The intraspecies diversity of *C. albicans* triggers qualitatively and temporally distinct host responses that determine the balance between commensalism and pathogenicity

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The host immune status is critical for preventing opportunistic infections with *Candida albicans*. Whether the natural fungal diversity that exists between *C. albicans* isolates also influences disease development remains unclear. Here, we used an experimental model of oral infection to probe the host response to diverse *C. albicans* isolates *in vivo* and found dramatic differences in their ability to persist in the oral mucosa, which inversely correlated with the degree and kinetics of immune activation in the host. Strikingly, the requirement of interleukin (IL)-17 signaling for fungal control was conserved between isolates, including isolates with delayed induction of IL-17. This underscores the relevance of IL-17 immunity in mucosal defense against *C. albicans*. In contrast, the accumulation of neutrophils and induction of inflammation in the infected tissue was strictly strain dependent. The dichotomy of the inflammatory neutrophil response was linked to the capacity of fungal strains to cause cellular damage and release of alarmins from the epithelium. The epithelium thus translates differences in the fungus into qualitatively distinct host responses. Altogether, this study provides a comprehensive understanding of the antifungal response in the oral mucosa and demonstrates the relevance of evaluating intraspecies differences for the outcome of fungus–host interactions *in vivo*.

INTRODUCTION

Candida albicans is an important pathobiont in humans that can cause diseases ranging from mild superficial symptoms affecting the skin and the mucosa to severe systemic infections associated with high mortality rates. The development of disease is generally attributed to defects in host resistance. Individuals with impaired cellular immunity such as AIDS patients with low CD4⁺ T cell counts or glucocorticoid-treated patients display an increased susceptibility to fungal infection and frequently display symptoms of oral thrush.¹ More

recently, primary immunodeficiencies affecting the T cell compartment and in particular the T helper (Th)17 subset of T cells, which are associated with chronic forms of mucocutaneous candidiasis, revealed a critical role of the interleukin (IL)-17 pathway, in particular the two IL-17 family members IL-17A and IL-17F, in host protection from *C. albicans*.²

The discovery of genetic defects associated with fungal infections and experimental work with mouse infection models helped to decipher the mechanisms of antifungal defense in the mucosal epithelium. IL-17A and IL-17F are rapidly induced

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in response to *C. albicans* by different tissue-specific innate cellular sources.^{3–5} They act in a synergistic manner^{3,6} and target the epithelium for induction of antimicrobial peptides.^{7,8} The IL-17-dependent epithelial response is complemented by neutrophils, which rapidly infiltrate the infected tissue in an IL-17-independent manner⁷ and prevent fungal growth and dissemination to underlying tissue.^{7,9} Altogether, the tight interplay between immune cells and the epithelium guarantees for efficient fungal control in the mucosa. In addition, IL-17 may exert direct effects on *C. albicans* and thereby affect its virulence.¹⁰

Infections with *C. albicans* are usually not contagious. Rather, the fungus is acquired through childbirth from the mother as a part of the normal microbiota. The *C. albicans* population within a given individual is largely clonal.^{11–13} The colonizing fungus itself may turn pathogenic and cause disease when host defenses are breached. Therefore, variations in fungal isolates that exist between individuals, in addition to host factors, may contribute to disease susceptibility, although this notion is often undervalued and not well understood. *C. albicans* displays a significant intra-species diversity both at a phylogenomic and phenotypic level.^{14–16} Phenotypic variations include differences in adherence, stress responses, gene expression, morphological plasticity and the capacity to form biofilm. The basis for this variation and its impact on the host response remain largely unclear. Strain-specific differences recently drew attention in the murine intravenous challenge model.^{15–17} No study so far analyzed differences in pathogenicity of natural fungal isolates at mucosal surfaces, which is the site where *C. albicans* usually resides, and how they determine the decision between commensalism and disease.

Here, we probed the interaction between diverse and randomly selected natural *C. albicans* isolates and the host *in vivo* using the well-established model of oropharyngeal candidiasis (OPC). This allowed us to assess in a uniform and *Candida*-naïve host background the spectrum of responses that are raised through natural variations in the pathogen and how they define the outcome of the interaction with the fungus. Irrespective of their mucosal or blood origin, we observed striking differences between isolates in their ability to persist in the host, which inversely correlated with their capacity to induce a rapid inflammatory response. While the accumulation of neutrophils in the infected tissue was strictly strain-dependent, the IL-17 pathway was essential across different strains to restrict fungal growth in the mucosal tissue. Strain-specific differences in the induction of an inflammatory response did not correlate with expression of known *C. albicans* virulence genes including *ECE1*, but rather associated with the capacity to cause epithelial damage and release IL-1 α from keratinocytes. Altogether, this study emphasizes the key role of IL-17 in host defense against *C. albicans*, which is uncoupled from the neutrophil response, and it sets the stage for evaluating fungal determinants associated with commensalism vs. pathogenicity of the fungus *in vivo*.

RESULTS

Fungal persistence in the host varies between isolates and inversely correlates with transient weight loss of infected animals at the onset of infection

The acute host response to OPC infection with *C. albicans* SC5314 in mice is characterized by an acute inflammatory response that comprises neutrophil infiltration and induction of IL-17 and antimicrobial effector molecules.^{7,18} The infection is accompanied with a transient loss in body weight. Weight recovery within 4–7 days generally correlates with fungal clearance in wildtype (WT) mice. In contrast, immunodeficient animals lacking these protective immune mechanisms are unable to control the fungus and suffer from prolonged cachexia.^{7,18}

To assess whether this response was general or affected by variation in the fungal strain, we selected a small number of *C. albicans* isolates from different origins that were genetically unrelated (**Supplementary Figure S1AB** online) and showed comparable growth in rich and minimal media *in vitro* (**Supplementary Figure S2AB**). The cell wall composition and degree of β -glucan exposure of these isolates was also comparable (**Supplementary Figure S2CD**). We performed OPC infection of WT mice with four isolates, namely SC5314, Cag, 529L, and 101. They were all equally capable of infecting the oral mucosa as evaluated by quantification of the fungal load in the tongue on day 1 post infection (p.i.; **Figure 1a**). Histology analysis revealed that all isolates filamented in the tissue and invaded the oral keratinized epithelium (**Figure 1b,c**). Transmission electron microscopy showed that they penetrated keratinocyte cell membranes in the stratum corneum comparably (**Figure 1c**). However, striking differences between the isolates were observed when analyzing the course of infection over time. The degree of weight loss induced in the mice by the different isolates varied greatly on day 1 to day 3 p.i. (**Figure 1d**). To determine how these differences were associated with fungal control in the host, we assessed the fungal burden in the tongue of infected mice on day 3 and day 7 p.i. In contrast to strain SC5314, which was rapidly cleared to undetectable levels, all other isolates persisted in the host and high fungal counts could still be recovered one week p.i. (**Figure 1e,f**). The high fungal load remained detectable for up to 4 weeks p.i., at least in case of strain 101 (**Supplementary Figure S3**). These data indicated that transient weight loss during the first days of OPC was not a consequence of high fungal burden in the mice, but rather that strong changes in weight during the initial phase of infection were associated with enhanced fungal control. Importantly, for the persistent fungal isolates no tissue pathology was observed despite the prolonged presence of high fungal loads (**Figure 1g**).

