

BRIEF REPORT

## Batf3-dependent CD103<sup>+</sup> dendritic cell accumulation is dispensable for mucosal and systemic antifungal host defense

Timothy J. Break<sup>a</sup>, Kevin W. Hoffman<sup>b</sup>, Muthulekha Swamydas<sup>a</sup>, Chyi-Chia Richard Lee<sup>c</sup>, Jean K. Lim<sup>b</sup>, and Michail S. Lionakis<sup>a</sup>

<sup>a</sup>Fungal Pathogenesis Unit, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; <sup>b</sup>Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; <sup>c</sup>Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

### ABSTRACT

Dendritic cells (DCs) are critical for defense against a variety of pathogens and the formation of adaptive immune responses. The transcription factor Batf3 is critical for the development of CD103<sup>+</sup>CD11b<sup>-</sup> DCs, which promote IL-12-dependent protective immunity during viral and parasitic infections, dampen Th2 immunity during helminth infection, and exert detrimental effects during bacterial infection. Whether CD103<sup>+</sup> DCs modulate immunity during systemic or mucosal fungal disease remains unknown. Herein, we report that Batf3 is critical for accumulation of CD103<sup>+</sup> DCs in the kidney and tongue at steady state, for their expansion during systemic and oropharyngeal candidiasis, and for tissue-specific production of IL-12 in kidney but not tongue during systemic and oropharyngeal candidiasis, respectively. Importantly, deficiency of CD103<sup>+</sup> DCs does not impair survival or fungal clearance during systemic or oropharyngeal candidiasis, indicating that Batf3-dependent CD103<sup>+</sup> DC accumulation mediates pathogen- and tissue-specific immune effects.

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## Introduction

Systemic candidiasis, caused by invasion of the commensal yeast *Candida* into the bloodstream, is a leading cause of nosocomial bloodstream infections in the United States.<sup>1</sup> Importantly, infected patients have a 30 – 40% mortality rate, despite antifungal therapy,<sup>2</sup> making systemic candidiasis an important unmet medical condition. Mucosal candidiasis, although not life-threatening, is more common than systemic candidiasis worldwide; oral thrush develops in the majority of AIDS patients,<sup>3</sup> and approximately 75% of all women will develop vaginal candidiasis at some point in their lifetime.<sup>4</sup> Furthermore, the emergence of azole- and echinocandin-resistant *Candida* strains makes treatment of these infections more difficult.<sup>5</sup> Therefore, it is important to understand cellular and molecular immunological factors that are necessary for protection against *Candida* infections in order to develop targeted immune-based therapies to improve patient outcomes.


Dendritic cells (DCs) are immune cells essential for the protection of the host against a wide variety of

pathogens and for the generation of both innate and adaptive immune responses.<sup>6</sup> With regard to *Candida*, DCs are able to phagocytose and kill ingested *Candida*, produce cytokines in response to *Candida* stimulation, and present *Candida*-specific antigens *in vitro*.<sup>7,8</sup> However, the importance of DCs during *C. albicans* infection *in vivo* is not well-defined, as only recent studies have attempted to address their role during systemic and mucosal candidiasis.<sup>9–11</sup>

There are several subsets of DCs, including classical, monocyte-derived, and plasmacytoid DCs. The classical DCs can be further subdivided, based on surface marker expression and/or function, with one of these subsets being CD103<sup>+</sup> DCs, which correspond to CD8 $\alpha$ <sup>+</sup> DCs in lymphoid tissues.<sup>6</sup> CD103<sup>+</sup> DCs are thought to be most important for cross-presentation of antigens and production of IL-12, and are dependent on several transcription factors, including Batf3, which is indispensable for their development.<sup>6</sup> CD103<sup>+</sup> DC-dependent IL-12 production can be beneficial or detrimental during infection with the helminths *Schistosoma mansoni* or *Heligmosomoides*

**CONTACT** Michail S. Lionakis, MD, ScD  [lionakism@mail.nih.gov](mailto:lionakism@mail.nih.gov)  National Institute of Allergy and Infectious Diseases, 9000 Rockville Pike, Building 10, Room 11C102, Bethesda, MD 20892, USA.

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*polygyrus*, respectively.<sup>12</sup> Furthermore, CD103<sup>+</sup> DCs are essential for protection against the parasitic protozoans *Cryptosporidium parvum* and *Toxoplasma gondii*<sup>13,14</sup> and during West Nile and Sendai viral infections,<sup>15,16</sup> but detrimental during infection with the intracellular bacterium *Listeria monocytogenes*.<sup>17</sup> No studies to date have determined the *in vivo* role of CD103<sup>+</sup> DCs on innate protection against fungal infections. Therefore, we sought to determine the impact of Batf3-deficiency on susceptibility to the commensal fungus, *C. albicans*, in both systemic and oral infection models in mice.

