversies in the IL-17 field, particularly in the area of anti-*Candida* immunity. For example, in oropharyngeal candidiasis (OPC), IL-17R signaling in oral epithelial cells drives the expression of antimicrobial peptides necessary for defense against candidiasis, and there is no role for the IL-17R signaling in the hematopoietic compartment (13). In disseminated candidiasis, we and others have shown that IL-17 is produced locally in the kidney upon infection and that kidney-resident cells

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respond to IL-17 directly (7, 14, 15). In contrast, another study suggested that IL-17 is not made within the kidney but acts on NK cells to drive the production of GM-CSF, with protective activities in this setting of candidiasis (8). To date, the *in vivo* significance of IL-17R signaling in the kidney compartments where IL-17 mediates immunity against *C. albicans* is unknown.

IL-17 is classically recognized as a regulator of neutrophils and antimicrobial peptides. This is reflected in the profile of IL-17–induced genes in OPC, where genes encoding CXC chemokines, β -defensins, calprotectin (S100A8/9), and lipocalin 2 are upregulated (16). In a recent study from our laboratory, we identified the kallikreins (*Klk*) as a distinct class of IL-17 target genes with renal protective functions in disseminated candidiasis (9). This unexpected observation highlighted that IL-17 induces expression of tissue-specific genes depending on target organs and disease context.

Tissue *Klk*, an essential component of *Klk*-kinin system (KKS), are a family of 15 related serine proteases. *Klk1*, in particular, plays a critical role in renal function and pathology (17). *Klk* act on kininogens to generate bradykinin and kallidin. Bradykinin signals through 2 G-protein coupled receptors: bradykinin receptor b2 (*Bdkrb2*) is constitutively expressed, and *Bdkrb1* is induced in inflammatory conditions (17). Accordingly, mice lacking components of the KKS and humans with polymorphisms in KKS-related genes exhibit exacerbated kidney pathology in acute or chronic kidney diseases (18–22).

In disseminated candidiasis, progressive loss of kidney function is directly related to the inability of the host to clear the fungus and repair injured tissue. Renal tubular epithelial cells (RTEC) play a particularly pivotal role in driving inflammation in the kidney following acute or chronic injury (23, 24). RTEC constitute the majority among the cell types in the kidney cortex, where hyphal invasion and immune cells infiltration are hallmarks of early-stage infection, followed by spread to the medullary region at a later time point (5, 25). RTEC are activated by pathogen-associated molecular patterns and inflammatory mediators to secrete cytokines, chemokines, and tissue protective growth factors (14, 23, 24). We have previously shown that *Klk1* is induced in RTEC in an IL-17–dependent manner following systemic infection (9). However, the contribution of IL-17R signaling in RTEC in mediating renal immunity to *C. albicans* is poorly understood.

Here, we provide evidence indicating that the contribution of IL-17RA signaling in hematopoietic cells in systemic infection is not required during immunity against disseminated candidiasis. Rather, IL-17 produced locally in the kidney upon infection mediates nonredundant renal-protective signals through IL-17RA on RTEC. Consequently, mice deficient in IL-17RA signaling in RTEC (*Il17ra*^{RTEC}) succumb to infection due to increased renal damage. Similar to *Il17ra*^{−/−} mice, expression of kidney-protective *Klk1* was markedly reduced in *Il17ra*^{RTEC} mice, and bradykinin-driven protection is mediated via *Bdkrb1*, not *Bdkrb2*. Our data indicate that IL-17R signaling in RTEC plays an essential role in activating renal-protective KKS in disseminated candidiasis.

Results

Innate TCR $\gamma\delta$ ⁺ T cells are the primary source of IL-17 in the C. albicans–infected kidney. First, we confirmed whether renal expression of IL-17 family cytokines were upregulated in the early stages of disseminated candidiasis. When transcript expression of *Il17a*, *Il17f*, *Il17c*, and *Il17e* were measured in the infected kidney at day 2 postinfection (p.i.), we observed a significant increase in *Il17a* — but not *Il17f*, *Il17c*, and *Il17e* — mRNA levels during disseminated infection (Figure 1A).

In the oral mucosa and skin, IL-17 from innate TCR $\alpha\beta$ ⁺ and TCR $\gamma\delta$ ⁺ T cells are required for resistance to *C. albicans* infection (26, 27). To identify the cellular source of IL-17 in the kidneys, we took advantage of the sensitive *Il17a*^{Cre}Rosa26R^{eYFP} (IL-17^{eYFP}) fate-tracking mice that permanently marks IL-17–expressing cells with eYFP (28). These mice were systemically infected with *C. albicans*, and percentage of IL-17–producing cells in the kidneys was determined based on eYFP expression at day 2 p.i. As expected, flow cytometry analysis revealed a 5- to 6-fold increase in the number of inflammatory cells (CD45⁺) following infection (Figure 1B). When evaluated for IL-17 producers within the inflammatory cells, we observed a significant increase in the number of eYFP⁺ cells in the *C. albicans*–infected kidneys than uninfected mice. We further gated on the eYFP⁺ cells to determine the absolute frequencies of IL-17–producing innate TCR $\gamma\delta$ ⁺ and TCR $\alpha\beta$ ⁺ T cells in the kidneys. In uninfected mice, we saw a small baseline population of innate TCR $\gamma\delta$ ⁺ and TCR $\alpha\beta$ ⁺ T cells showing evidence of present or prior IL-17 production. Following infection, there is a 6- to 7-fold increase in the number of CD45⁺eYFP⁺TCR δ ⁺ cells, indicating that innate TCR $\gamma\delta$ ⁺ T cells expand and are the major source of IL-17 in the kidneys. While the frequency of IL-17–producing innate TCR $\alpha\beta$ ⁺ cells was less compared with TCR $\gamma\delta$ ⁺ T cells, there was a reproducible 2-fold