Persistent fungal isolates fail to induce an acute inflammatory response in the host

To investigate the cause of these differences in colonization vs. clearance with different *C. albicans* isolates, we assessed the response of the host in the four infected groups. Histology of the infected tissue revealed striking differences in the degree of

Figure 1 Fungal persistence in the host inversely correlates with transient weight loss of infected animals at the onset of infection. WT mice were infected with *C. albicans* SC5314, Cag, 529L, or 101. **(a)** Tongue fungal burden on day 1. **(b)** Representative images of sagittal tongue sections (day 1) stained with PAS. Arrows: inflammatory infiltrates. Scale bars: 50 μ m. Inset: 2-fold magnification. **(c)** Representative transmission electron microscopy images of infected tongues (day 1). Red circles: fungal penetration of cell membranes in the stratum corneum. Scale bars: 5 μ m. **(d)** Body weight of infected mice. **(e,f)** Tongue fungal burden on day 3 and day 7. **(g)** Representative images of sagittal tongue sections (day 7) stained with PAS. Scale bars: 100 μ m. In **a**, **e**, **f**, each symbol represents one mouse. Data are the pool of two independent experiments. The geometric mean and significant differences between each group and the SC5314-infected group are indicated. In **d**, data are the mean $\pm$ s.d., $n=6$, pooled from two independent experiments. Significant differences between groups infected with SC5314 and Cag, 529L or 101 are indicated with #, $\dagger$ or *, respectively. PAS, periodic-acid Schiff; WT, wild type.

inflammation induced on day 1 p.i. (**Figure 1b**). Although large infiltrates of inflammatory cells, which consist mainly of neutrophils (**Supplementary Figure S4A**),⁷ were observed in the tongue of strain SC5314-infected mice, barely any cellular infiltrates were observed in strain 101-infected animals and only few and small infiltrates were found in the other groups on day 1 p.i. Quantification of neutrophils by flow cytometry of single-cell suspensions prepared from the infected tongues confirmed these results (**Figure 2a** and **Supplementary Figure S4A**). Importantly, the neutrophil response to strain SC5314 was strongest, even if the infection dose was lowered 25-fold (**Supplementary Figure S4B**). The blunted neutrophil response to infection with the persistent *C. albicans* isolates correlated with their limited capacity to induce neutrophil-recruiting chemokines such as CXCL2 and the granulopoietic factor granulocyte-colony stimulating factor (G-CSF) (**Figure 2b,c**). G-CSF was measured in the serum accounting for the endothelial source of this factor.¹⁹ Of note, we did not observe dissemination of the persistent isolates away from the mucosal epithelium despite the fact that neutrophils are critical for preventing fungal dissemination in case of strain

SC5314.⁷ These results demonstrate that the role of neutrophils during OPC is strain specific. Induction of IL-6 and TNF was also differentially induced in response to the different isolates, albeit differences for TNF did not reach statistical significance (**Figure 2d,e**). Next, we assessed the activation of the IL-17 pathway and downstream targets on day 1 p.i. IL-17 expression was strongly reduced in the tongue of mice infected with the persistent *C. albicans* isolates compared to strain SC5314 (**Figure 2f,g**). Similarly, *S100a9* transcripts were very weak if at all induced (**Figure 2h**).

Altogether, this indicated that natural *C. albicans* isolates stimulated a highly variable response in the host, whereby the extent of inflammation induction correlated with fungal clearance (see below).

The variation in the pathogenicity of *C. albicans* isolates does not correlate with their clinical origin

All isolates tested so far, which displayed low pathogenicity and thus persisted in the host, were collected from the oral mucosa of patients and healthy individuals, while SC5314 was originally obtained from a blood culture. To test whether the origin of the

Figure 2 Persistent *C. albicans* isolates induce a limited inflammatory response in the host. WT mice were infected with *C. albicans* SC5314, Cag, 529L or 101 and analyzed after 1 day. **(a)** Quantification of tongue CD11b⁺ Ly6C⁺ Ly6G⁺ neutrophils by flow cytometry. **(b)** *Cxcl2* expression in the tongue was assessed by qRT-PCR. **(c)** Serum G-CSF was quantified by ELISA. **(d–h)** *Il6*, *Tnf*, *Il17a*, *Il17f* and *S100a9* expression in the tongue was assessed by qRT-PCR. Each symbol represents one mouse. Data are the pool of two independent experiments. The mean **(c)** or the geometric mean **(a, b, d–h)** of each group and significant differences between each group and the SC5314-infected group are indicated. Solid line: (geometric) mean of a naive control group. *IL6*, interleukin-6; *Tnf*, tumor necrosis factor; G-CSF, granulocyte-colony stimulating factor; qRT-PCR, quantitative reverse transcriptase-PCR; WT, wild type.

isolates correlated with their pathogenicity, we examined the behavior of two additional independent blood isolates (UC820 and ATCC14053) in the OPC mouse model. These isolates displayed similar growth kinetics, cell wall composition and β -glucan exposure *in vitro* as strain SC5314 (Supplementary

Figure S2). Irrespective of their origin, they readily colonized the oral mucosa and persisted for over a week without inducing significant weight loss in the host (Figure 3a–c). Similar to the oral isolates analyzed before (Figures 1 and 2), they induced an attenuated inflammatory response (Figure 3d–f).

Figure 3 The variation in the pathogenicity of *C. albicans* isolates did not correlate with their origin. WT mice were infected with *C. albicans* SC5314, ATCC14053, UC820 or CEC3605. (a) Body weight of infected mice. Data are the mean $\pm$ s.d. ($n = 6$, pooled from two independent experiments). (b,c) Tongue fungal burden on day 3 and day 7. (d) Quantification of tongue CD11b⁺ Ly6G⁺ neutrophils (day 1) by flow cytometry. (e,f) *Il17a* and *S100a9* expression in the tongue (day 1) was assessed by qRT–PCR. (g) Representative images of sagittal tongue sections (day 1) stained with PAS. Arrows: inflammatory infiltrates. Scale bars: 50 μ m. Inset: twofold magnification. (h–j) *Cxcl2*, *Il6*, and *Tnf* expression in the tongue on day 1 was assessed by qRT–PCR. (k) Serum G-CSF on day 1 was quantified by ELISA. (l) *Il17f* expression in the tongue (day 1) was assessed by qRT–PCR. (m) Scatter plot with data from Figures 1f, 2a and 3d. Color code: mucosal isolates (grey), blood isolates (black). In b–f and h–l, each symbol represents one mouse. Data are the pool of two independent experiments (except for the CEC3605 group in b, which was from one experiment). The mean (a, k) or geometric mean (b–f, h–j, l) of each group and significant differences between each group and the SC5314-infected group are indicated. Solid line in d–f, h–l: (geo)mean of a naive control group. *Il6*, interleukin-6; *Tnf*, tumor necrosis factor; G-CSF, granulocyte-colony stimulating factor; qRT–PCR, quantitative reverse transcriptase–PCR; WT, wild type.

Within a larger collection of natural isolates (see below), we identified additional strains of high pathogenicity similar to strain SC5314. One of them, strain CEC3605, which was collected from the vaginal mucosa and displayed a normal phenotype *in vitro* (Supplementary Figure S2), was then tested for its behavior *in vivo*. Similar to SC5314, mice infected with CEC3605 displayed a strong transient weight loss (Figure 3a) and rapid fungal control (Figure 3b,c). As expected from the similar behavior of CEC3605 to SC5314, CEC3605 triggered a very strong and rapid inflammatory response in the oral mucosa on day 1 p.i. with large neutrophilic infiltrates (Figure 3d,g) and high levels of neutrophil-recruiting chemokines, *Il6* and *Tnf* transcripts in the epithelial tissue (Figure 3h–j) as well as high G-CSF levels in the serum (Figure 3k). Moreover, *Il17a*, *Il17f* and IL-17 target genes were strongly induced by strain CEC3605 on day 1 p.i. (Figure 3e,f and l).