## Results

We first investigated the role of CD103<sup>+</sup> DCs in mediating control of systemic *C. albicans* infection by employing the well-established mouse model of systemic candidiasis in *Batf3*<sup>-/-</sup> mice. The kidney is the target organ in the model, which is thought to mimic skin-derived disease in humans.<sup>2</sup> Although CD103<sup>+</sup> (or CD8α<sup>+</sup>) DCs are absent in several peripheral organs of *Batf3*<sup>-/-</sup> mice, including the lung, intestines, lymph nodes, dermis, spleen, thymus, and liver,<sup>12,15,16</sup> they have been shown to develop in the absence of Batf3 in the spleens and lungs during infection with intracellular pathogens, in the spleen upon IL-12 administration, or in the spleen and lymph nodes during short-term bone marrow reconstitution; Batf3-independent, Batf/Batf2-dependent mechanisms have been implicated in some of these settings.<sup>18,19</sup> Therefore, we first examined whether CD103<sup>+</sup> DCs are present in the *Batf3*<sup>-/-</sup> kidney both prior to, and during *C. albicans* infection. We utilized flow cytometry and defined this population as CD45<sup>+</sup>MHCII<sup>high</sup>F4/80<sup>-</sup>CD11c<sup>high</sup>CD103<sup>+</sup>CD11b<sup>-</sup> cells (Figure S1A), as previously described.<sup>20</sup> CD103<sup>+</sup> DCs were present in the *Batf3*<sup>+/+</sup> kidney, and their number significantly increased during *C. albicans* infection ( $p < 0.001$ ) (Fig. 1A–C). In contrast, *Batf3*<sup>-/-</sup> kidneys were devoid of CD103<sup>+</sup> DCs both under homeostatic conditions and during infection, indicating that CD103<sup>+</sup> DC development is strictly Batf3-dependent in this organ in the model (Fig. 1C); the paucity of CD103<sup>+</sup> DCs was further highlighted by the decreased percentage of CD103<sup>+</sup> DCs out of all DCs in uninfected and infected *Batf3*<sup>-/-</sup> kidneys (Fig. 1D). In contrast, Batf3 deficiency did not significantly affect the accumulation of other myeloid cells (CD11b<sup>+</sup>CD103<sup>-</sup> DCs, neutrophils, macrophages, Ly6C<sup>high</sup> monocytes) in the infected kidney (Figure S2A). These data show that *Batf3*<sup>-/-</sup> kidneys lack CD103<sup>+</sup> DCs both under homeostatic conditions and during systemic *C. albicans* infection.

To determine whether lack of renal CD103<sup>+</sup> DCs affects susceptibility to systemic candidiasis, mice were

injected intravenously with 10<sup>5</sup> *C. albicans* and survival was monitored for 4 weeks. We found no difference between wild-type and *Batf3*<sup>-/-</sup> mice with regard to survival (Fig. 1E), control of fungal proliferation in the kidney (Fig. 1F), or serum creatinine levels (Fig. 1G), which is an indicator of kidney damage. In addition, no differences were noted in the histological examination of *C. albicans*-infected *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> kidneys (Fig. 1H). Because *Candida* strain-specific differences in antifungal immune responses have been reported *in vivo*,<sup>21</sup> we infected *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> with another strain isolated from a patient with systemic candidiasis (UC820),<sup>20</sup> and found no increased susceptibility of *Batf3*<sup>-/-</sup> mice, as shown by similar survival (Figure S3A) and CFUs at day 4 p.i (Figure S3B). Taken together, these data show that, although Batf3 is critical for renal accumulation of CD103<sup>+</sup> DCs during systemic candidiasis, their deficiency does not impair the control of the infection *in vivo*.

We next sought to examine the *in vivo* role of Batf3-dependent CD103<sup>+</sup> DCs during oropharyngeal candidiasis (OPC), as the immune factors that mediate protection during systemic versus mucosal *Candida* infections are typically non-overlapping.<sup>22</sup> As with the kidney, we used flow cytometry to first determine whether Batf3 is indispensable for accumulation of CD103<sup>+</sup> DCs in the mouse tongue (see Figure S1B for gating strategy). Indeed, we found a small population of CD103<sup>+</sup> DCs in the uninfected tongue of *Batf3*<sup>+/+</sup> mice that was absent in *Batf3*<sup>-/-</sup> tongues (Fig. 2A–C). We then used a previously reported sublingual *C. albicans* infection model,<sup>23,24</sup> and found a significant expansion in the number and percentage of CD103<sup>+</sup> DCs out of all DCs in *Batf3*<sup>+/+</sup> tongues ( $p < 0.01$  and  $p < 0.0001$ , respectively), while CD103<sup>+</sup> DCs remained absent in the tongue of *Batf3*<sup>-/-</sup> mice during fungal infection (Fig. 2C–2D). As with the kidney, Batf3 deficiency did not impair the accumulation of other myeloid cells (CD11b<sup>+</sup>CD103<sup>-</sup> DCs, neutrophils, macrophages, Ly6C<sup>high</sup> monocytes) in the infected tongue (Figure S2B). Collectively, these data show that, similar to the kidney, *Batf3*<sup>-/-</sup> tongues lack CD103<sup>+</sup> DCs both under homeostatic conditions and during mucosal *C. albicans* infection.

To determine whether lack of oral mucosal CD103<sup>+</sup> DCs plays a role during OPC, *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice were sublingually infected with *C. albicans*, and fungal load and histology were examined after infection. We found no difference in *C. albicans* growth at day 1 early post-infection (p.i.) between wild-type and *Batf3*<sup>-/-</sup> tongues. By day 5 p.i., the vast majority of *C. albicans* was cleared from both *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> tongues (Fig. 2E). In addition, the extent of epithelial involvement by abscess formation was similar between

*Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice at day 1 p.i. (Fig. 2F-H); abscesses were localized in the epithelial layer without penetration into deeper tissues and were cleared in both strains of mice by day 5 p.i. (Fig. 2G-H). Furthermore, *Batf3*<sup>-/-</sup> mice did not exhibit increased fungal

proliferation in the tongue at day 5 p.i., compared with *Batf3*<sup>+/+</sup> mice, when we used the *C. albicans* strains 529L (Figure S4A) and Y72 (Figure S4B), which were obtained from patients with oral candidiasis.<sup>23,25</sup> Taken together, these data show that although *Batf3* is critical