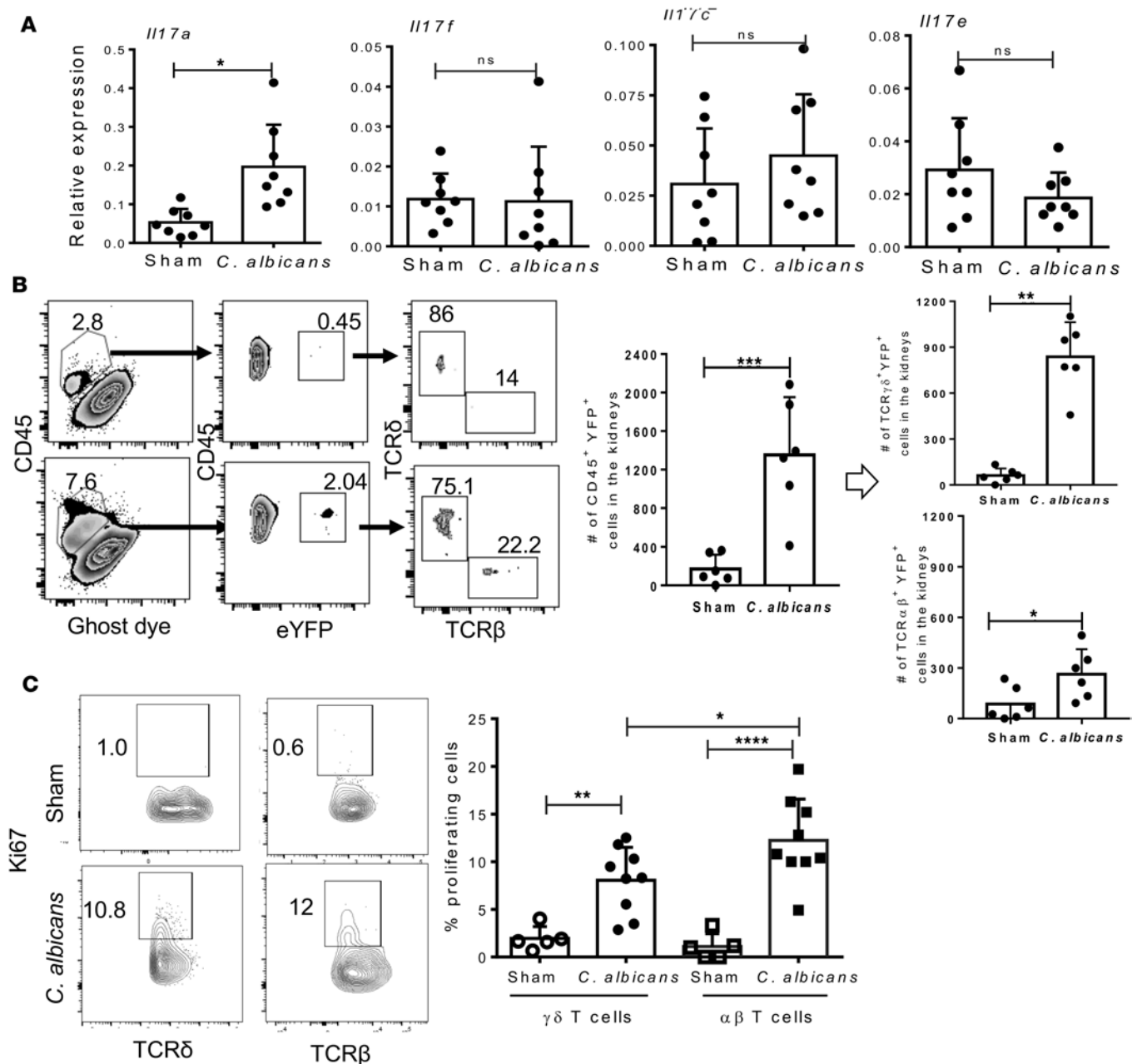


Figure 1. Innate TCR $\gamma\delta^+$ T cells produce IL-17 in *C. albicans*-infected kidney. (A) WT mice were either subjected to disseminated *C. albicans* infection or left uninfected (sham) ($n = 8$). At day 2 p.i., renal mRNA levels of *Il17a*, *Il17f*, *Il17c*, and *Il17e* were quantified by qPCR. (B) Single cell suspensions from the perfused kidneys of *C. albicans* infected or sham IL-17^{eYFP} mice ($n = 6$) were used to determine the number of eYFP⁺ cells (gated on live CD45⁺) at day 2 p.i. Absolute numbers of innate TCR $\gamma\delta^+$ and TCR $\alpha\beta^+$ T cells producing IL-17 were evaluated by gating on live CD45⁺eYFP⁺ cells. (C) Percentages of renal Ki-67⁺TCR $\gamma\delta^+$ and Ki-67⁺TCR $\alpha\beta^+$ cells from *C. albicans*-infected ($n = 9$) or sham mice ($n = 5$) (gated on live CD45⁺ cells) at day 2 p.i. Numbers in the zebra (B) and contour (C) plots reflect percentages of cells. In the dot plots, each dot represents an individual mouse, and data are represented as mean \pm SD and compared by 2-tailed Student's *t* test for A and B and by 1-way ANOVA for C. Data are pooled from 2 independent experiments for A-C. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

increase in the number of TCR $\alpha\beta^+$ cells in the infected kidneys compared with uninfected animals. This observation is in line with a previous report showing that IL-17-producing TCR $\gamma\delta^+$ T cells play a vital role in the early host resistance against systemic *C. albicans* infection in the lung (29). Notably, we were unable to detect any eYFP-expressing ILC3 cells in the sham or infected kidney at this time point (data not shown).

To explain the increase in innate IL-17 producers in the fungal infected kidney, we next evaluated the proliferation of these cells by performing Ki-67 staining in non-fate-tracking mice. Systemic infection with *C. albicans* resulted in a strong proliferation of innate TCR $\gamma\delta^+$ and TCR $\alpha\beta^+$ T cells, as evidenced by the increased number of

Ki-67⁺ cells after infection (Figure 1C). The innate TCR $\alpha\beta$ ⁺ cells showed marginally increased proliferation than TCR $\gamma\delta$ ⁺ T cells. These results indicate that kidney-resident or infiltrating innate TCR $\gamma\delta$ ⁺ T cells are the primary cell types responsible for IL-17 production in the kidney during disseminated *C. albicans* infection.

IL-17RA signaling in nonhematopoietic cells is required for protection against disseminated candidiasis. Renal antifungal response is mediated by infiltrating cellular effectors (hematopoietic cells) and kidney-resident cells (nonhematopoietic cells). A previous study has shown that IL-17RA is required only in nonhematopoietic cells for oral defense against OPC (13). We hypothesized that there is likewise an essential contribution of IL-17RA in the nonhematopoietic compartment in disseminated candidiasis. To test this idea, BM cells from *Il17ra*^{-/-} (CD45.2⁺) and WT (CD45.1⁺) mice were adoptively transferred into lethally irradiated *Il17ra*^{-/-} or WT recipients. Several studies have shown that most hematopoietic cells in the kidney are radiosensitive, thus allowing us to probe the contribution of hematopoietic vs. nonhematopoietic cells in renal defense against disseminated candidiasis (30–33). Baseline serum blood urea nitrogen (BUN), creatinine, and neutrophil gelatinase-associated lipocalin (NGAL) expression in the kidney revealed no overt difference in irradiation-induced kidney dysfunction or injury between WT and *Il17ra*^{-/-} mice after BM reconstitution (Supplemental Figure 1, A and B; supplemental material available online with this article; <https://doi.org/10.1172/jci.insight.98241DS1>). Similar observations were also noted before the transfer of BM cells (24 hours after irradiation) between WT and *Il17ra*^{-/-} mice (Supplemental Figure 1, C and D). Six weeks later, the recipient mice were tested for reconstitution by staining for CD45.1⁺ or CD45.2⁺ cells in the peripheral blood by FACS (Supplemental Figure 1E). Successfully reconstituted mice were subjected to disseminated candidiasis, and survival was evaluated over 14 days. *Il17ra*^{-/-} mice reconstituted with *Il17ra*^{-/-} BM succumbed to infection at an early time point (100% mortality by day 7 p.i.), phenocopying *Il17ra*^{-/-} mice (Figure 2A). WT mice receiving WT or *Il17ra*^{-/-} BM exhibited increased survival (50% survival at day 14 p.i.) than *Il17ra*^{-/-} mice reconstituted with *Il17ra*^{-/-} BM, indicating that IL-17RA expression on hematopoietic cells is not required for antifungal defense. Interestingly, *Il17ra*^{-/-} mice receiving WT BM showed increased susceptibility (100% mortality around day 10 p.i.) in comparison with mice lacking *Il17ra* on hematopoietic cells. Remarkably, mortality in mice where IL-17RA signaling is absent in nonhematopoietic cells was almost similar to *Il17ra*^{-/-} mice reconstituted with *Il17ra*^{-/-} BM. These data establish that IL-17RA signaling is exclusively required in nonhematopoietic cells for antifungal immunity in disseminated candidiasis.

IL-17RA signaling in RTEC is critical for anti-Candida immunity in the kidney. The major kidney-resident cells implicated in renal inflammation belong to the glomerular, tubular, and vascular compartments, including podocytes, mesangial cells, endothelial cells, and proximal and distal RTEC (34). RTEC constitute approximately 70% of cells in the cortex, where they are in close proximity to *C. albicans* and inflammatory cells (34). RTEC express functional IL-17RA and produce cytokines and chemokines in response to IL-17 under in vitro condition (14, 24). Based on these findings, we sought to define the contribution of IL-17RA signaling specifically in RTEC during systemic infection. We generated mice lacking IL-17RA signaling in RTEC by crossing *Il17ra* ^{β/β} mice to well-characterized *Cdh16*^{Cre} (*Ksp1.3*^{Cre}) transgenic mice where the Cre recombinase is exclusively expressed in RTEC (35, 36). Flow cytometry analysis of *Il17ra* ^{β/β} *Cdh16*^{Cre+} (henceforth referred to as *Il17ra*^{RTEC}) mice confirmed effective deletion of IL-17RA in RTEC (CD45⁺CD133⁺) (Figure 2B). The kidney histology of *Il17ra*^{RTEC} mice exhibit no overt changes in the renal architecture at the baseline (Supplemental Figure 2A). Overall, these data provided verification for the deletion of the *Il17ra* alleles in RTEC.

To assess the role of IL-17RA in RTEC, *Il17ra*^{RTEC} and *Il17ra* ^{β/β} *Cdh16*^{Cre-} (hereafter referred to as control) mice were either infected with *C. albicans* or left uninfected, and survival was assessed over 14 days. *Il17ra*^{RTEC} mice showed significantly increased susceptibility to systemic infection compared with control animals (Figure 2C). Nevertheless, *Il17ra*^{RTEC} mice demonstrated marginally increased resistance to *Il17ra*^{-/-} mice, indicating that IL-17RA signaling in kidney-resident cell types apart from RTEC may play a minor yet distinguishable role in renal immunity. Additionally, female and male mice demonstrated comparable susceptibility to disseminated candidiasis (Supplemental Figure 2B). Due to *C. albicans* strain-specific differences in antifungal immunity, we next assessed the susceptibility of *Il17ra*^{RTEC} mice following systemic infection with a different strain of *C. albicans* (HUN96) (37). *Il17ra*^{RTEC} mice infected with HUN96 strain (5×10^5 CFU) showed early mortality compared with the control group (Figure 2D). To address the contribution of IL-17 signaling in other renal epithelial cell types, we generated mice lacking *Il17ra* in podocytes by crossing *Il17ra* ^{β/β} mice to *Nphs2*^{Cre} transgenic mice, in which Cre recombinase is solely expressed in podocytes (termed *Il17ra*^{POD}) (38). Unlike *Il17ra*^{RTEC} mice, there was no difference in susceptibility between *Il17ra*^{POD} and control animals to disseminated candidiasis (Figure 2E). These results indicate that IL-17RA signaling in RTEC play an important and likely sufficient role for renal protection against disseminated candidiasis.