Altogether, these results suggested that the host compartment, from which *C. albicans* isolates were collected, was not decisive for their behavior in the host. Rather the intrinsic capacity of individual isolates to promote a response in a previously *C. albicans*-naïve host determined whether they persisted in the epithelium or were rapidly cleared. Moreover, the degree of early inflammation induced by a strain seemed to determine the outcome of infection, with an inverse correlation between the inflammatory response triggered in the host and fungal persistence (Figure 3m). This is consistent with what was observed before in different experimental systems.^{20,21}

Filamentation *in vitro* is not a good predictor of the pathogenicity of *C. albicans* isolates in the oral mucosa *in vivo*

To explore the underlying cause of the variability in the outcome of the interaction of the *C. albicans* isolates with the host, we compared the fungal morphology under different

conditions. *C. albicans* is able to undergo morphological switching between budding yeast, pseudohyphal and hyphal forms. The ability to filament is generally thought to be a critical feature of pathogenicity.²² All strains filamented in FCS-containing liquid medium in a comparable manner with small differences in hyphal length after 3 h (Supplementary Figure S5A). Differences in filamentation between the *C. albicans* isolates were much more pronounced when grown on Spider medium for 10 days. Although filamentation was observed reproducibly with strains SC5314, CEC3605 and UC820, while strains Cag, 529L and ATCC14053 had a more variable phenotype and no filamentation was observed for strain 101 in this assay (Supplementary Figure S5BC). When growing the fungal strains on TR146 keratinocytes, all isolates were found to filament, albeit the proportion of cells forming pseudohyphae or hyphae, the hyphal length and the degree of branching varied (Supplementary Figure S5D). Importantly, no correlation was found between hyphal growth on TR146 and the behavior of the fungal isolates *in vivo*: ATCC14053, which induced a similar degree of inflammation as Cag, formed mostly short pseudohyphae, while long hyphae were observed in case of Cag (Supplementary Figure S5D). Likewise, 101, but not UC820 formed typical hyphal structures on TR146 cells, although none of the two induced significant inflammation *in vivo* (Supplementary Figure S5D).

Finally, we assessed fungal morphology *in vivo* using two pathogenic and two persistent isolates as examples. They all formed hyphal filaments in the infected tongue, which were hardly distinguishable in shape and length (Figures 1b and 3g). This observation was confirmed when analyzing histology in top view of horizontally cut tongues at 24 h p.i. (Figure 4a). Determining the localization of *C. albicans* in the third dimension of the oral epithelium was not possible 1 day p.i. when large neutrophil infiltrates were present in the mucosa of mice infected with SC5314 and CEC3605 (Figures 1b

Figure 4 Representative images of tongue sections from WT mice infected with SC5314, 529L, 101, or CEC3605 stained with PAS. Top view of dorso-ventral sections on day 1 (a) and sagittal sections after 12 h post-infection (b). Inset in a: fourfold magnification. Filled arrows: boundary between stratum corneum and stratum granulosum; open arrows: stratum basale. PAS, periodic-acid Schiff; WT, wild type.

and 3g). We therefore shifted our analysis to an earlier time point during infection. This revealed important differences in the invasion depth of *C. albicans* isolates in the oral epithelium, which reflected the differential inflammatory response that they elicited (Figure 4b). While the pathogenic isolates SC5314 and CEC3605 penetrated into the stratum spinosum and granulosum, the persistent isolates 529L and 101 were found exclusively in the amorphous stratum corneum at this early (Figure 4b) as well as at later time points (Figure 1g). Together, these results indicated that the variation in pathogenicity between *C. albicans* isolates were not easily explained by differences in fungal morphology, but rather by their differential localization within the squamous mucosal epithelium.

Induction of damage and IL-1 α in keratinocytes reflects the pathogenicity of *C. albicans* in vivo

As a first contact point, epithelial cells play a key role in the initial interaction between *C. albicans* and the host during superficial infections. *C. albicans* can adhere, invade and damage the epithelium, all parameters associated with enhanced virulence.²² All isolates adhered to the keratinocyte cell line TR146, albeit to variable degrees (Supplementary Figure S6A). Likewise, the capacity of the isolates to form biofilm varied and did not show a pattern that could be associated with the observed phenotypes *in vivo* (Supplementary Figure S6B). In contrast, the extent to which the different isolates caused epithelial cell damage, as assessed by lactate dehydrogenase (LDH) release, reflected their differential induction of inflammation *in vivo* (Figure 5ab). Importantly, the differences in damage induced by the isolates were conserved at different infection doses (Supplementary Figure S6C). The release of LDH by cultured keratinocytes in response to *C. albicans* *in vitro* was thus a good correlate for fungal pathogenicity *in vivo* (Figure 5cd).

In the following, we used the LDH release assay as a surrogate to examine the variation in pathogenicity of *C. albicans* within a larger collection of 76 natural isolates. Although largely diverse, they all ranked between the two extremes identified as high damage inducers (>10% LDH release after 8 h of stimulation, exemplified by SC5314 and CEC3605) and low-damage inducers (<10% LDH release after 24 h of stimulation, exemplified by 101, 529L and UC820). A small proportion of isolates fell into the group of intermediate-damage inducers (<10% LDH release after 8 h, >10% after 24 h of stimulation; Figure 5e). Confirming our previous results (Figure 3m), the degree of damage induced by different isolates did not correlate with their source, namely mucosal tissues or blood cultures (Figure 5f).

The induction of cellular damage by *C. albicans* was recently associated with the virulence factor *ECE1*, which gives rise to a pore forming toxin Candidalysin.²³ We therefore assessed *ECE1* expression by *C. albicans* isolates in contact with TR146 cells. Although we detected significant differences in *ECE1* expression between the different isolates (Figure 5g), they did not correlate with the degree of LDH release (Figure 5h) or the *in vivo* pathogenicity induced. Moreover, we did not detect

significant differences in expression of *KEX1* and *KEX2*, two proteinases involved in the processing of *ECE1* (ref. 23) between the isolates tested (Supplementary Figure S6D). Therefore, mechanisms beyond those involving *ECE1* seem to determine the capacity of *C. albicans* to cause host damage when moving away from strain SC5314. Expression of other well-known virulence factors, such as *ALS3*, *SAP4*, *SAP5*, *SAP6*, *SAP9*, and *HWPI* and the virulence-associated transcriptional regulators *EFG1* and *CPH1* were not significantly altered between the isolates (Supplementary Figure S6E,F).

Induction of cellular damage is linked to the release of cytoplasmic molecules, some of which can act as alarmins and promote inflammation if freed into the extracellular space where they do not usually reside. Indeed, IL-1 α release by keratinocytes in response to *C. albicans* isolates mirrored the levels of LDH release (Figure 5i,j). In contrast, keratinocyte secretion of other cytokines such as IL-6 and IL-8 did not follow the same pattern (Supplementary Figure S6G). The differential IL-1 α response by pathogenic and non-pathogenic isolates was also observed in the infected tongue, as shown for a prototypic isolate of each type, SC5314 and 101 (Figure 5k).

Altogether, these data indicate that the induction of damage and release of danger-associated molecules from cultured keratinocytes *in vitro* is a good predictor of *C. albicans* pathogenicity *in vivo*. Moreover, they suggest that the epithelium translates differences in the fungus into differential host responses.

Persistent *C. albicans* isolates induce a delayed and qualitatively altered response in the oral mucosa

Our data suggested that non-pathogenic *C. albicans* isolates persisting in the stratum corneum of the oral mucosa induce a very limited host response. However, the observation that they did not overgrow the host or disseminate, indicated that they were not completely uncontrolled by the host. We, therefore, examined the antifungal response to the persistent strains 101 and 529L at later time points. In contrast to the strong and transient neutrophil response induced by pathogenic isolates (Figure 6a), 529L and 101 induced small and isolated neutrophil infiltrates in the infected tongue on day 3 and 5 p.i. (Figure 6a). This attenuated response was paralleled by a strongly impaired induction of G-CSF and *Cxcl2* expression (Figure 6bc). In contrast, expression of IL-17 and IL-17 target genes was strongly induced in the tongue of 529L- and 101-infected mice on day 3 and 5 that reached comparable levels to those found in SC5314-infected tongues on day 1 p.i. (Figure 7a–c). These data were further confirmed by measuring IL-17A and IL-17F protein induction in response to persistent isolates at later time points (Figure 7de). Likewise, *C. albicans*-specific Th17 cells were induced comparably by all isolates tested in the draining lymph nodes on day 7 p.i. (Supplementary Figure S7).