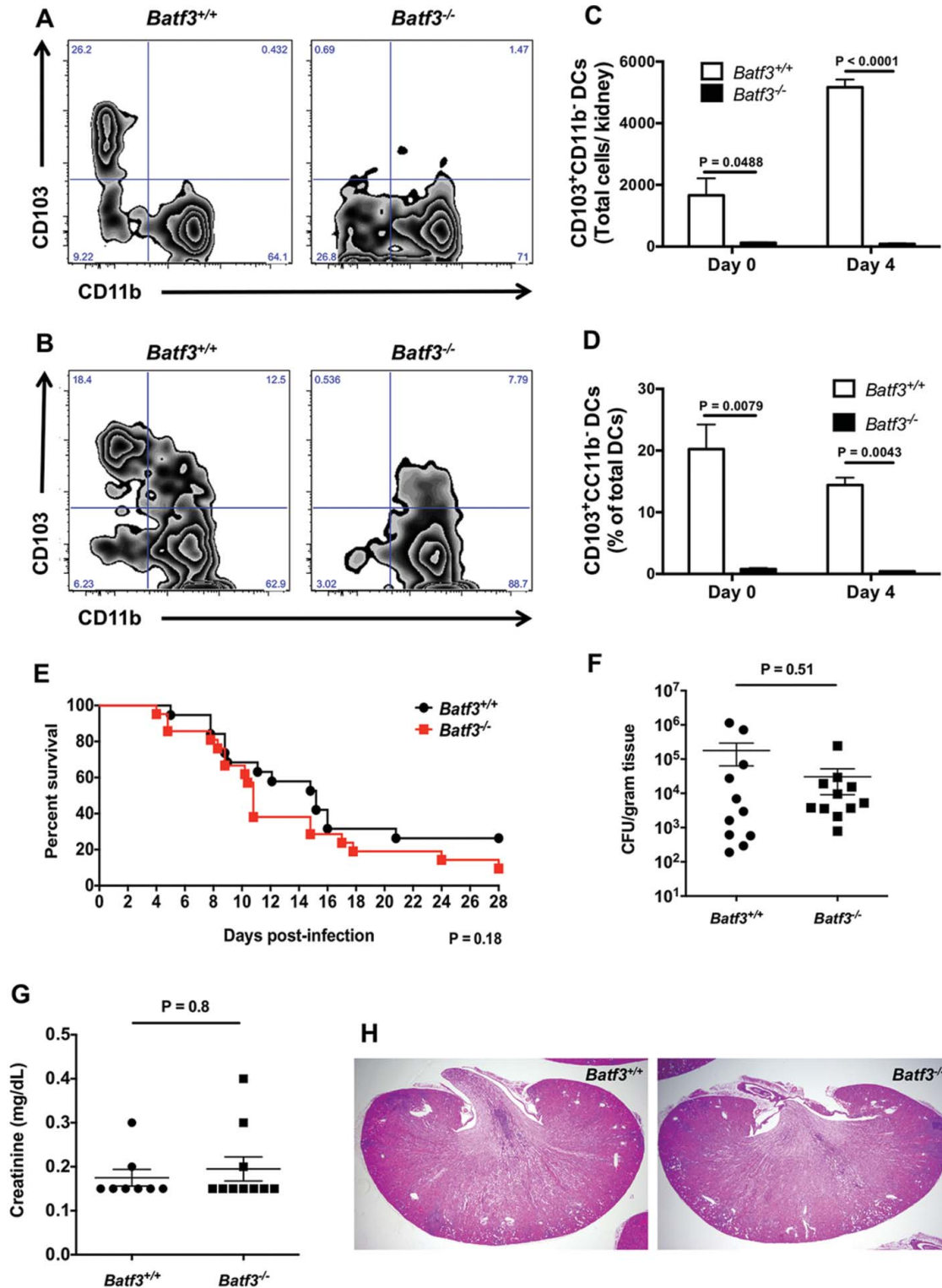


Figure 1. (For figure legend, see page 829.)

for accumulation of CD103<sup>+</sup> DCs in the oral mucosa, these cells are dispensable for protection against OPC *in vivo*.

As CD103<sup>+</sup> DCs are known to produce a wide array of cytokines and chemokines upon recognition of microbial ligands, we measured levels of 13 cytokines and chemokines via Luminex array in kidney and tongue homogenates during systemic and mucosal infection with *C. albicans*, respectively. We found that CD103<sup>+</sup> DCs are not major cellular sources for production of IL-1 $\beta$ , IL-4, IL-6, IL-17A, IL-22, CCL2, CCL3, CCL4, CCL5, CXCL1, or CXCL2 in the kidney and tongue during systemic and mucosal candidiasis, respectively (Tables 1 and 2). Instead, in the tongue, the anti-inflammatory cytokine, IL-10, was ~35% reduced in *Batf3*<sup>-/-</sup> mice after infection (Table 2). Importantly, production of both IL-17A and IL-22, which is essential for control of OPC,<sup>26</sup> was not impaired in *Batf3*-deficient tongues.

A cytokine that has been shown to be integrally dependent on the presence of CD103<sup>+</sup> DCs is IL-12.<sup>14,27</sup> Interestingly, we found that IL-12p70 production was significantly reduced in the kidneys of systemically infected *Batf3*<sup>-/-</sup> mice, whereas IL-12p70 levels were similar in *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> tongues during mucosal *Candida* infection (Fig. 3A–B). Therefore, these data indicate that IL-12 production by CD103<sup>+</sup> DCs, in the setting of *Candida* infection, is tissue-specific.