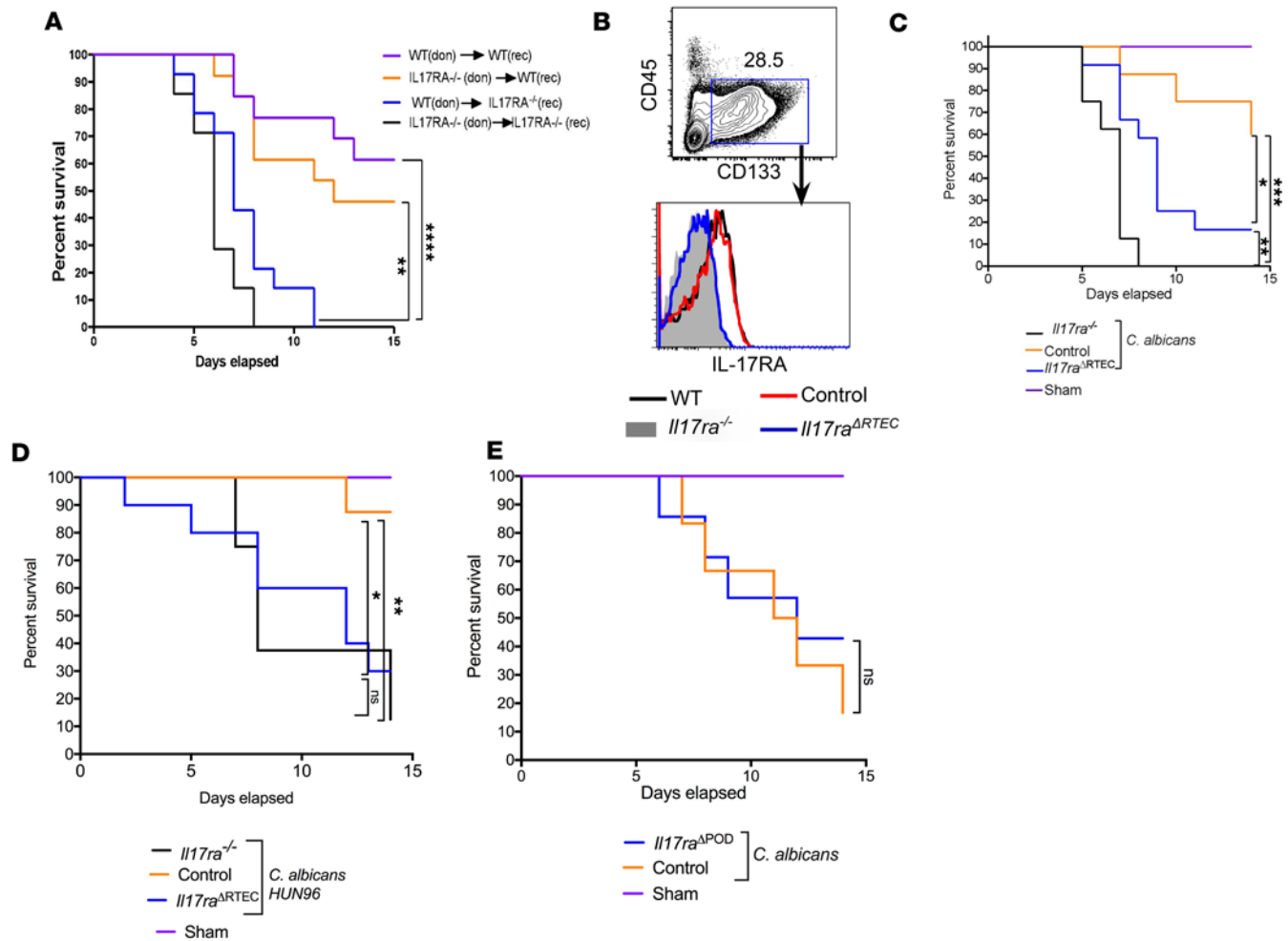


Figure 2. RTEC-specific expression of IL-17RA is required for antifungal immunity in the kidney. (A) BM cells from *Il17ra*^{-/-} (CD45.2⁺) and WT (CD45.1⁺) mice were adoptively transferred into irradiated (9 Gy) *Il17ra*^{-/-} or WT recipients ($n = 14$). Six weeks later, mice were systemically infected with *C. albicans*, and survival was evaluated over 14 days. Don, donor; rec, recipients. (B) Total kidney cells from WT, *Il17ra*^{-/-}, control, and *Il17ra*^{ΔRTEC} mice ($n = 3$) were evaluated for IL-17RA expression on RTEC (live CD45⁺CD133⁺). The number in the contour plot reflects percentage of cells. Histogram is representative of 1 of 3 independent experiments. (C) Control, *Il17ra*^{ΔRTEC}, and *Il17ra*^{-/-} mice were systemically infected with *C. albicans* (CAF2-1 strain; 1×10^5 CFU) ($n = 15-16$) or left uninfected ($n = 3-5$). Mice were evaluated for survival over 14 days. (D) Control, *Il17ra*^{ΔRTEC}, and *Il17ra*^{-/-} mice were systemically infected with *C. albicans* (HUN96 strain; 5×10^5 CFU) ($n = 8-11$) or left uninfected ($n = 2$). Mice were evaluated for survival over 14 days. (E) *Il17ra*^{ΔPOD} (*Il17ra*^{ΔPOD}) mice were either subjected to infection ($n = 8-10$) or left uninfected ($n = 3$) and were evaluated for survival over 14 days. Data are pooled from 3 independent experiments for A, C, and E and 2 independent experiments for D. The comparison of survival curves were performed by Log-rank (Mantel-Cox) test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

NK cells are functionally normal in mice with RTEC-specific deletion of IL-17RA. A previous report has suggested a critical role for IL-17RA in NK cell mediated antifungal immunity following disseminated candidiasis (8). In this study, *Il17ra*^{-/-} mice demonstrated increased fungal load in the kidney due to a cell-intrinsic defect in the development of functional NK cells. Although *Cdh16*^{Cre} transgenic mice has been widely used for RTEC-specific gene targeting (39), we wanted to rule out any defects in NK cell function due to leaky expression of Cre recombinase that could account for the increased susceptibility of *Il17ra*^{ΔPOD} mice to fungal infection. Flow cytometry analysis of BM cells showed comparable IL-17RA expression on immature NK (iNK) and precursor NK (NKP) cells between *Il17ra*^{ΔPOD} and control mice (Figure 3A and Supplemental Figure 3).

We further assessed the functionality of NK cells from *Il17ra*^{ΔRTEC} and control mice by measuring IFN γ production from splenic NK cells following LPS injection. In line with the previous report (8), NK cells from *Il17ra*^{-/-} mice produced less IFN γ than WT mice (Figure 3B). In contrast, there was no difference in the percentage of IFN γ -producing NK cells between the *Il17ra*^{ΔRTEC} and control animals. Notably, we were unable to detect IFN γ or GM-CSF from splenic NK cells in mice following systemic *C. albicans* infection

at 24 hours p.i. in our experiments (Supplemental Figure 4, A and B). These results suggest that increased susceptibility of *Il17ra*^{ΔRTEC} mice to disseminated candidiasis is not due to defects in NK cell function.

Kidney fungal clearance is moderately impaired in mice with RTEC-specific deletion of IL-17RA. We next determined the impact of RTEC-specific IL-17RA signaling in fungal clearance and early inflammatory events in the kidney. *Il17ra*^{ΔRTEC} and control kidney surprisingly exhibited comparable fungal load at day 2 p.i., a time point at which *Il17ra*^{-/-} mice showed increased fungal burden (Figure 4A) (9). However, *Il17ra*^{ΔRTEC} mice showed half a log increase in the fungal load compared with control kidney at day 5 p.i. These results indicate that IL-17RA signaling in RTEC marginally affects fungal clearance, albeit at a delayed time point.

We also compared the frequency of kidney-infiltrating innate cells in the *Il17ra*^{ΔRTEC} and control mice following fungal infection. In agreement with the fungal clearance data, the percentages of kidney-infiltrating total inflammatory cells (CD45⁺), neutrophils, macrophages, and NK cells were comparable between the groups at day 2 p.i. (Figure 4B). Renal transcript expression of *Cxcl5*, *S100a8*, and *Defb1* was significantly downregulated in the kidney of *Il17ra*^{ΔRTEC} mice (Figure 4C). However, other IL-17-responsive genes such as *Lcn2* and *Cxcl1* showed no change in the *Il17ra*^{ΔRTEC} mice, similar to what has been shown in *Il17ra*^{-/-} mice (Supplemental Figure 5) (9). These data suggest that reduced expression of antimicrobial peptides in *Il17ra*^{ΔRTEC} mice may account for the defect in fungal clearance at a delayed time point.