Together, these results indicated that *C. albicans* isolates, which initially induced a restrained response, did not remain undetected by the immune system and an IL-17 signature response was readily triggered after a few days of fungal

Figure 5 Induction of damage and IL-1 α in keratinocytes reflects the pathogenicity of *C. albicans* in vivo. (**a,b**) LDH release from TR146 cells stimulated with *C. albicans* SC5314, Cag, 529L, 101, ATCC14053, UC820, CEC3605, or the mutant strain *efg1* Δ/Δ , which was included as a negative control, for 8 h (**a**) or 24 h (**b**). (**c,d**) Scatter plot of data from **Figures 5a, 2a and 3d** (**c**) or **Figures 5b, 2a and 3d** (**d**). Color code: mucosal isolates (green), blood isolates (red). (**e,f**) Damage induction by 76 *C. albicans* isolates (including the seven isolates used in this study) classified as low (<10% LDH release at 24 h), mid (<10% at 8 h/>10% after 24 h) and high (>10% after 8 h) damage inducers. In **f**, the number of isolates in each category is shown separately for isolates from mucosal surfaces (superficial) and blood cultures (invasive). (**g**) *ECE1* mRNA expression by *C. albicans* isolates upon contact with TR146 keratinocytes for 24 h. (**h**) Scatter plot of data from **Figure 5b** and **g**. (**i**) IL-1 α levels in the samples from (**b**). Solid line: basal IL-1 α levels in the supernatant of unstimulated TR146 cells. (**j**) Scatter plot of data from **Figure 5b** and **i**. (**k**) Representative images of sagittal tongue sections from WT mice infected with SC5314 or 101 for 1 day and stained with anti-IL-1 α (red) and DAPI (blue) (top) or PAS (bottom). Scale bars: 100 μ m. In **a, b** and **i**, each symbol represents a separate well, the mean of each group is indicated. Data are a pool of two independent experiments. In **g**, data are the pool of three independent experiments, the geomean of each group is indicated. IL-1 α , interleukin-1 α ; PAS, periodic-acid Shift; WT, wild type.

presence in the host. In contrast, the recruitment of neutrophils remained very limited, even at later time points when the fungal load was still high.

IL-17 signaling is essential for preventing fungal overgrowth irrespective of the degree of fungal pathogenicity

To evaluate whether the IL-17 response to OPC infection with persistent strains was relevant for host defense and fungal control in the oral mucosa despite the delayed induction, we

infected mice with a genetic defect in IL-17 signaling. Both IL-17RA- and IL-17RC-deficient mice showed increased fungal loads after infection with strains 101 and 529L if compared to WT control hosts (**Figure 7fg**). Groups of mice infected with strain SC5314 were also included in this experiment for comparison (**Figure 7fg**). The relevance of the IL-17 pathway for limiting the growth of all fungal isolates was also obvious when analyzing infected tongues by histology (**Figure 7h**). Infection of IL-17 receptor deficient mice with strains 101 or 529L did not result in major changes in body weight

Figure 6 The neutrophil response to persistent isolates is strongly attenuated. **(a)** Representative images of sagittal tongue sections from WT mice infected with SC5314, 529L, 101, or CEC3605 for 1, 3, or 5 days and stained with anti-Ly6G. Scale bars: 1 mm. **(b,c)** Serum G-CSF **(b)** and tongue *Cxcl2* mRNA **(c)** of WT mice infected with SC5314, 529L, or 101 for 1, 3, or 5 days. Each symbol represents one mouse. The mean **(b)** or geometric mean **(c)** of each group is indicated. Data in **b** and **c** are pooled from two independent experiments. Solid line: (geo)mean of a naive control group. Significant differences between each group and the SC5314-infected group (day 1) or between naive and infected groups for each strain are indicated by asterisks or by hashes, respectively. G-CSF, granulocyte-colony stimulating factor; WT, wild type.

(Supplementary Figure S8), re-emphasizing that the weight loss during the early phase of OPC, as seen with pathogenic strains of *C. albicans*, was not directly associated with the fungal burden.

In conclusion, these results showed that IL-17 signaling plays a non-redundant role for mucosal immunity against *C. albicans*, irrespective of the nature of the infecting strain, whereas epithelial cell damage, mucosal inflammation and neutrophil infiltration display a strain-specific dichotomy and are associated with fungal pathogenicity.

DISCUSSION

C. albicans is a common pathobiont and one of the most important disease-causing fungi in humans. Disease symptoms occur if host defenses are breached. Specific immune mechanisms that protect from different forms of diseases have been characterized. However, little attention has been paid so far to the contribution of the natural diversity of *C. albicans* to the decision between health and disease. In this study, we took

advantage of a mouse model of OPC to probe the differential interaction of the host with diverse fungal isolates in a complex but uniform and *C. albicans*-naïve environment *in vivo*. We identified major qualitative and temporal differences in immune activation by distinct fungal isolates. The capacity of *C. albicans* to trigger a response by the epithelium such as the release of alarmins/IL-1 α correlates with the induction of an inflammatory response and the rapid control of the fungus by the host. Thereby, the functional variability of *C. albicans* natural isolates determines a fine balance between commensalism and pathogenicity and thus the long-term outcome of the interaction with the host. In contrast, the requirement of the IL-17 pathway for preventing fungal outgrowth is conserved among all isolates tested, highlighting the key role of this cytokine in host protection from *C. albicans*, irrespective of the fungal pathogenicity.

Significant diversity exists between natural isolates of *C. albicans*, both from a phylogenomic as well as a phenotypic perspective. Population studies of *C. albicans* support a

Figure 7 IL-17 signaling is essential for preventing fungal overgrowth irrespective of the degree of fungal pathogenicity. (**a–c**) *Il17a*, *S100a9* or *Defb3* expression in the tongue of WT mice infected with SC5314, 529L, or 101 for 1, 3, or 5 days. Each symbol represents one mouse. The geometric mean of each group is indicated. Solid line: geometric mean of a naive control group. Data are pooled from two independent experiments. (**d,e**) IL-17A and IL-17F protein levels in the tongue of WT mice infected with 529L or 101 for 1 or 3 days compared to the respective levels induced by SC5314 on day 1 post-infection. In A–E, significant differences between each group and the SC5314-infected group (day 1) or between naive and infected groups for each strain are indicated by asterisks or by hashes, respectively. (**f,g**) Tongue fungal burden on day 7 in WT and *Il17ra*^{-/-} (**f**) or *Il17rc*^{-/-} mice (**g**) infected with SC5314, 529L, or 101. Each symbol represents one mouse. The geometric mean of each group is indicated. Significant differences of the knockout to the corresponding WT control group for each fungal strain are indicated. (**h**) Representative images of sagittal tongue sections of 101-infected WT and *Il17ra*^{-/-} mice stained with PAS. Scale bars: 250 μ m. Inset: fourfold magnification, scale bars: 50 μ m. IL-17, interleukin-17; PAS, periodic-acid Schiff; WT, wild type.

predominantly clonal mode of reproduction of this diploid and largely heterozygous organism.^{11–13} Examples of phenotypic variation across *C. albicans* isolates have been studied most extensively in the context of antifungal drug resistance.^{24,25} Differences between natural *C. albicans* isolates that affect their interaction with the host have also been reported.¹⁵ A striking example of phenotypic intraspecies variation is provided by the

differential behavior of two unrelated natural isolates in mice lacking the β -1,3-glucan receptor Dectin-1, which was based on differences in the composition and nature of their cell wall and recognition by innate immune cells *in vivo*.¹⁷ Variation may also exist in the extent to which TLR4 recognizes different *C. albicans* isolates.²⁶ In all these examples, the genetic basis for the phenotypic differences remains unexplored. This also

applies to the isolates analyzed in this report. Future research will be needed to decipher the genetics underlying natural differences in the virulence traits of *C. albicans*, although the high degree of sequence variation between individual isolates makes this endeavor a challenge.^{16,27}

Existing intraspecies variation however is likely contributing to the probability of disease development and/or the severity of symptoms when comparing individuals with a comparable immune status. *C. albicans* infections are generally not transmissible, but rather caused by the fungal strain that was already present as a component of the microbial flora prior to the outbreak of symptoms. This is consistent with the observation that the origin of the isolates from mucosal surfaces or blood cultures did not segregate by differences in pathogenicity.