## Discussion

In the present study, we examined for the first time the *in vivo* role of *Batf3*-dependent CD103<sup>+</sup> DCs in host defense during systemic and mucosal fungal infections in mice. During parasitic, viral and bacterial infection, CD103<sup>+</sup> DCs have been reported to play crucial roles, whether protective or deleterious. Specifically, CD103<sup>+</sup> DCs are essential for control of intestinal parasitic infections caused by *Toxoplasma gondii*<sup>14</sup> and

*Cryptosporidium parvum*,<sup>13</sup> for mediating protective immunity against viruses, and for control of syngeneic fibrosarcomas.<sup>15,16</sup> On the other hand, during infection with the intracellular bacterium *Listeria monocytogenes*, CD103<sup>+</sup> DCs promote the development of disease by serving as an essential pathogen entry point.<sup>17</sup> Interestingly, CD103<sup>+</sup> DCs play differential roles during helminthic infections; they are protective during infection with *Schistosoma mansoni*, but deleterious during infection with *Heligmosomoides polygyrus*.<sup>12</sup> Our data demonstrate that CD103<sup>+</sup> DCs are redundant for the control of mucosal and systemic infection with *C. albicans*, indicating that *Batf3*-dependent CD103<sup>+</sup> DCs promote pathogen-specific immune responses. Future studies will be required to evaluate whether CD103<sup>+</sup> DCs mediate anti-fungal host defense during infection caused by other fungi such as dimorphic fungi or ubiquitous inhaled molds.

It has been shown that protective IL-12 production is highly dependent on CD103<sup>+</sup> DCs during infection with *Toxoplasma gondii*<sup>14</sup> and *Encephalitozoon cuniculi*.<sup>27</sup> Furthermore, CD103<sup>+</sup> DC-dependent production of IL-12 exacerbates chronic intestinal infection with *Heligmosomoides polygyrus*, and ameliorates IL-13-mediated liver fibrosis during infection with *Schistosoma mansoni*.<sup>12</sup> We show here that IL-12 production is dependent on CD103<sup>+</sup> DCs during systemic, but not oral *C. albicans* infection. Decreased IL-12 production in the kidney during systemic candidiasis in *Batf3*<sup>-/-</sup> mice does not impair susceptibility to the infection, in agreement with the redundant role of IL-12 in the control of systemic candidiasis.<sup>28</sup> Instead, while IL-12 is known to mediate early control of OPC,<sup>29</sup> our data show that *Batf3*-dependent CD103<sup>+</sup> DCs are dispensable for IL-12 production during OPC and infection control at the oral mucosa. These findings further highlight the dichotomy of immune factors that are necessary for control of mucosal vs. systemic candidiasis and underscore the organ-specific

**Figure 1.** (see previous page) The transcription factor *Batf3* is critical for the accumulation of CD103<sup>+</sup> DCs in the mouse kidney but dispensable for protection against systemic candidiasis. (A–D) Kidney leukocytes were isolated from *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice left uninfected or infected intravenously with *C. albicans* 4 d prior to harvest, and analyzed using flow cytometry. (A–B) Representative zebra FACS plots from uninfected mice (A) or *Candida*-infected mice at day 4 p.i. (B) after gating on live CD45<sup>+</sup>MHCII<sup>high</sup>F4/80<sup>-</sup>CD11c<sup>+</sup> DCs, and plotting based on CD103 and CD11b expression. (C) Total number of CD103<sup>+</sup>CD11b<sup>-</sup> DCs in the uninfected and *Candida*-infected kidney. (D) Percentage of CD103<sup>+</sup>CD11b<sup>-</sup> cells within all DCs in the uninfected and *Candida*-infected kidney. (E) Survival of *Candida*-infected *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice. (F) Kidney fungal burden in *Candida*-infected *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice determined at day 4 p.i. as the number of CFUs/gram of tissue. (G) Serum creatinine levels of *Candida*-infected *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice at day 4 p.i. (H) Representative H&E stained kidney sections from *Candida*-infected *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice at day 4 p.i. Data in (C, D, F, and G) were analyzed using unpaired t-tests or Mann-Whitney U tests, where appropriate. Data in (E) were analyzed using a log-rank (Mantel-Cox) test. Data in (A–D) are representative FACS plots or combined data from 2 independent experiments with a total n of 5–6 mice/group. Data in (E) are combined from 2 independent experiments with a total n of 19–21 mice/group. Data in (F–G) are combined from 2–3 independent experiments with a total n of 8–11 mice/group. Histology sections in (H) are representative images from 2 independent experiments with a total of 4–5 mice/group.

nature of the cellular immune response against *Candida* challenge. Future studies should aim to define the organ-specific microenvironment cues that dictate

Batf3-dependent CD103<sup>+</sup> DC-mediated cytokine production and determine the cellular source(s) of IL-12 in the *Candida*-infected oral mucosa.