IL-17RA signaling in RTEC prevents kidney damage following disseminated candidiasis. Based on our survival data (Figure 2C), we wanted to determine whether the absence of IL-17RA signaling in RTEC is associated with increased renal damage, leading to loss of kidney function. H&E staining of serial kidney sections revealed increased tissue pathology in the cortex and inner and outer medullary regions of *Il17ra*^{ΔRTEC} at day 7 p.i. (Figure 5A and Supplemental Figure 5B). Consequently, renal function was compromised in *Il17ra*^{ΔRTEC} mice as evident from increased serum BUN levels at day 7 p.i. (Figure 5B). *Il17ra*^{ΔRTEC} kidney demonstrated a marked increase in the expression of NGAL and kidney-injury marker 1 (KIM1), classical kidney injury markers (Figure 5C, Supplemental Figure 5B, and Supplemental Figure 6, A and B). Collectively, these results demonstrate that IL-17RA signaling in RTEC prevents kidney damage during disseminated candidiasis.

We have previously shown that IL-17RA signaling alleviates renal injury during hyphal invasion by preventing the apoptosis of kidney-resident cells (9). Therefore, we compared the apoptosis of tubular epithelial cells between *Il17ra*^{ΔRTEC} and control kidneys following *C. albicans* infection. At day 7 p.i., we observed a significantly increased frequency of apoptotic kidney-resident tubular epithelial cells (CD45⁺CD133⁺) in *Il17ra*^{ΔRTEC} mice compared with control kidney (Figure 6A). Supporting our apoptosis data, absence of IL-17RA in RTEC also resulted in a striking increase in the number of active Caspase-3⁺ tubular epithelial cells (CD45⁺CD133⁺) (Figure 6B). Thus, activation of IL-17RA signaling in RTEC limits renal damage during systemic fungal infection by limiting the apoptosis of kidney-resident cells.

IL-17RA-dependent expression of Klk1 in RTEC protects mice against systemic C. albicans infection. Our published study identified an intriguing link between IL-17/KKS axis-mediated renal protection against disseminated candidiasis (9). We were the first to our knowledge to show that kidney-protective Klk1 is expressed only in RTEC following fungal infection in an IL-17-dependent manner (9). To confirm that Klk1 is indeed produced specifically from RTEC, we measured the transcript expression of *Klk1* in the kidneys of infected *Il17ra*^{ΔRTEC} and control mice. Similar to what has been shown in *Il17ra*^{-/-} mice (9), *Il17ra*^{ΔRTEC} kidney demonstrated a significant reduction in the mRNA levels of *Klk1* and *Klk1b26* compared with control mice at day 2 p.i. (Figure 7A). Consequently, RTEC in the cortex and medullary region of the infected kidney expressed diminished levels of Klk1 in the absence of IL-17RA at day 7 p.i. (Figure 7B and Supplemental Figure 6C). These results highlight the essential contribution of IL-17RA signaling in RTEC for the production of renal-protective Klk1 in disseminated infection.

Klk1 mediates cleavage of kininogens to generate bradykinin, which signals through *Bdkrb1* and *Bdkrb2* (17). To define the role of RTEC-derived Klk1 in renal immunity, *Il17ra*^{ΔRTEC} mice were either treated with bradykinin or left untreated, and survival was evaluated following systemic infection. Bradykinin-treated *Il17ra*^{ΔRTEC} mice exhibited significantly increased survival benefit in comparison with the untreated group (Figure 7C). Collectively, these results extend our previous finding and indicate that the IL-17/Klk1 axis in RTEC plays a critical role in the renal host defense in disseminated candidiasis.

We next wanted to define the individual contribution of *Bdkrb1* and *Bdkrb2* in renal defense against systemic fungal infection. First, we evaluated *Bdkrb1* and *Bdkrb2* mRNA expression in the infected kidney of WT mice. The fungal infection resulted in a significant upregulation of *Bdkrb1* but not *Bdkrb2* transcript expression at day 2 p.i. (Figure 7D). This result indicates that the inflammatory milieu in the kidney contributes to the induction

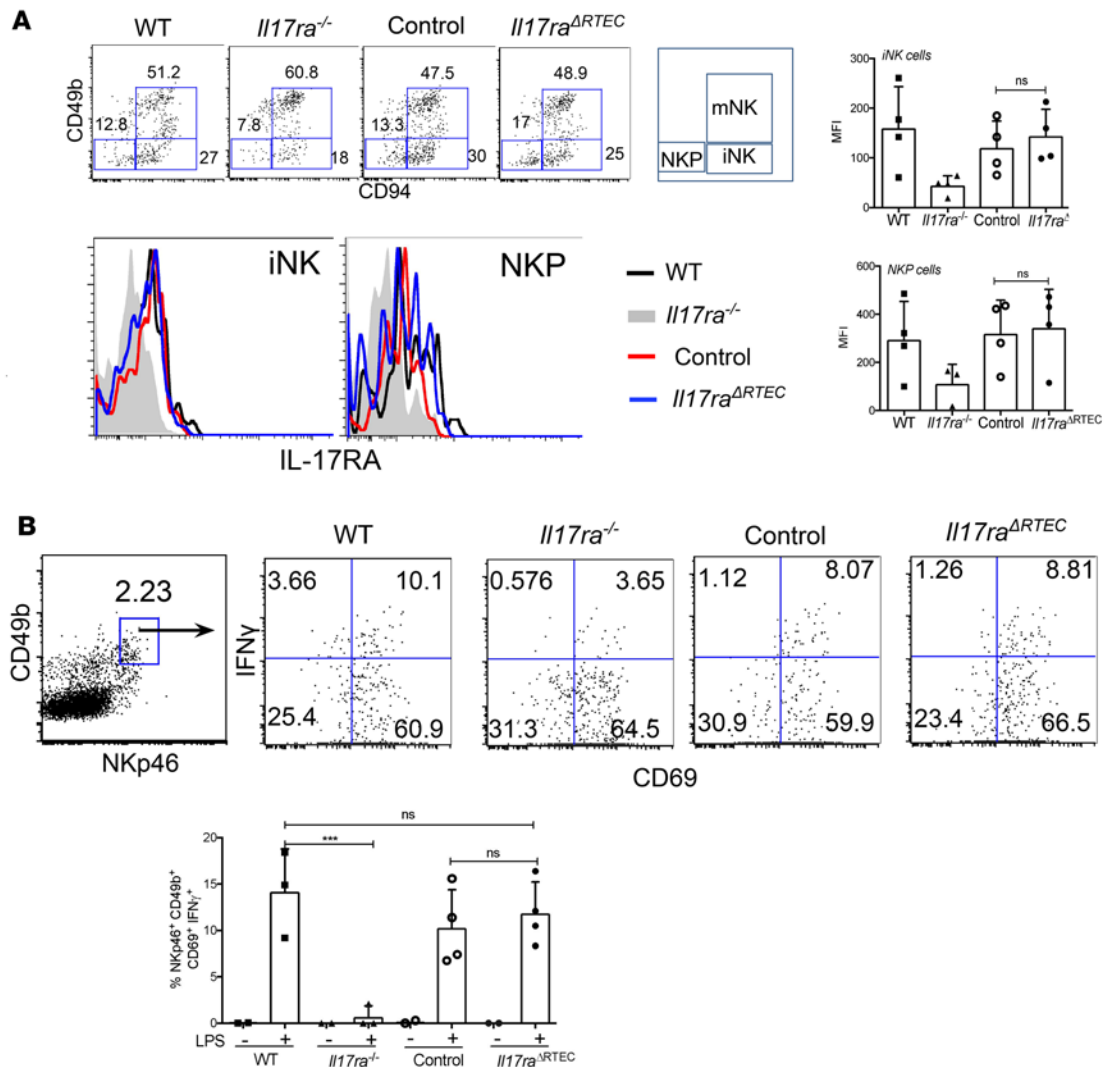


Figure 3. NK cells are functional in *Il17ra*^{dRTEC} mice. (A) BM cells from WT, *Il17ra*^{-/-}, control, and *Il17ra*^{dRTEC} mice (*n* = 4) were evaluated for IL-17RA expression on mature NK (mNK) (liveCD45⁺CD3⁺NKG2d⁺CD49b⁺CD94⁺), immature NK (iNK) (liveCD45⁺CD3⁺NKG2d⁺CD49b⁻CD94⁺), and precursor NK (NKP) (liveCD45⁺CD3⁺NKG2d⁺CD49b⁻CD94⁻) cells by flow cytometry. (B) WT, *Il17ra*^{-/-}, control, and *Il17ra*^{dRTEC} mice were either injected i.p. with LPS (1.5 ng/ml) (*n* = 4) or left untreated (*n* = 2). Five hours later, splenic NK cells (gated on liveCD45⁺CD3⁺NKp46⁺CD49b⁺CD69⁻) were evaluated for IFN γ production by intracellular cytokine staining. Numbers in the dot plots indicate percentages of cells. In the dot plots, each dot represents individual mouse, and data are represented as mean \pm SD. Data were pooled from 3 and 2 independent experiments for A and B, respectively, and analyzed by 1-way ANOVA. ****P* < 0.001.

of *Bdkerb1* expression, similar to what has been shown in other inflammatory kidney diseases (18). To determine the specific role of *Bdkerb1* and *Bdkerb2* in IL-17/Klk1 axis-driven renal defense, anti-*Bdkerb1*- or anti-*Bdkerb2* antagonist-treated WT mice were subjected to disseminated candidiasis, and survival was evaluated over 14 days. Mice treated with selective *Bdkerb1* antagonist succumb to infection earlier (100% mortality around day 7 p.i.) compared with the untreated or *Bdkerb2* antagonist-treated groups (Figure 7E). The survival of *Bdkerb2* antagonist-treated mice was comparable with untreated mice. Collectively, these data suggest that *Bdkerb1* activation is a key downstream mediator in the IL-17/Klk1-mediated protection against disseminated candidiasis.