In the current study, we focused on how the fungal diversity affects the host response *in vivo*. Infection of mice via the sublingual route revealed a spectrum of temporally and qualitatively different immune responses to different isolates with the two extremes exemplified by strains SC5314 and 101, and various intermediate cases. While some isolates triggered a massive infiltration of neutrophils and a strong expression of IL-17 in the infected tissue within one day p.i., as it was observed previously for strain SC5314 (refs 3,7), the response to other isolates was delayed with induction of IL-17 from day 3 p.i., but only very limited neutrophil recruitment. The former scenario was associated with rapid fungal elimination from the host, whereas the latter allowed persistent colonization of the host mucosal epithelium by the isolate. Rapid induction of an inflammatory response appeared thus to be critical for the decision between fungal colonization vs. elimination and the contribution of infiltrating neutrophils to the response is strain dependent.

The segregated host response was paralleled by very distinct patterns of weight loss and recovery during infection with different isolates. Our data suggest that not the fungal load, as previously thought,^{7,18} but rather the rapid inflammatory response, and possibly the associated damage caused to the host, elicits the drop in weight on day 1–2 p.i. It remains unclear whether specific host factor(s) account for it, and if so, which one(s). IL-1 or TNF, which can cause cachexia in other contexts, do not seem to be involved in the experimental setting used here. According to our preliminary data, IL-1 receptor deficiency did not relieve the drop in weight, and no significant increase in systemic TNF levels were measured upon infection with strain SC5314.

The qualitative differences in the host response between different *C. albicans* isolates, and in particular the observation that the delayed induction of IL-17 by persistent isolates was not accompanied by significant neutrophil infiltration, underlines the notion that the two pathways are uncoupled during OPC.⁷ Although genes associated with neutrophil trafficking and function can be affected by IL-17 in some cases,^{28–30} defective IL-17 signaling does not impair neutrophil recruitment to the site of infection during OPC,⁷ and the induction of IL-17 does not entail an enhanced neutrophil response as shown here.

The IL-17 pathway has emerged as a key player in protection from superficial candidiasis, as evidenced by the strong association of chronic forms of mucocutaneous candidiasis with defects in genes linked to IL-17 production or signaling.² Likewise, the critical role of the IL-17 pathway in preventing uncontrolled fungal overgrowth in the epithelium was conserved in mice irrespective of the pathogenicity of the infecting strain, albeit the delayed induction of IL-17 and IL-17 target genes appeared insufficient for eliminating colonizing fungus from the murine host.

C. albicans-specific Th17 cells can be found in the circulation of most individuals irrespective of the fungal diversity within the human population.³¹ *C. albicans*-specific Th17 cells are also induced in mouse models of superficial candidiasis.^{32–34} In this study we compared the Th17 response to different *C. albicans* isolates and found that it was induced to comparable degrees in all cases. Thus, the drastic differences in inflammation during the initial acute host response do not translate in differences of adaptive immunity. This suggests that the initial inflammatory response to some isolates is redundant for the initiation of adaptive antifungal immunity. Alternatively, the increased antigen load in case of infection with persistent isolates may compensate for the initially limited induction of T cell activating/polarizing cytokines.

During OPC infection with highly virulent *C. albicans* strains, such as SC5314, IL-17 was induced rapidly and prior to the initiation of cytokine production by conventional Th17 cells.^{3–5} Lymphocytes of the innate immune system including innate TCR $\alpha\beta$ ⁺ T cells, TCR $\gamma\delta$ ⁺ T cells and innate lymphoid cells (ILCs) have been identified as the source of the immediate production of IL-17 in the skin and oral mucosa within one day p.i.^{3–5} The cell type(s) responsible for tongue IL-17 production 3–5 days after infection of mice with colonizing isolates of *C. albicans* and the signals responsible for the delayed IL-17 production in this situation remain to be identified.

Our data demonstrate that the differential induction of an inflammatory response by diverse *C. albicans* isolates in the oral mucosa correlate closely with the degree of damage that these isolates caused in keratinocyte cultures. Consistent with previous reports,^{23,35} LDH release as a readout for damage induction also correlates closely with the release IL-1 α by keratinocytes. We show that this applies also *in vivo*, where induction of IL-1 α in the oral epithelium was greatly increased in response to strain SC5314 compared to strain 101. IL-1 signaling is linked to the regulation of neutrophil recruitment in response to *C. albicans*.^{19,36} Therefore, the epithelium is capable of sensing differences between fungal isolates that it translates into an inflammatory response, and thereby determines the overall degree of the (innate) host response. The measure of LDH and IL-1 α release by keratinocytes in response to *C. albicans* isolates thus serves as a surrogate for determining the degree of inflammation induced in the host tissue *in vivo* and the efficiency of fungal control, which in turn are linked to differences between commensalism and pathogenicity. The keratinocyte assay may thus be used to predict the outcome of the interaction between *C. albicans*

isolates and the host epithelial/superficial tissues *in vivo*. It may serve for screening collections of fungal isolates from cohorts of patients with specific immune defects to assess a possible correlation between the pathogenicity of the isolates and the severity of symptoms.

Epithelial damage caused by *C. albicans* appears to result from a combination of different mechanisms. Filamentation is generally thought to be a prerequisite for damage induction, and expression of many virulence genes is associated with fungal morphology.³⁷ More recent results however have challenged the dogma that there is a precise correlation between morphology and virulence/damage, and filamentation *per se* appears not sufficient for damage induction or virulence.^{23,38} Similarly, the degree of filamentation of different *C. albicans* isolates analyzed in this study did not correlate well with the degree of damage they induced in keratinocytes. Moreover, the expression of well-known virulence genes such as *ALS3*, *SAP4*, *SAP5*, *SAP6*, *SAP9*, *HWP1*, and *EFG1* upon fungal contact with the epithelium did also not match the functional response of the epithelium to the different isolates. Among all examined virulence genes, the largest variations in expression between the isolates were observed for *ECE1*. Proteolytic cleavage of the Ece1 protein leads to the release of a peptide toxin called Candidalysin, which was recently described to be sufficient and required for induction of epithelial cell damage by *C. albicans* strain SC5314 (ref. 23). Although we could confirm strong expression of *ECE1* by strain SC5314 in contact with keratinocytes, *ECE1* expression did not correlate with keratinocyte damage induction in all cases. A limitation of our study may be that *ECE1* expression was measured at the transcript level, and polymorphisms in the *ECE1* gene were not analyzed, which may add to the complexity in the system. The molecular and genetic basis for the diversity among *C. albicans* isolates triggering epithelial cell damage and inflammation, and thus for the variation between pathogenicity and commensalism in the host remains to be determined in the future. Importantly, of all the different parameters analyzed *in vitro* in this study, only very few could be linked closely to the behavior of *C. albicans* isolates *in vivo* in the living host organism. Results from *in vitro* studies, such as the assessment of fungal growth on Spider medium, should thus be interpreted with care and not extrapolated to fungal pathogenicity *in vivo*. This is reminiscent of strain-specific differences in virulence that manifest in Dectin-1-deficient hosts, which could only be revealed *in vivo*.¹⁷

Together, our extensive study with different unrelated *C. albicans* isolates in contact with the host offered detailed insights into the intricate interplay between the fungus and host *in vivo*, and emphasized the limitations of results obtained from studies with a single (random) strain. This study taught us how not only different immune pathways differ in their contribution to antifungal immunity, but also how variations in the fungus result in selective activation of distinct defense mechanisms and thereby influence the outcome of the microbe-host interaction. While the requirement of IL-17 for fungal control in the mucosa is highly conserved, inflammation and neutrophil infiltrates are only triggered by highly pathogenic strains, which

thereby induce their rapid clearance. Accordingly, the natural diversity of *C. albicans* is an important determinant of the fine balance between commensalism and pathogenicity *in vivo*.