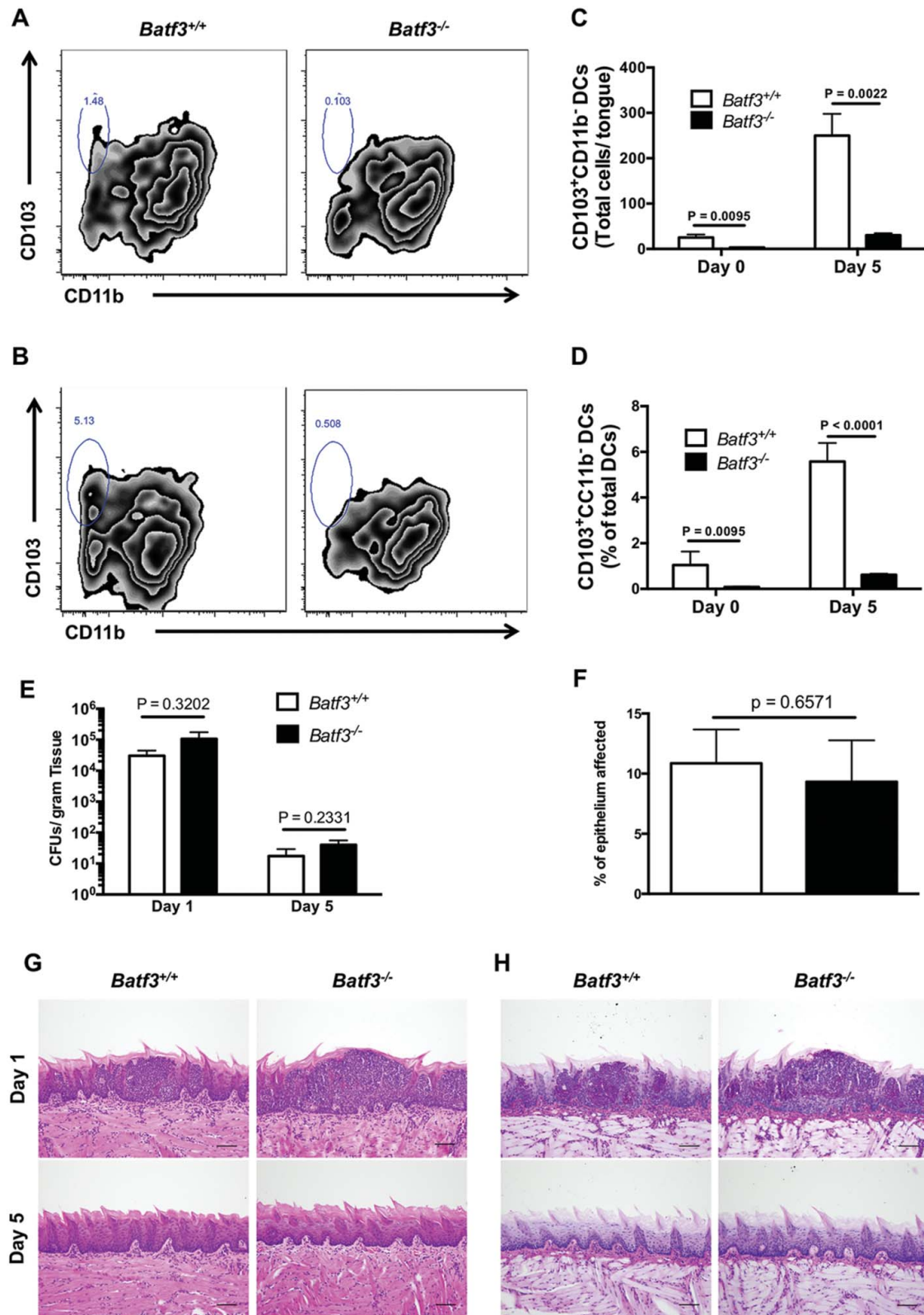


Figure 2. (For figure legend, see page 831.)

It has been shown that CD103<sup>+</sup> DCs can expand in a Batf3-independent manner in the spleens and lungs during intracellular bacterial infection, in the spleen upon IL-12 administration, or in the spleen and lymph nodes during short-term bone marrow reconstitution; Batf/Batf2 have been shown to promote Batf3-independent DC expansion in some of these conditions.<sup>18,19</sup> Our data show that CD103<sup>+</sup> DC development is strictly Batf3-dependent in the kidney and tongue both at steady-state and during acute *C. albicans* infection. More work will be required to define whether generation of CD103<sup>+</sup> DCs is Batf3-dependent in other *Candida*-infected tissues and during infection with other fungal pathogens, and to examine the tissue- and pathogen-specific cues that modulate Batf3-dependent and independent CD103<sup>+</sup> DC development.

Besides their non-redundant roles in the innate control against infection, including systemic candidiasis,<sup>9</sup> DCs have well-characterized adaptive functions via presentation of antigens to T cells for the development of adaptive immune responses. Interestingly, on one hand, CD103<sup>+</sup> DCs were not necessary for priming of Th17 cells during primary *Candida* infection.<sup>10</sup> Instead, a recent elegant study found that, during a secondary *Candida* infection, Batf3<sup>-/-</sup> mice had elevated Th17 responses and decreased CFUs in the skin upon epicutaneous priming and secondary epicutaneous challenge. This effect was not seen in the kidney upon epicutaneous priming and secondary systemic challenge,<sup>11</sup> underscoring the compartmentalized effects of CD103<sup>+</sup> DCs during a secondary fungal infection. Therefore, future research should elucidate the role of CD103<sup>+</sup> DCs in antifungal host defense during secondary and chronic *Candida* infection via oral, gastrointestinal, or systemic challenges.