Discussion

While it is evident that IL-17 is needed for renal immunity to candidiasis, the underlying mechanisms are poorly understood. In the past decade, most studies in the IL-17 field have focused on the generation of IL-17-producing cells. With the development of mice with conditional deletion of IL-17R, studies defining the downstream effects of IL-17 on target cells have just begun to appear. For example, oral epithelial cells drive IL-17R-dependent responses against OPC through regulation of antimicrobial peptides (13). On the other hand, IL-17R sig-

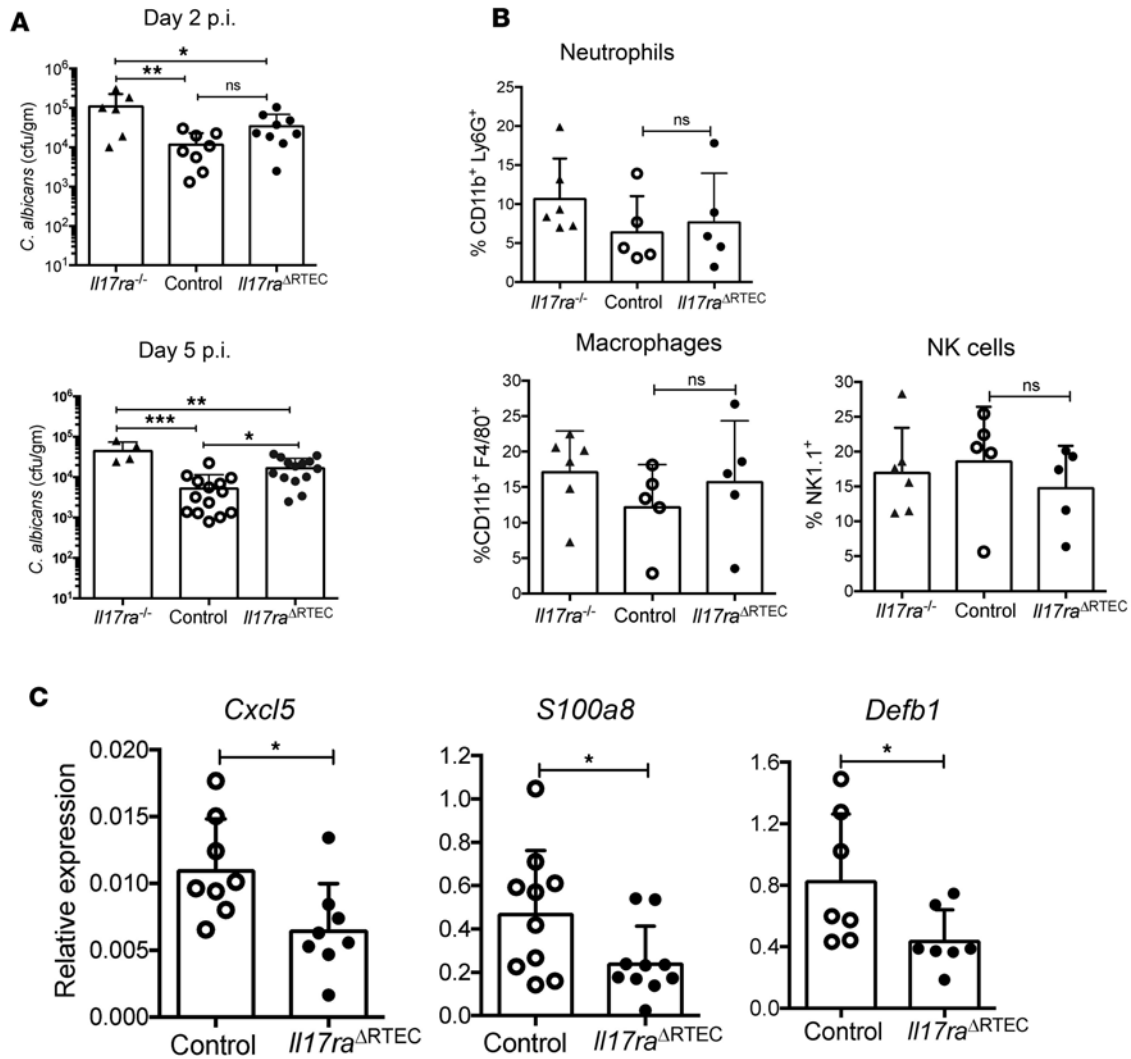


Figure 4. Early renal innate response in *Il17ra*^{ARTEC} mice after disseminated candidiasis. Control, *Il17ra*^{ARTEC} ($n = 8-14$) and *Il17ra*^{-/-} mice ($n = 6$) were subjected to systemic *C. albicans* infection. **(A)** Fungal burden was measured in the kidneys at days 2 and 5 p.i. Data for surviving *Il17ra*^{-/-} mice ($n = 4$) are shown for day 5 p.i. **(B)** The kidneys of *Il17ra*^{-/-}, control, and *Il17ra*^{ARTEC} mice ($n = 5-6$) were evaluated for kidney infiltrating neutrophils (live CD45⁺Ly6G⁺CD11b⁺), macrophages (live CD45⁺F4/80⁺CD11b⁺), and NK cells (live CD45⁺CD3⁺NK1.1⁺) at day 2 p.i. **(C)** Renal transcript expression of *Cxcl5*, *S100a8*, and *Defb1* was measured by qPCR in control and *Il17ra*^{ARTEC} mice ($n = 7-10$) at day 2 p.i. Each dot represents an individual mouse, and data are represented as mean \pm SD. Data are pooled from 2 independent experiments for **A** and **B** and 3 independent experiments for **C** and were analyzed by 1-way ANOVA. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

naling in the lung epithelium establishes chemokine gradients essential for antibacterial immunity (40). The common emergent theme from these studies is that IL-17-responsive genes are differentially regulated in similar cell types from different organs, with profound implications in designing organ-targeted therapeutic strategies to combat systemic fungal infections. Thus, lessons from mucosal sites do not necessarily translate to solid organs, such as the kidney. Here, we show that IL-17R signaling in RTEC drives the activation of KKS, critical for renal protection against fungal invasion. Despite being a fatal systemic infection, the IL-17R signaling is markedly restricted in the kidney and not in the cells of immune system during antifungal immune response. This observation contrasts with TNF α , where activities on neutrophils and other myeloid cells are required for antifungal immunity (41). The study presented here is the first systematic dissection of cytokine signaling in the kidney to our knowledge and reveals insights into the renal activities of IL-17 in antifungal immunity.

IL-17RA serves as a receptor for several IL-17 family members, including IL-17, IL-17F, IL-17C, and IL-17E (12). Therefore, the increased susceptibility observed in *Il17ra*^{ARTEC} mice could be due to defective signaling of several IL-17 family members other than IL-17. Since IL-17F, IL-17C, and IL-17E were unable to induce Klk1 expression in RTEC (9), it is unlikely that these members contribute to the protection in