METHODS

Fungal strains and media. The *C. albicans* blood isolates SC5314 (ref. 39), UC820 (ATCC MYA-3573 (ref. 40)), ATCC14053 (ref. 41), the oral isolate 529L (isolated from an oral candidiasis patient,²⁰ a kind gift from J. Naglik), the oral isolates 101 (isolated from a healthy volunteer during an epidemiological clinical trial (approved by the cantonal ethics committee of the Canton of Ticino, Switzerland, approval number CE 2669)), Cag (isolated from a chronic mucocutaneous candidiasis patient with unknown etiology, kindly obtained from D. Firinu) and the vaginal isolate CEC3605, (ref. 42 isolated from a healthy carrier) were included in this study. Additional 67 isolates of different origin (mainly Europe and North Africa) were from the collection of the d'Enfert laboratory.^{14,42–46} *C. albicans* isolates were obtained after written informed consent by adult subjects or by their parent/guardian in case of children and were analyzed anonymously. The isogenic mutant strain *efg1Δ/Δ*, (ref. 47 a kind gift from A. Mitchell) was used as a control in some experiment.

All isolates were maintained on Sabouraud or Yeast Peptone Dextrose (YPD) agar plates for short term and in glycerol-supplemented medium at -80°C for long term storage. For determining fungal growth *in vitro*, Yeast Nitrogen Base (YNB) with 2% Glucose or Hams's Nutrient Mixture F12 medium (Sigma, Buchs, Switzerland) supplemented with L-Glutamine, Penicillin, Streptomycin, and 1% FCS was used. For other *in vitro* experiments and for *in vivo* and cell culture infection experiments, *C. albicans* was inoculated at an $\text{OD}_{595} = 0.1$ and grown in YPD medium at 30°C and 180 r.p.m. for 15–18 h. For induction of hyphae in liquid medium, Hams's Nutrient Mixture F12 medium (Sigma) supplemented with L-Glutamine, Penicillin, Streptomycin, and 10% FCS (filamentation assay) or 1% FCS (RNA-Isolation, damage induction) was used. For determination of the fungal cell wall composition and β -glucan exposure, *C. albicans* was grown in YPD medium at 37°C overnight and subcultured into fresh YPD at $\text{OD}_{600} = 2$ for 3.5 h at 37°C to obtain logarithmic-growing cells.

Microsatellite analysis. For microsatellite analysis, *C. albicans* was grown overnight on Sabouraud agar plates at 37°C and DNA was extracted using InstaGene matrix following the manufacturer's instructions. Primers were *Cdc3* fwd, 5'-CAGATGATTTTTTGTA TGAGAAGAA-3', *Cdc3* rev 5'-CAGTACAAGATTTAAATGTTCAAG-3', *Ef3* fwd 5'-TTTCTCTCTCTTCATATAGAA-3', *Ef3* rev 5'-GGATTCAGTACAGCAGACA-3', *His3* fwd 5'-TGGCAAAAA TGATATTCCAA-3', *His3* rev 5'-TACACTATGCCCCAAACAC A-3', *CaV* fwd 5'-TGCCAAATCTTGAGATACAAGTG-3', *CaV* rev 5'-CTTGCTTCTCTTGCTTTAAATTG-3', *CaVII* fwd 5'-GGGGAT AGAAATGGCATCAA-3', *CaVII* rev 5'-TGTGAAACAATTCTCTCTTGC-3'.^{48,49} One primer of each set was 5' labelled with Hexachloro-fluorescein (for CDC3, HIS3 and CAVII) fluorescein amidite (for EF3, CAV), respectively. PCR reactions were done according to standard protocols using HotStarTaq Polymerase (Qiagen, Hombrechtikon, Switzerland) and a 2720 Thermal Cycler (Applied Biosystems, Zug, Switzerland). Gene products were analyzed on a 3500 Genetic Analyzer using a GeneScan 600 Liz dye size standard and GeneMapper v4.1 software (Applied Biosystems).

Multilocus sequence typing. Multilocus sequence typing was performed as published previously.⁵⁰ Allele and diploid sequence type (DST) assignments were determined using the *C. albicans* multilocus sequence typing database (<http://pubmlst.org/calbicans>). A UPGMA dendrogram was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) v7 software (<http://www.megasoftware.net>).

C. albicans biofilm formation. Biofilm formation was assessed as described⁵¹ with the following modifications. After overnight culture in YPD, *C. albicans* yeast cells were washed twice in PBS and resuspended at 10^6 cells/ml in RPMI medium (Sigma) supplemented with 10% fetal calf serum (FCS). The cell solution was added to 1 cm²-sized FCS-treated adhesive silicone gel sheets (Smith and Nephew) placed into a 24-well cell culture plate and incubated for 90 min at 37 °C, 60 r.p.m. for initial cell adhesion. Silicone gel sheets were then washed with PBS to remove non-adherent cells and moved to a new 24-well plate containing 1 ml fresh medium. After 48 h at 37 °C in a humidified incubator without shaking, the squares were washed with PBS and biofilm was assessed at OD₄₉₀ after addition of 0.5 g/l filtered XTT (Sigma) in PBS.

C. albicans filamentation assays. After overnight culture in YPD, *C. albicans* yeast cells were washed twice in PBS and adjusted to 10^7 cells/ml in Hams's Nutrient Mixture F12 medium (Sigma) supplemented with L-Glutamine, Penicillin, Streptomycin and 10% FCS. One ml of this cell suspension was incubated for 3 h at 37 °C on a glass coverslips placed in a 24-well plate. After three time washing with PBS, cells were stained with Calcofluor white. Hyphae length was determined with an Axiolab Microscope (Carl Zeiss, Feldbach, Switzerland) and AxioVisionRel4.6 software.

For testing the colony morphology on Spider medium *C. albicans* cells were grown in YPD and washed in PBS as described above, and adjusted to 10^7 cells/ml in PBS. 5 µl of this cell suspension and of a 1:100 dilution was spotted on Spider medium plates and incubated for 10 days at 37 °C. Pictures were taken with a Dinolite Digital Microscope and the Dinocapture 2.0 software (www.dinolite.eu).

Analysis of the fungal cell wall composition. Logarithmically-growing *C. albicans* was washed three times in 50 ml of 1 mM cold phenylmethanesulfonyl fluoride (PMSF). 3 volumes of glass beads were then added to one volume of pellet and vortexed for 10–15 times at 1-minute intervals to break the cells. The cell lysate was again washed three times with 50 ml cold PMSF, before addition of 3 volumes of 2% SDS to the pellet and incubation for 10 min at 100 °C for two rounds. SDS was removed by washing the pellet in 1 mM PMSF and the pellet was dried overnight at 55 °C before re-suspension in 1 mM PMSF to a solution of 0.01 g dry weight/ml and storage at –20 °C. Total chitin was extracted and measured as described.⁵² Total mannan were extracted from isolated cell walls by alkali treatment and further precipitated with Fehling's solution as a copper complex as described.⁵³ Total glucan was extracted as described⁵⁴ and the extracted glucan and mannan components were then measured by the Dubois method⁵⁵ using glucose as a standard.