In humans, autosomal recessive IRF8 deficiency predisposes to OPC<sup>30</sup> and is associated with complete lack of all monocyte and DC subsets, including CD141<sup>+</sup> DCs, which correspond to mouse CD103<sup>+</sup>CD11b<sup>-</sup> DCs.<sup>31</sup> Since we show that Batf3<sup>-/-</sup>

mice are not susceptible to OPC, our data suggest that monocytes and/or CD11b<sup>+</sup> DC subsets, not CD103<sup>+</sup>CD11b<sup>-</sup> DCs, are likely important for control of OPC in humans. Mice lacking Irf8 have defective development of CD8α<sup>+</sup> DCs, plasmacytoid DCs, and monocytes,<sup>32</sup> and studies should evaluate their susceptibility to OPC and define the mechanisms by which Irf8 promotes mucosal antifungal immunity.

In summary, we show that the transcription factor Batf3 is crucial for CD103<sup>+</sup> DC accumulation in the oral mucosa and kidney at steady state and during fungal infection, and for promotion of tissue-specific IL-12 production during fungal challenge. Importantly, in contrast to bacterial, parasitic and viral infections, we demonstrate that Batf3-dependent tissue accumulation of CD103<sup>+</sup> DCs is redundant for protective host defense during acute mucosal and systemic fungal disease. More work will be required to define the tissue- and pathogen-specific roles of Batf3-dependent CD103<sup>+</sup> DCs in innate and adaptive immunity and to determine the differential roles of resident mononuclear phagocytes other than CD103<sup>+</sup> DCs in mediating protection against mucosal and systemic fungal disease in mice and humans.<sup>9,20</sup>

## Materials and methods

### Mice

Batf3<sup>-/-</sup> mice were a kind gift from Dr. Brian Kelsall (NIAID, NIH) and C57Bl/6 (Batf3<sup>+/+</sup>) mice were purchased from Jackson Laboratories. Batf3<sup>-/-</sup> mice were developed as previously described.<sup>15</sup> Age- and sex-matched mice were used and kept 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 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.

**Figure 2.** (see previous page) Batf3 mediates CD103<sup>+</sup> DC accumulation, but not protection from *Candida* infection, in the mouse oral mucosa. (A–D) Tongue leukocytes were harvested from Batf3<sup>+/+</sup> and Batf3<sup>-/-</sup> mice left uninfected or sublingually infected with *C. albicans* 5 d prior to harvest, and analyzed using flow cytometry. (A–B) Representative zebra FACS plots from uninfected mice (A) or *Candida*-infected mice at day 5 p.i. (B) after gating on live CD45<sup>+</sup>MHCII<sup>high</sup>CD11c<sup>high</sup> DCs, and plotting based on CD103 and CD11b expression. (C) Total number of CD103<sup>+</sup>CD11b<sup>-</sup> DCs in the uninfected and *Candida*-infected tongue. (D) Percentage of CD103<sup>+</sup>CD11b<sup>-</sup> cells within all DCs in the uninfected and *Candida*-infected tongue. (E) Fungal burden in *Candida*-infected Batf3<sup>+/+</sup> and Batf3<sup>-/-</sup> tongue determined at days 1 and 5 p.i., expressed as the number of CFUs/gram of tissue. (F) Percentage of tongue epithelium with abscess formation at day 1 p.i. after oral *Candida* infection. (G–H) Representative images from H&E (G) and PAS (H) stained tongue sections are shown at days 1 (upper panels) and 5 (lower panels) p.i. Data in (C–F) were analyzed using unpaired t-tests or Mann-Whitney U tests, where appropriate. Data in (A–D) are representative FACS plots or combined data from 2 independent experiments with a total n of 4–6 mice/group. Data in (E) are combined from 2 independent experiments with a total n of 6–7 mice/group. Data in (F–H) are representative images or combined data from 2 independent experiments with a total n of 4 mice/group.

**Table 1.** CD103<sup>+</sup> DCs are dispensable for the production of a variety of pro-inflammatory cytokines and chemokines in the kidney during systemic candidiasis.

Cytokine/Chemokine	Batf3 <sup>+/+</sup>	Batf3 <sup>-/-</sup>	P-value
IL-1 $\beta$	10609 $\pm$ 1466	11617 $\pm$ 2468	0.7168
IL-4	3059 $\pm$ 294.6	2839 $\pm$ 500.2	0.6958
IL-6	25941 $\pm$ 5651	60713 $\pm$ 21928	0.1784
IL-10	963.3 $\pm$ 83.55	844.3 $\pm$ 114.5	0.4057
IL-17A	346.7 $\pm$ 18.85	303.5 $\pm$ 45.90	0.3551
IL-22	107.9 $\pm$ 12.42	103.2 $\pm$ 19.58	0.8340
CCL2	75973 $\pm$ 8268	50446 $\pm$ 8056	0.0522
CCL3	1386 $\pm$ 312.4	1451 $\pm$ 491.7	0.9084
CCL4	1365 $\pm$ 351.3	1869 $\pm$ 552.5	0.4356
CCL5	9314 $\pm$ 632.8	8031 $\pm$ 1468	>0.9999
CXCL1	8107 $\pm$ 1334	9585 $\pm$ 2284	0.5644
CXCL2	13535 $\pm$ 2550	11766 $\pm$ 2559	0.6405

Notes. Cytokine and chemokine concentrations are expressed as pg/g of kidney at day 4 post-infection. Data represent mean values  $\pm$  SEM and are combined from 2 independent experiments with a total n of 6–8 mice/group. Statistical analysis was performed using an unpaired t-test or Mann-Whitney U test, where appropriate.