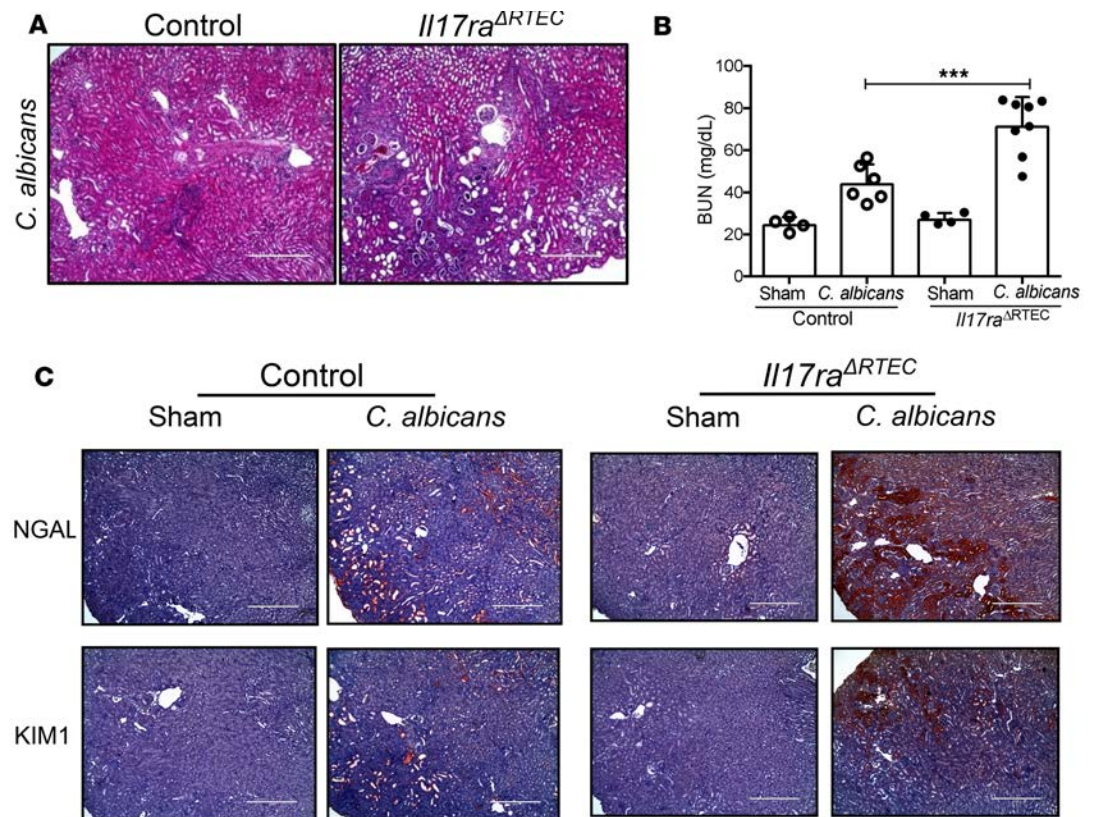


Figure 5. *Il17ra*^{ΔRTEC} mice demonstrated aggravated renal damage following systemic *C. albicans* infection. Control and *Il17ra*^{ΔRTEC} mice were either subjected to systemic *C. albicans* infection ($n = 6-8$) or left uninfected ($n = 4$). At day 7 p.i., mice were evaluated for (A) histopathology and inflammatory cell influx by H&E staining (original magnification, 100 \times); (B) serum BUN level at day 7 p.i.; and (C) NGAL and KIM1 expression by IHC. Representative photomicrographs from 2 independent experiments for A and C. Original magnification, 100 \times . Pooled results from 2 independent experiments for B. Each dot represents an individual mouse, and data are represented as mean \pm SD and analyzed by 1-way ANOVA. *** $P < 0.001$. Scale bars: 400 μ m.

fungal infection. However, there is lack of conclusive data using targeted genetic approaches to rule out the potential involvement of IL-17C signaling in RTEC. Future studies with mice harboring RTEC-specific deletion of IL-17RE, an IL-17 receptor subunit that is solely utilized by IL-17C, will shed light on the role of this cytokine in antifungal immunity in the kidney (42).

A published study suggests that IL-17 may be important for driving NK cell function in disseminated infection through a mechanism dependent on GM-CSF-mediated neutrophil killing of *C. albicans* (8). Subsequently, a recent report has highlighted the essential role of type I IFN-driven IL-15 from inflammatory monocytes in inducing GM-CSF production from NK cells, necessary to enhance anti-*Candida* activity of neutrophils (43). However, discrepancies exist regarding the role of NK cells in disseminated infection. Quintin et al. showed that NK cells are redundant for antifungal defense in immunocompetent hosts (44). In fact, NK-depleted animals showed increased survival compared with the control group. Additionally, NK-depleted mice with severe combined immunodeficiency did not show enhanced susceptibility to systemic *C. albicans* infection (45). Although NK cells lack direct candidacidal capacity (46, 47), these cells can activate phagocytes by secreting IFN γ (48, 49). Interestingly, *C. albicans* suppresses NK cell function via induction of myeloid-derived suppressor cells, thus questioning the functional stability of NK cells in this system (50, 51). It is still possible that NK cells contribute to neutrophil-mediated early fungal clearance in the kidney, as reported by Bär et al. (8). However, experimental data showing a head-to-head comparison between cells of hematopoietic and nonhematopoietic origin deficient in IL-17R in anti-*Candida* immunity are currently lacking. Using BM chimera mice, we now show that the contribution of IL-17R signaling in hematopoietic cells is quite minimal in antifungal immunity. Accordingly, mice deficient in IL-17RA signaling in RTEC exhibited normal NK cell function, indicating that increased susceptibility of *Il17ra*^{ΔRTEC}

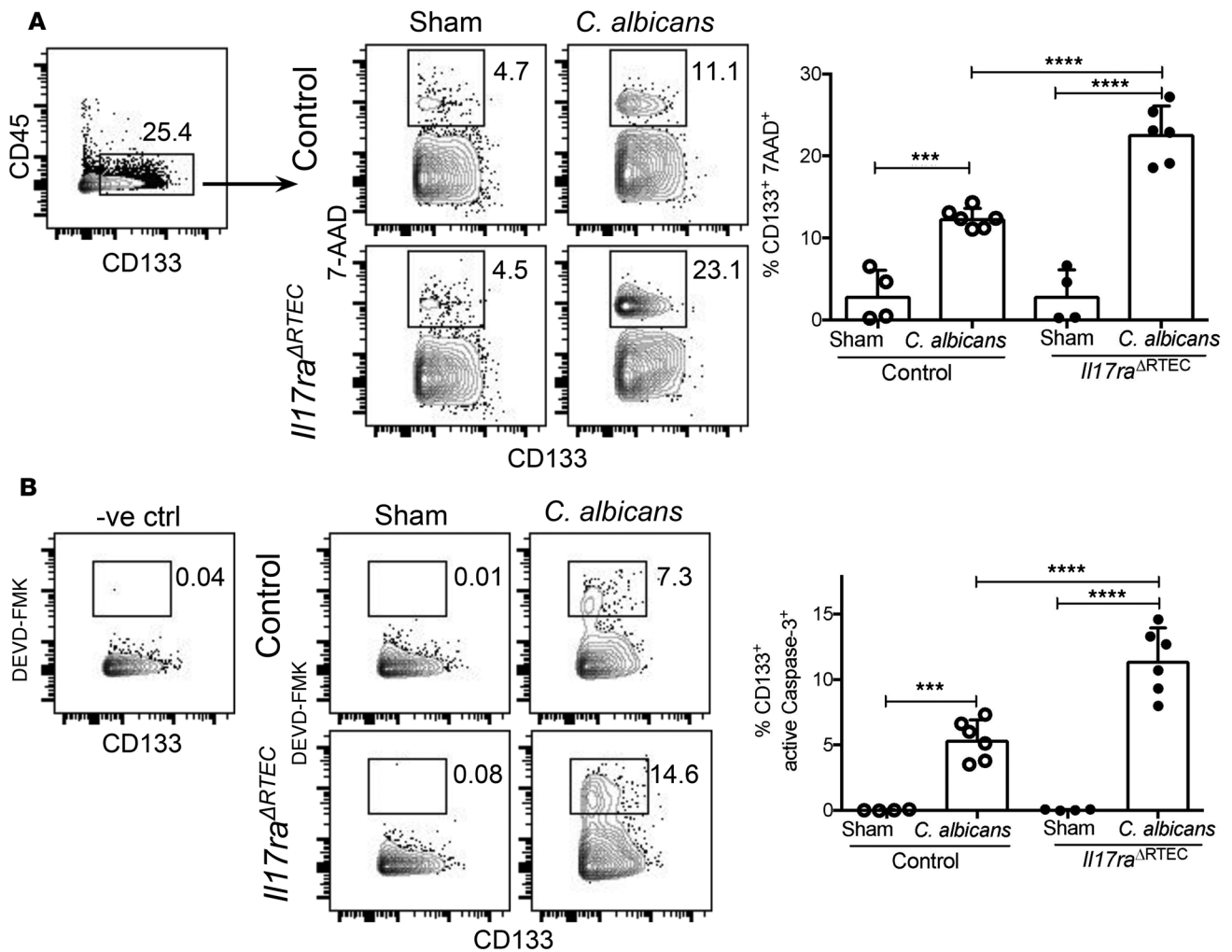


Figure 6. Increased apoptosis of RTEC in the absence of IL-17RA signaling following disseminated candidiasis. Control and *Il17ra*^{ΔRTEC} mice were subjected to systemic *C. albicans* infection ($n = 6$) or left uninfected ($n = 4$). The percentages of (A) apoptotic kidney-resident tubular epithelial cells (gated on CD45⁺CD133⁺ cells) were detected by 7-AAD staining, and (B) percentages of active Caspase-3⁺ kidney-resident tubular epithelial cells (gated on CD45⁺CD133⁺ cells) were quantified by DEVD-FMK staining. Numbers in the contour plot reflect percentages of cells. In the dot plots, each dot represents an individual mouse, and data are represented as mean \pm SD. Pooled data are from 2 independent experiments and analyzed by 1-way ANOVA. *** $P < 0.001$; **** $P < 0.0001$.

mice to disseminated infection is not due to NK cell defect. Although *Il17ra*^{-/-} mice demonstrated a defect in NK cell function, we were unable to detect any IFN γ or GM-CSF production from NK cells following systemic *C. albicans* infection. The apparent discordant between our findings and others are poorly understood. It is possible that differences in the microbiome between animal colonies may account for the dissimilarities observed and requires careful consideration in the future.