Determination of β-glucan exposure of C. albicans. Quantification of β-1,3-glucan exposure on the surface of *C. albicans* was done as previously published,⁵⁶ with some modifications. 200 µl of logarithmically-growing *C. albicans* suspension was pelleted in a 96-well plate by centrifugation at 3500 rpm for 5 min. Cells were fixed with 1% formaldehyde, washed twice with PBS and blocked with 1% BSA for 1 h at room temperature with gentle shaking, before incubation with an anti-β-1,3-glucan monoclonal antibody (mouse IgG, Biosupplies; 1:600 dilution in 1% BSA) overnight at 4 °C with gentle shaking, followed by three washing steps in PBS, incubation with an Alexa Fluor 488-coupled anti-mouse IgG (Molecular Probes; 1:300 dilution) for 1 h at room temperature with gentle shaking and three final washing steps in PBS. Relative fluorescent intensities were then calculated by taking the ratio of the tested sample stained with/without secondary Alexa Fluor 488-coupled anti-mouse IgG antibody.

Cell lines. The oral keratinocyte cell line TR146 (ref. 57, Sigma) was grown in Hams's F12 nutrient mixture supplemented with L-Glutamine, Penicillin, Streptomycin and 10% FCS if not indicated otherwise. For experiments, cells were seeded at 2×10^5 cells/well in

6-well plates or at 2×10^4 cells/well in 96-well plates, respectively, and grown to confluent monolayers for 2–3 days prior to infection as described below for the individual assays.

Filamentation of C. albicans on TR146 cells. For assessing *C. albicans* filamentation, TR146 cells were grown to confluency on cover slips placed in a cell culture dish. *C. albicans* was grown in YPD overnight, washed twice in PBS and added onto the keratinocytes in cell culture medium at 10^3 cells/ml. After 24 h, the medium was removed and the cells fixed with acetone followed by Calcofluor white staining. Microscopic pictures were taken with an Axiolab Microscope and AxioVisionRel4.6 software (Carl Zeiss).

Adherence of C. albicans to TR146 cells. Adherence assay was performed as described⁵⁸ with the following modifications. Monolayers of TR146 cells in 6-well plates were incubated with 10^2 *Candida* yeast cells for 2 h at 37 °C. The supernatant was then removed and distributed on YPD agar plates to determine the number of non-adherent fungal cells. After rinsing the 6-well plates with PBS the wells were overlaid with melted Wort agar at 40 °C and incubated for 1 day at 37 °C to count fungal colonies. Adherence was determined as (# of colonies grown on Wort agar)/(# of colonies grown on Wort agar) + (# of colonies grown from culture supernatant).

Cytokine secretion by TR146 cells. TR146 cells were grown to confluency in 96-well plates in Hams's F12 nutrient mixture with 1% FCS and stimulated with 2×10^4 *C. albicans* yeast cells per well for 24 h. Human IL-6, IL-8 and IL-1α secretion by TR146 cells was quantified with the ELISA MAX Deluxe Set kits (Biolegend, Fell, Germany) according to manufacturer's instructions.

LDH release assay. The damage assay was performed as described.⁵⁹ Briefly, TR146 cells were grown to confluency in a 96-well plate in grown in Hams's F12 nutrient mixture with 1% FCS and stimulated with 2×10^4 *C. albicans* yeast cells per well for the indicated time period. Control wells were incubated with medium only or lysed with 1% Triton-X-100 at the end of the incubation time to determine 100% damage. LDH release into the supernatant was quantified with the LDH cytotoxicity kit (Roche / Sigma) according to the manufacturer's instructions.

RNA isolation and quantitative RT-PCR from C. albicans. Fungal cells were disrupted in Trizol reagent (Sigma) with glass beads (0.5 mm) in a FastPrep24 (MP Biomedicals / Lucerna Chem AG, Luzern, Switzerland) for 5 cycles of 1 min at 50 Hz with cooling between cycles. RNA was isolated with Direct-zol RNA Mini Prep spin columns (Zymo research / Lucerna Chem AG, Luzern, Switzerland) and cDNA was generated by RevertAid reverse transcriptase (Thermo Scientific, Zug, Switzerland). Quantitative PCR was performed using Fast SYBR Green Master Mix and a FAST 3500 Genetic Analyzer (both Applied Biosystems). The primers were *Efb1* fwd 5'-CATTGATG GTACTACTGCCAC-3', *Efb1* rev 5'-TTTACCGGCTGGCAAGTC TT-3', *Kex1* fwd 5'-CTGATTCCGATTCCACCAAGT-3', *Kex1* rev 5'-ATGAGCGAGTGACATTGCT-3', *Kex2* fwd 5'-ATCGGCATCAC AACAACAAA-3', *Kex2* rev 5'-GCTGATTGTTGTCCCTCCTC-3', *Hwp1* fwd 5'-CGGAATCTAGTGTCTGCTCT-3', *Hwp1* rev 5'-TAGGAGCGACACTTGAGTAATTGG-3', *Ece1* fwd 5'-CGTTCC AGATGTTGGCCTTAATCT-3', *Ece1* rev 5'-CTGAGCCGGCATCT CTTTAACTG-3', *Cph1* fwd 5'-GGTGGCGGCAGTGATAGTG-3', *Cph1* rev 5'-GTGTACTCCGGTGACGATTTTTC-3', *Als3* fwd 5'-TC TCGTCCTCATTACCAACCAT-3', *Als3* rev 5'-GGGGATTGTA AAGTGGATTCTGTG-3', *Sap4* fwd 5'-GCCGATGGTTCTGTTGC AC-3', *Sap4* rev 5'-GAGCCGCTATACTGGCCTT-3', *Sap5* fwd 5'-TGGTTTTCAAAGCGGCGAAG-3', *Sap5* rev 5'-AGCATCAACA TTTCGTCCCC-3', *Sap6* fwd 5'-CGTGGTGACAGAGGTGACTT-3', *Sap6* rev 5'-TGGTAGCTTCGTTGGCTTGG-3', *Sap9* fwd 5'-TTTC AGCGACACTTTGCAGC-3', *Sap9* rev 5'-TGTCGACTGTTCTGC TGGAG-3', *Efg1* fwd 5'-TCCCACCACATGTATCGACAA-3', *Efg1* rev 5'-GAAGTGCTCGAGGCGTTCA-3'.

Mice. Wildtype (WT) C57BL/6J mice were purchased from Janvier Elevage. *Il17ra*^{-/-} and *Il17rc*^{-/-} mice were obtained from Amgen (Thousand Oaks, CA) and bred at the Laboratory Animal Service Center (University of Zürich, Switzerland). All mice were on the C57BL/6 background, kept in specific pathogen-free conditions and used in sex- and age-matched groups at 6–12 weeks of age. All mouse experiments described in this study were conducted in strict accordance with the guidelines of the Swiss Animal Protection Law and were performed under protocols approved by the veterinary office of the Canton Zürich, Switzerland (license number 201/2012 and 183/2015). All efforts were made to minimize suffering and ensure the highest ethical and humane standards.

OPC infection model. Mice were infected sublingually with 2.5×10^6 cfu *C. albicans* yeast cells as described⁶⁰ without immunosuppression. Mice were weighed daily. For determination of fungal burden, the tongue of euthanized animals was removed, homogenized in sterile 0.05% NP40 in H₂O for 3 min at 25 Hz using a Tissue Lyzer (Qiagen) and serial dilutions were plated on YPD agar containing 100 µg/ml Ampicillin.

Isolation of tongue cells. Mice were anaesthetized with a sublethal dose of Ketamine (100 mg/kg), Xylazine (20 mg/kg) and Acepromazine (2.9 mg/kg), and perfused by injection of PBS into the right heart ventricle prior to removing the tongue. Tongues were cut into fine pieces and digested with DNase I (200 µg/ml, Roche) and Collagenase IV (4.8 mg/ml, Invitrogen, Zug, Switzerland) in PBS at 37 °C for 45–60 min. Single cell suspensions were passed through a 70 µm strainer using ice-cold PBS supplemented with 1% FCS and 2 mM EDTA and analyzed by flow cytometry (see below).