### Fungal strains and mouse models of *C. albicans* infection

*C. albicans* strain SC5314 was used in all experiments, unless stated otherwise. In some systemic infection experiments, the *C. albicans* strain UC820, which was isolated from a patient with systemic candidiasis, was used.<sup>20</sup> In some OPC infections, the *C. albicans* strains 529L or Y72, which were isolated from patients with oral candidiasis, were used.<sup>23,25</sup> *C. albicans* was grown in yeast extract (BD, Cat. #212750), peptone (BD, Cat. #211677), and dextrose (Avantor, Cat. #4912) media that contained penicillin and streptomycin (Corning, Cat. #30-002-Cl) in a shaking incubator at 30°C. Prior to infection, *C. albicans* was centrifuged, washed with PBS, and counted with a hemocytometer. For systemic *C.*

**Table 2.** Induction of mucosal IL-17/IL-22 is Batf3-independent after oral *C. albicans* infection.

Cytokine/Chemokine	Batf3 <sup>+/+</sup>	Batf3 <sup>-/-</sup>	P-value
IL-1 $\beta$	8500 $\pm$ 3137	9773 $\pm$ 2616	0.3874
IL-4	1380 $\pm$ 208.1	985.7 $\pm$ 81.49	0.1078
IL-6	22112 $\pm$ 5407	22190 $\pm$ 3522	0.4740
IL-10	1755 $\pm$ 225.5	1166 $\pm$ 95.44	0.0370*
IL-17A	298.0 $\pm$ 48.83	270.4 $\pm$ 20.97	0.6145
IL-22	312.6 $\pm$ 162.1	304.3 $\pm$ 112.1	>0.9999
CCL2	52548 $\pm$ 18743	61327 $\pm$ 14405	0.3874
CCL3	632.4 $\pm$ 359.8	989.4 $\pm$ 455.6	0.3052
CCL4	286.3 $\pm$ 175.2	888.0 $\pm$ 423.8	0.1320
CCL5	139.5 $\pm$ 33.43	197.6 $\pm$ 59.90	0.4166
CXCL1	6557 $\pm$ 3242	5361 $\pm$ 1599	0.7476
CXCL2	9158 $\pm$ 4423	10430 $\pm$ 3757	0.8308

Notes. Cytokine and chemokine concentrations are expressed as pg/g of tongue at day 1 post-infection. Data represent mean values  $\pm$  SEM and are combined from 2 independent experiments with a total n of 6 mice/group. Statistical analysis was performed using an unpaired t-test or Mann-Whitney U test, where appropriate. An \* indicates that the values are significantly different from one another.

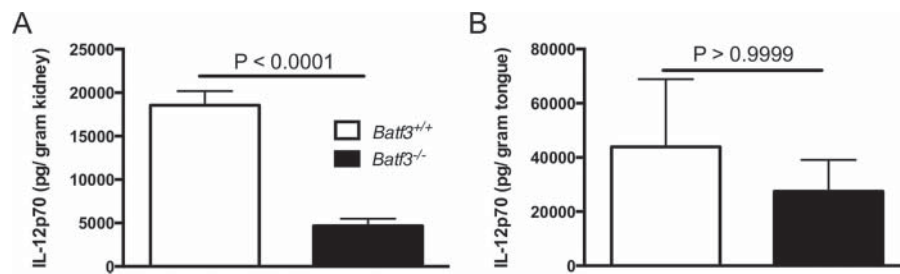
*albicans* infections with SC5314, 10<sup>5</sup> *C. albicans* cells were injected into the lateral tail vein. For systemic *C. albicans* infections with UC820, 1.5  $\times$  10<sup>5</sup> or 3  $\times$  10<sup>5</sup> *C. albicans* cells were injected into the lateral tail vein for assessment of CFUs or survival, respectively. For OPC, sublingual infections were performed using cotton swabs saturated in media containing 10<sup>7</sup> *C. albicans*/ml, as previously described.<sup>23,24</sup>

### Determination of fungal colony forming units (CFUs)

To assess tissue fungal load after infection, the tongues or kidneys of mice were homogenized in PBS using an Omni tissue homogenizer. The homogenate was plated undiluted or after serial dilutions on yeast, peptone, and dextrose agar plates containing penicillin and streptomycin. The plates were incubated for 24–48 hours at 37°C, the colonies were counted, and the data were presented as the total number of CFUs per gram of tissue. To lower the limit of detection for fungal burden determination in the tongue, the entire homogenate was plated. If no colonies were counted, a value of 0 was assigned.

### Single cell suspensions from the kidney and tongue

Single-cell suspensions were obtained from the kidney using a previously described technique.<sup>33</sup> Briefly, mice were perfused with PBS and kidneys were minced and incubated in a solution containing liberase TL (Roche Cat. #05989132001) and DNase I (Roche Cat. #10104159001) for 20 min in a shaking water bath at 37°C. Digested tissue was passed through a 70  $\mu$ m filter (Greiner Bio-One, Cat. # 542070) and red blood cells lysed with ACK lysis buffer (Quality Biological Cat. #118-156-721). Cells were then passed through a 40  $\mu$ m filter (Greiner Bio-One, Cat. # 542040), resuspended in 40% Percoll (GE Healthcare, Cat. #17-0891-01), and overlaid onto 70% Percoll. The gradient was centrifuged at 872 g for 30 min at room temperature, and the cells at the interface between the 40% and 70% layers were collected and stained for flow cytometry. Single-cell suspensions were obtained from the tongue based on a previously described technique.<sup>10</sup> The tongue was finely minced and digested in a solution containing 385 U/ml collagenase type 4 (Worthington Cat. #LS004189), 2 U/ml dispase II (Gibco, Cat. # 17105-041), and 50  $\mu$ g/ml DNase I in 10 ml RPMI for 45 min in a shaking water bath at 37°C. After 45 min, the solution was quenched with PBS + 5 mM EDTA (Quality Biological, Cat. # 351-027). The cells were serially filtered through a 70  $\mu$ m filter and a 40  $\mu$ m filter. The cells were washed, resuspended in 40% Percoll, and overlaid onto 70%



**Figure 3.** *Batf3*-dependent accumulation of CD103<sup>+</sup> DCs mediates tissue-specific production of IL-12 during systemic, but not oropharyngeal candidiasis. IL-12 levels were measured in *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice in the kidney at day 4 after intravenous *C. albicans* infection (A) or in the tongue at day 1 after sublingual *C. albicans* infection (B). Data were analyzed using unpaired t-test (A) or Mann-Whitney U test (B). Data are combined from 2 independent experiments with a total n of 6–8 mice/group.