RTEC constitute the major cell types (70%) in the renal cortex, the site for hyphal invasion and immune response in the course of a systemic infection (34). RTEC express various pattern recognition and cytokine receptors and are, thus, uniquely positioned to interact with both pathogen and immune cells (34). We previously showed that RTEC are responsive to IL-17 (14). IL-17 in synergy with TNF α induced the expression of Klk1 in RTEC, but not in glomerular and vascular cells (9). However, there is a paucity of data using targeted genetic approaches that can identify specific cell types responsible for Klk1 production. We now show that mice with conditional deletion of IL-17RA in RTEC express diminished Klk1 and succumb to infection earlier than control animals. Interestingly, *Il17ra*^{-/-} mice demonstrated a more severe phenotype than mice with conditional deletion of IL-17RA in RTEC. Since expression of the *Cdh16* promoter varies between RTEC of proximal and distal tubules, it is possible that *Cdh16*^{Cre} recombines and deletes IL-17RA in the majority but not all RTEC (35). Additionally, the absence of IL-17R signaling in podocytes, an epithelial cell type in

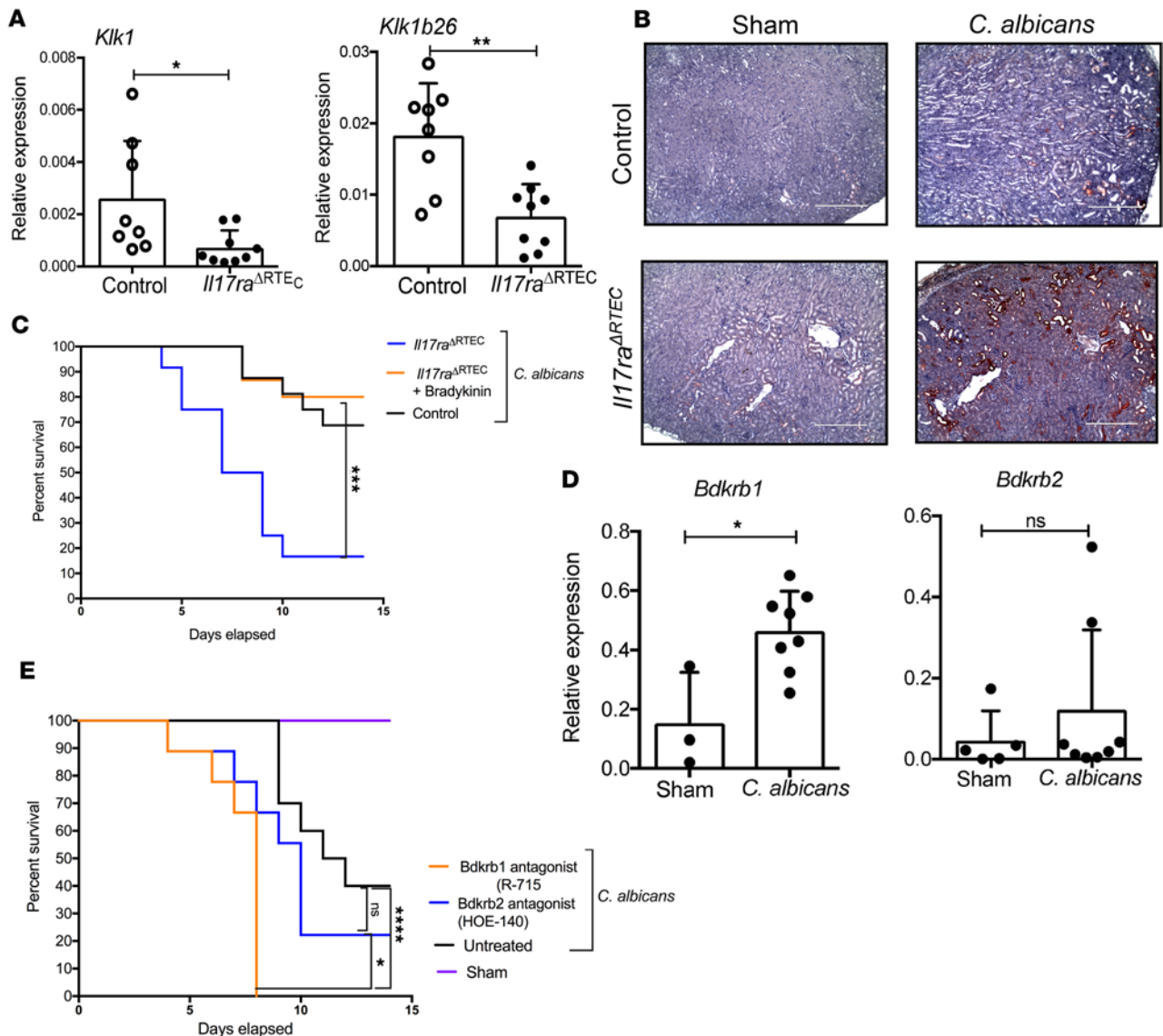


Figure 7. Diminished activation of KKS in the *C. albicans*-infected kidney of *Il17ra*^{ΔRTEC} mice. Control and *Il17ra*^{ΔRTEC} mice ($n = 8-9$) were subjected to systemic *C. albicans* infection. **(A)** At day 2 p.i., renal transcript expression of *Kik1* and *Kik1b26* was measured by qPCR. Each dot represents an individual mouse, and the data are represented as mean \pm SD. Data were pooled from 2 independent experiments and analyzed by 2-tailed Student's *t* test. **(B)** Serial kidney sections ($n = 6$) were stained for Kik1. Original magnification, 100 \times ; scale bars: 400 μ m. Representative photomicrographs from 2 independent experiments. **(C)** *Il17ra*^{ΔRTEC} mice ($n = 12-15$) were treated \pm bradykinin (300 nmol/kg/day) starting day -1 (relative to infection) and then daily over 14 days. Control mice were left untreated. Both *Il17ra*^{ΔRTEC} and control mice were infected systemically with *C. albicans*. Sham mice were treated \pm bradykinin ($n = 3-5$). Survival was assessed over 14 days. Data are pooled from 4 independent experiments and analyzed by Log-rank (Mantel Cox) test. **(D)** WT mice ($n = 3-8$) were subjected to systemic *C. albicans* infection. At day 2 p.i., renal transcript expression of *Bdkrb1* and *Bdkrb2* was quantified by qPCR. Each dot represents individual mouse, and data are represented as mean \pm SD. Data are pooled from 2 independent experiments and analyzed by 2-tailed Student's *t* test **(E)** WT mice ($n = 10-11$) were treated with either *Bdkrb1* (R-715; 1 mg/kg/day) or *Bdkrb2* (HOE-140; 1 mg/kg/day) antagonists or PBS starting day -1 relative to infection and then daily over 14 days. Sham mice were treated with the antagonists only ($n = 3$). Mice were evaluated for survival over 14 days. The data are pooled from 2 independent experiments and analyzed by Log-rank (Mantel Cox) test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

glomerulus, has no impact on antifungal immunity in the kidney. Nevertheless, we do not rule out the contribution of IL-17R signaling on other cell types in renal protection against disseminated candidiasis. Therefore, additional studies using mice with conditional deletion of *Kik1* in RTEC warrants careful consideration.

We show that IL-17RA expression in RTEC plays a minor yet distinguishable delayed role in the renal fungal clearance. This data is in stark contrast to *Il17ra*^{-/-} mice, indicating that global IL-17RA signaling, but not RTEC-specific IL-17RA signaling, contributes to control of early fungal growth following systemic *C. albicans* infection. How IL-17RA signaling in RTEC drives kidney fungal clearance at a delayed time

point is poorly understood. Our data clearly show that antimicrobial peptides such as β -defensin 1 and S100a8/9 were significantly downregulated in the kidneys of fungal-infected *Il17ra*^{RTEC} mice. It is possible that IL-17R signaling in non-RTEC cell types may drive neutrophil influx and early fungal clearance, while late fungal control is mediated by IL-17–driven expression of AMPs from RTEC. Future studies beyond the scope of this manuscript should focus on the sequential events in the kidney that drive early and late fungal clearance in an IL-17–dependent manner.