Analysis of Th17 priming. Cervical lymph nodes were removed on day 7 post-infection and single cell suspensions were prepared by digested with DNase I (2.4 mg/ml, Roche) and Collagenase I (2.4 mg/ml, Invitrogen) in PBS for 15 min at 37 °C. For inducing cytokine secretion by primed T cells, 10^6 cervical lymph node cells were re-stimulated for 6 h with 10^5 DC1940 cells pulsed with 2.5×10^5 /ml heat-killed *C. albicans* or left unpulsed. Brefeldin A (10 µg/ml, AppliChem, Baden-Dättwil, Switzerland) was added for the last 5 h. IL-17 production by CD3⁺ CD4⁺ T cells was analyzed by intracellular cytokine staining and flow cytometry (see below).

Flow cytometry. All antibodies were from BioLegend, if not stated otherwise. Single cell suspensions of tongues were stained in ice-cold PBS supplemented with 1% FCS, 5 mM EDTA, and 0.02% NaN₃ with LIVE/DEAD Fixable Near-IR Stain (Invitrogen, Zug, Switzerland), anti-CD45.2 (clone 104), anti-CD11b (clone M1/70), anti-Ly6C (clone AL-21, BD Biosciences, Allschwil, Switzerland) and anti-Ly6G (clone 1A8). For intracellular cytokine staining, T cells were first incubated in ice-cold PBS containing LIVE/DEAD Fixable Near-IR Stain, anti-CD4 (clone RM4-5) and anti-CD3ε (clone 145-2C11). After fixation and permeabilization using BD Cytofix/Cytoperm (BD Biosciences) the cells were then incubated in Perm/Wash buffer (BD Biosciences) containing anti-IL-17A (clone TC11-18H10.1) and anti-IFNγ (clone XMGI.2) antibodies. Data were acquired on a FACS LSRII (BD Biosciences) or on a FACS Gallios (Becton Coulter, Nyon, Switzerland) and analyzed with FlowJo software (FlowJo LLC, www.flowjo.com). For all experiments, the data were pre-gated on live single cells.

RNA isolation and quantitative RT-PCR from mouse tissues. Isolation of total RNA from bulk tongues was carried out according to standard protocols using Trizol Reagent (Sigma). cDNA was generated by RevertAid reverse transcriptase (Thermo Scientific). Quantitative PCR was performed using SYBR Green (Roche) and a Rotor-Gene 3000 (Qiagen) or a QuantStudio 7 Flex (Invitrogen). The primers were *Actb* fwd 5'-CCCTGAAGTACCCCATTTGAAC-3', *Actb* rev 5'-CTTTTCACGGTTGGCCTTAG-3'; *Cxcl2* fwd 5'-AGTGAAGTCCGCTGTCAATGC-3', *Cxcl2* rev 5'-GCAACCTTTTGACCCGCT-3'; *S100a9* fwd 5'-GTCCAGGTCTCCATGATGT-3' and *S100a9* rev

5'-TCAGACAAATGGTGAAGCA-3'; *Il17a* fwd 5'-GCTCCAGAA GGCCCTCAGA-3' and *Il17a* rev 5'-AGCTTTCCTCCGCATTGA-3'; *Defb3* fwd 5'-GTCTCCACCTGCAGCTTTTAG-3' and *Defb3* rev 5'-ACTGCCAATCTG ACGAGTGTT-3'; *Il17f* fwd 5'-GAGGATAAC ACTGTGAGAGTTGAC-3' and *Il17f* rev 5'-GAGTTCATGGTG CTGTCTTCC-3'; *Il6* fwd 5'-GAGGATACCACTCCCAACAGA CC-3' and *Il6* rev 5'-AAGTGCATCATCGTTGTTTCATACA-3'; *Tnfa* fwd 5'-CATCTTCTCAAAATTCGAGTGACAA-3' and *Tnfa* rev 5'-TGGGAGTAGACAAGGTACAACCC-3'. All qPCR assays were performed in duplicates and the relative gene expression (rel. expr.) of each gene was determined after normalization with β-actin transcript levels.

Protein quantification by ELISA. Serum G-CSF were determined by sandwich ELISA according to standard protocols. The antibodies used were anti-G-CSF (clone 67604, R&D Systems, Zug, Switzerland) for coating and biotinylated polyclonal rabbit anti-G-CSF (Peprotech, London, UK) for detection, respectively. IL-17A and IL-17F cytokines were quantified by cytometric bead array (BD Biosciences) in tissue homogenates using the mouse IL-17F and IL-17A flex sets according to the manufacturer's instructions. For the preparation of tongue homogenates, the organ was cut in fine pieces and homogenated in 100 µl PBS supplemented with 1 x complete protease inhibitor cocktail (Roche) and 1% Triton-X-100 for 3 min at 25 Hz using a Tissue Lyzer (Qiagen). Data were acquired on a FACS Gallios (Becton Coulter) and analyzed with FloJo software.

Histology and immunofluorescence. For histology, tissues were fixed in 4% PBS buffered paraformaldehyde overnight and embedded in paraffin, or embedded in Tissue-Tek O.C.T compound (VWR International GmbH, Dietikon, Switzerland), snap-frozen in liquid nitrogen and stored at -20 °C. Sagittal sections (9 µm) were stained with periodic-acid Schiff (PAS), counterstained with Haematoxylin and mounted with Pertex (Biosystems, Switzerland) according to standard protocols. For immunofluorescence staining, tissues were embedded in Tissue-Tek O.C.T compound, snap-frozen in liquid nitrogen and stored at -20 °C. Sagittal cryosections (9 µm), were fixed with acetone for 10 min at room temperature, stained with anti-IL-1α (clone ALF-161, BioXCell, West Lebanon, NH, USA), anti-hamster-Cy3 (Jackson ImmunoResearch, Newmarket, UK) and 4',6'-Diamidino-2-phenylindole dihydrochloride (DAPI, Sigma) and mounted with Mowiol (VWR International GmbH, Dietikon, Switzerland). All slides were scanned with a NanoZoomer 2.0 HT (Hamamatsu Photonics K.K., Solothurn, Switzerland) using a 20x objective, and analyzed using NDP.scan 2.5.88 software.

Transmission electron microscopy. Transmission electron tissue samples were fixed in 2.5% glutaraldehyde (EMS/Lucerna-Chem AG, Luzern, Switzerland) buffered in 0.1 M Na-P-buffer overnight, washed three times in 0.1 M buffer, post fixed in 1% osmium tetroxide (Sigma) and dehydrated in ascending concentrations of ethanol followed by propyleneoxide and infiltration in 30 and 50% Epon (Sigma). At least three 0.9 µm toluidine blue stained semi-thin sections per localisation were produced. Representative areas were trimmed and subsequently 90 nm, lead citrate (Sigma) and uranyl acetate (Sigma) contrasted ultrathin sections were produced and viewed under Phillips CM10, operating with Gatan Orius Sc1000 (832) digital camera, Gatan Microscopical Suite, Digital Micrograph, Version 230.540.

Statistics. Statistical significance was determined by one-way analysis of variance with Dunnett's or Sidak's multiple comparison test, or by Pearson correlation coefficient and linear regression as appropriate using GraphPad Prism (GraphPad Software Inc., La Jolla, CA) with **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. Data displayed on a logarithmic scale were log-transformed before statistical analysis.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at <http://www.nature.com/mi>

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AUTHOR CONTRIBUTIONS

F.A.S. and S.L.L. designed the study and wrote the paper. F.A.S., E.G., A.G., N.S., F.K., F.S. and K.T.W. performed experiments and analyzed data. U.H. performed transmission electron microscopy. G.T.L. and N.P. performed experiments on fungal cell wall composition and β -glucan exposure. M.E.B. and C.D. provided fungal strains and provided advice. C.C.F. and S.L.L. supervised the project.

DISCLOSURE

The authors declared no conflict of interest.

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