Percoll. The gradient was centrifuged at 872 g for 30 min at room temperature, and the cells at the interface between the 40% and 70% layers were collected, washed with FACS buffer (PBS + 0.5% BSA + 0.01% NaN<sub>3</sub>), and resuspended in PBS for flow staining.

### Flow cytometry

Single-cell suspensions from the kidney or tongue were stained with LIVE/DEAD fluorescent dye (Invitrogen, Cat. # L-23105) for 10 min in PBS at 4°C. Next, the cells were incubated with rat anti-mouse CD16/32 (eBioscience, Cat. #14-0161) for 10 min at 4°C to block Fc receptors, and stained for cell-surface markers for 30 min at 4°C. To identify CD103<sup>+</sup> DCs and other myeloid populations, antibodies conjugated to eFluor 450, biotin/streptavidin BV570, PE, PerCP-Cy5.5, eFluor 605 NC, PE-Cy7, APC, AF700, or APC-Cy7 from eBioscience, Biolegend, or BD PharMingen were used. Antibodies were directed against MHC-II (Clone M5/114.15.2), CD103 (2E7), CD3 (17A2 or 145-2C11), B220 (RA3-6B2), CD19 (eBio1D3), NK1.1 (PK136), Ly6G (1A8), CD45 (30-F11), F4/80 (BM8), CD11c (N418), Ly6C (AL-21), and CD11b (M1/70). After washing with FACS buffer, cells were analyzed using a 5-laser LSR Fortessa (BD Biosciences, San Jose, CA), and data were analyzed using FlowJo software (FlowJo, Ashland, OR). Cell numbers were quantified using PE-conjugated counting beads (Spherotech, Cat. #ACFP-70-10), as previously described.<sup>33</sup>

### Determination of creatinine levels

Blood was collected from *Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice 4 d p.i. via cardiac puncture and placed in Serum Gel Z/1.1 tubes (Sarstedt, Cat. #41.1378.005), which were spun at ~16,000 g for 10 minutes at 4°C. Serum was collected and stored at -80°C until analysis of creatinine levels by the NIH Clinical Chemistry Laboratory.

### Histology

Kidneys were harvested at day 4 p.i., and tongues were harvested at days 1 and 5 p.i. following intravenous or sublingual *C. albicans* infection, respectively. The tissues were fixed in 10% formalin and embedded in paraffin. Longitudinal sections of the kidney or tongue were prepared for H&E or PAS staining (Histoserv, Inc., Germantown, MD). Sections were viewed under a microscope, and representative pictures were taken. For determination of the area affected by *Candida* infection, the entire tongue was photographed from the PAS-stained sections and the following formula was used: [(area of *Candida* abscesses/total area of the epithelium of the tongue) × 100%] using the Fiji image-processing program. This assessment was performed in a blinded manner with regard to the mouse genotype.

### Determination of cytokine and chemokine protein concentrations

*Batf3*<sup>+/+</sup> and *Batf3*<sup>-/-</sup> mice were infected with *C. albicans* intravenously or sublingually as described above, and kidneys or tongues were harvested at day 4 or day 1 p.i., respectively. Organs were homogenized with an Omni Tissue Homogenizer (Omni International) in PBS with 0.5% Tween-20 (Sigma-Aldrich Cat. #P7949) and a protease inhibitor cocktail (Roche Applied Science, Cat. #11836170001). The homogenate was then centrifuged at ~16,000 g for 10 minutes at 4°C, supernatants were filtered through a 22 μm filter (EMD Millipore, Cat. #SLGV033RS), and frozen at 80°C until use. To determine cytokine and chemokine protein concentrations, a multiplex bead array assay was utilized as previously described.<sup>23</sup> Antibodies and cytokine standards were purchased from R&D Systems or Peprotech as antibody pairs. Capture antibodies were coupled to individual Luminex bead sets for each cytokine or chemokine measured. Biotinylated antibodies were then added at twice



the recommended concentration for 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). The plates were read on a Luminex MAGPIX platform, and at least 50 beads were collected for each cytokine/chemokine per sample. The median fluorescence intensity for each bead was determined for analysis with the Milliplex software using a 5P regression algorithm.

### Statistical analyses

For the survival studies, log-rank (Mantel-Cox) tests were used. For all other experiments, unpaired *t*-tests or Mann-Whitney U tests were performed, where appropriate. All statistical analyses were performed using Prism 6 software (GraphPad). Quantitative data are presented as mean  $\pm$  standard errors of the mean (SEM), and a *P* value of  $<0.05$  was considered significant.

### Abbreviations

CFUs	colony-forming units
DCs	Dendritic cells
OPC	oropharyngeal candidiasis
PAS	Periodic acid-Schiff
p.i.	post-infection

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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