Our data indicate that *Bdkrb1* activation is required for antifungal defense in systemic *C. albicans* infection. This observation is in line with the CNS protective effect of *Bdkrb1* in IL-17–dependent experimental autoimmune encephalomyelitis (52, 53). However, these results are quite surprising in context with kidney diseases, since *Bdkrb1* activation aggravates renal damage following acute and chronic injury (54–56). It is also not clear why activation of *Bdkrb2*, which is constitutively expressed in the kidney and is associated with protection against kidney damage, showed a minimal effect on renal defense against systemic infection (57, 58). Follow-up studies to dissect signaling events downstream of *Bdkrb1* activation in the kidney are required to investigate these unanswered questions. Chiefly, understanding how *Bdkrb1* activation alters pro- vs. antiapoptotic signaling events in RTEC following fungal infection may pave the path for developing novel therapeutic approaches to prevent RTEC death and renal insufficiency in the infectious settings.

Overall, our study demonstrates that RTEC-specific IL-17R signaling is critical in regulating KKS in vivo — particularly *Klk1* expression and subsequent protection against renal damage in the kidney. Thus, targeting this molecular pathway may provide clinical benefits for patients with disseminated candidiasis in conjunction with antifungal therapy. We also show the essential role of *Bdkrb1* in conferring renal protection downstream of the IL-17/*Klk1* axis. These observations have potential translational implications, as selective agonists of the *Bdkrb1* are in clinical trials. Thus, these data can guide decisions in selecting appropriate pharmacological bradykinin receptor agonists for treating disseminated infection without compromising safety and efficacy.

Methods

Mice. C57BL/6J (WT) and *Cdh16*^{Cre} mice were purchased from the Jackson Laboratory. *Il17ra*^{-/-} mice were provided by Amgen and bred in-house. *Il17ra*^{fl/fl} mice were provided by Jay Kolls (University of Pittsburgh, Pittsburgh, Pennsylvania, USA) (27). *Il17*^{Cre} mice, created by Brigitta Stockinger (The Francis Crick Institute, London, United Kingdom), were crossed to Rosa26eYFP (The Jackson Laboratory) (28). All the experiments included age- and sex-matched controls.

Mouse model of disseminated candidiasis. *C. albicans* (strains CAF2-1 or HUN96) (provided by Sarah Gaffen, University of Pittsburgh) was grown on yeast extract peptone dextrose (YPD) plates (MilliporeSigma) at 30°C for 18–24 hours. *C. albicans* yeast cells were suspended in sterile PBS, and mice were injected via the lateral tail vein with PBS (sham-infected) or 1×10^5 CFU *C. albicans* (CAF2-1) or 5×10^5 CFU *C. albicans* (HUN96). Mice were weighed and monitored daily. Mice were sacrificed if they showed >20% weight loss or signs of severe pain or distress.

Evaluation of fungal burden in the kidney. At sacrifice on days 2 and 5 p.i., kidneys were weighed and homogenized in sterile PBS, using a GentleMACS (Miltenyi Biotec). Serial dilutions of organ homogenates were plated on YPD agar with antibiotics (MilliporeSigma), and fungal burden was represented as CFU per gram of tissue.

Measurement of serum BUN. Serum was collected by retro-orbital bleeding at day 7 p.i. Serum BUN levels were measured using Blood Urea Nitrogen Enzymatic kit (Bio Scientific Corp.).

Spleen and kidney cell suspensions. Spleens were harvested from mice and subjected to mechanical dissociation to prepare single cell suspensions, followed by RBC lysis by Mouse Erythrocyte Lysing Kit (R&D Systems). For kidneys, mice were perfused with PBS containing EDTA (MilliporeSigma) before harvesting the organ. Kidneys were cut into small pieces and digested at 37°C in 1 mg/ml collagenase IV (Worthington) in complete RPMI for 30 minutes. Cells were filtered through 70-mm cell strainers and washed twice in PBS.

Bradykinin and bradykinin receptor antagonists. Mice were injected i.p. with 200 l of bradykinin: 300 nmol/kg/day (R&D Systems) 1 day prior to infection and then daily for 14 days (9). Mice received i.p. injection of *Bdkrb1* antagonist (R-715, 1 mg/kg/day, R&D Systems), *Bdkrb2* antagonist (HOE-140, 1 mg/kg/day, R&D Systems) starting day -1 (relative to infection) and daily for 14 days, as described previously (9). Control mice received equal volume of PBS.

H&E and immunohistochemical staining. For kidney histology, formalin-fixed paraffin embedded kidney sections were subjected to H&E staining.

Immunohistochemical staining was done on formalin-fixed paraffin embedded sections. Sections were rehydrated, and antigen retrieval was performed with heated citrate. Primary antibodies against the following proteins were used: Klk1 (LS-C312857-100, LifeSpan Biosciences), NGAL (AF1757, R&D Biosystems), and KIM1 (Ab47635, Abcam). Secondary antibodies used were horseradish peroxidase-coupled antibodies (705-035-003 and 111-035-144, Jackson ImmunoResearch). Images were obtained with EVOS FL Auto microscope (Invitrogen).

Flow cytometry. At sacrifice on days 2, 5, or 7 p.i., as indicated in the text, kidneys were harvested following perfusion with PBS. The single cell suspensions were slowly layered over 5-ml Lympholyte (Cedarlane). The tubes were spun for 1,300 g for 30 minutes at room temperature. The cell layer at the interface of the media and Lympholyte was collected and washed twice with PBS and used for FACS staining. To identify the proliferating IL-17-producing cells, cells were stained with the following fluorescent conjugated antibodies: CD45 (30-F11, BioLegend), TCR β (H57-597, BioLegend), and TCR δ (GL3, BioLegend) and CD4 (GK1.5, eBiosciences), followed by intracellular staining with Ki-67 (B56, BD Biosciences). For flow cytometry staining of kidney-infiltrating inflammatory cells and tubular epithelial cells, cell suspensions were stained with antibodies against CD45 (30-F11), Ly6G (IA8; 1A8-Ly6g), CD11b (M1/70), F4/80 (BM8), CD3 (145-2C11), and NK1.1 (PK136; all from eBiosciences). For the detection of IFN γ production by NK cells in spleen suspensions, cells were first stained with antibodies against CD45, CD3 (145-2C11), CD69 (H1.2F3), NKp46 (29A1.4), and CD49b (DX5; all from BioLegend) and were subsequently stained with anti-IFN γ and anti-GM-CSF antibodies (BD Pharmingen) after fixation and permeabilization using intracellular cytokine staining kit (BD Pharmingen, San Jose CA). For the detection of IL-17RA expression by NK cells in BM cell suspensions, cells were stained with antibodies against CD45, CD3, CD122 (TM- β 1), CD94 (18d3), and CD49b (all from BioLegend) and IL-17R (PAJ-17R, Thermo Fisher Scientific). Data were acquired on a BD LSRFortessa cytometer (BD Biosciences) and analyzed with FlowJo (Tree Star Inc.).

To determine the frequency of dead and active Caspase3⁺ cells, perfused kidneys were harvested at day 7 p.i. Single cell suspension were prepared by treating minced kidney tissues in 1 mg/ml collagenase IV (Worthington) in complete RPMI for 30 minutes. Total kidney cells were surface stained with anti-CD45 and anti-CD133 antibodies (13A4, eBiosciences). To detect apoptotic cells and active Caspase-3 expression, cells were stained with 7-AAD (BD Pharmingen) and CaspGLOW Fluorescein active Caspase-3 staining kit (Thermo Fisher Scientific), respectively, as per the manufacturer's instructions.

For determination of absolute numbers of IL-17-producing cells, 50 μ l of CountBright absolute counting beads (Thermo Fisher Scientific) were added to 400 μ l stained cell samples. The samples were analyzed on BD LSRFortessa cytometer (BD Biosciences). The absolute number IL-17-producing cells in samples were calculated by comparing the ratio of bead events to cell events.

RNA extraction and real-time PCR. For real-time PCR analyses, RNA from homogenized kidneys of sham or infected mice was extracted using the RNeasy Mini kit (Qiagen). RNA was reverse-transcribed using the Superscript III First-strand kit (Invitrogen). Real-time PCR was performed using PerfeCTa SYBR Green FastMix ROX (Quanta BioSciences) on a 7300 Real-Time PCR System (Applied Biosystems). Primers were purchased from Quantitect (Qiagen). The expression of each gene was normalized to *Gapdh*.

Statistics. Data are presented as mean \pm SD, as indicated in the legend of each figure. The significance of the differences between groups was evaluated using unpaired 2-tailed *t* test or 1-way ANOVA wherever appropriate; $P < 0.05$ was considered significant. Survival curve data are presented as Kaplan-Maier plots with a log rank test to compare susceptibility between experimental groups. In figures, asterisks are used to denote statistical significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). Statistical analysis was performed in GraphPad PRISM 7. All experiments were performed at least twice to ensure reproducibility.

Study approval. The animal protocols used in this work were evaluated and approved by the University of Pittsburgh IACUC (protocols 14094427 and 14043544) and adhered to the guidelines in the *Guide for the Care and Use of Laboratory Animals* of the NIH (National Academies Press, 2011).

Author contributions

KR, CVJ, AHV, BMC, and PSB conducted experiments and acquired and analyzed data; JKK provided reagents; KR and PSB designed research studies and wrote the manuscript with input from all of the coauthors.

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