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Candidalysin Sets Off the Innate Alarm:

Candidalysin-induced epithelial cell damage promotes expansion of innate TCRαβ$^+$ cells during Oropharyngeal Candidiasis.

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Genetic evidence and experimental studies have underlined the importance of IL-17-mediated immunity in the protection against mucosal fungal infections. Specifically, the role of IL-17 in antifungal immunity has been extensively studied in the mouse model of oropharyngeal candidiasis (OPC). CD4$^+$ T helper 17 (Th17) cells represent the most abundant source of IL-17 upon activation of adaptive antifungal immune responses. However, innate sources such as γδ T cells and the newly identified innate TCRαβ$^+$ cells have been shown to rapidly produce IL-17 at the early stages of oral *Candida albicans* infection (1). It was previously demonstrated that this rapid innate Type-17 response is essential for fungal clearance (1). However, the triggers that activate innate immunity in response to *C. albicans* invasion of the oral mucosa are not fully understood. Transition from the yeast to the hyphal form has been shown to be essential for *C. albicans* invasion of the oral epithelium (OEC) and leads to barrier injury (2). It was recently discovered that Candidalysin, a pore-forming peptide toxin secreted by *C. albicans* hyphae, is a crucial molecular determinant of epithelial cell damage (3). In this issue of *Science Immunology*, *Verma* et. al (4) have identified Candidalysin as the missing link between *C. albicans* invasion and the expansion of innate TCRαβ$^+$ cells in the oral mucosa.

Given that *C. albicans* is a human commensal, but is absent in rodents, mice are immunologically naïve to this fungus (1), thus providing a neat experimental system to study the rapid activation of innate immunity in response to *Candida*. Here, *Verma* et al. have studied the sequence of events that lead to activation of CD4$^+$CD44^{hi}CCR6$^+$ innate TCRαβ$^+$ cells in response to primary *Candida* infection in the mouse OPC model. Despite high levels of CCR6 expression, *Verma* et al. now show that innate TCRαβ$^+$ cells expansion during *C. albicans* infection is not due to CCR6-CCL20 mediated recruitment to the oral mucosa, but rather to local proliferation or recruitment through other mechanisms (4). Upon secondary *C. albicans* infection, activation of phagocytic cells through the Dectin-1/Syk/CARD9 and/or TLR2 pathways synergizes with *Candida*-specific memory Th17 cells to contain the

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infection (5–7). Here, by studying the activation of innate innate TCRαβ$^+$ cells to primary *C. albicans* infection, *Verma* et al. demonstrate that proliferation of TCRαβ$^+$ cells does not rely on the activation of TCR signaling or on signaling through Dectin-1 or TLR2. This suggests that antigen specificity or cell activation through these pattern recognition receptors is dispensable for innate TCRαβ$^+$ cells response in the OPC model.

How do innate TCRαβ$^+$ cells sense *C. albicans* invasion? Hyphal formation is essential for *C. albicans* mucosal invasion and epithelial cell damage, and the authors demonstrate that yeast locked *C. albicans* mutant was not able to activate IL-17 production in the oral mucosa. The hyphae-associated virulence factor Candidalysin, has been previously shown to be secreted exclusively by invasive hyphae (3). Thus, *Verma* et al. explored whether Candidalysin might be the trigger of the innate Type-17 response by TCRαβ$^+$ cells. Indeed, when mice were infected with a *C. albicans ece1*Δ/Δ, a strain lacking the Candidalysin-encoding extent of cell elongation 1 gene (*ECE1*), both the expansion of innate TCRαβ$^+$ cells and the expression of *Il17* were strongly reduced. In contrast to earlier observation by Moyes et al. (3), the authors now report a comparable fungal load between the wild type and the *C. albicans ece1*Δ/Δ, suggesting that the reduced innate TCRαβ$^+$ cells proliferation was not due to reduced fungal exposure.

To explain the observed phenotype, the authors next focused on understanding the mechanisms behind the Candidalysin-mediated initiation of the innate IL-17 response in the oral mucosa. The induction of rapid innate responses to *Candida* infection has been previously linked to epithelial damage (8). Candidalysin is essential to trigger such damage through the activation of a MAPK phosphatase MKP1/c-Fos dependent danger response, inducing pro-inflammatory IL-1β and IL-6 production (3). Pro-IL-1α stored in oral keratinocytes allows for the rapid release of active IL-1α during *C. albicans* infection (8); a mechanism conserved in response to other pathogens causing EC damage (9). *Verma* et al. found that *Il1β* expression was induced in a Candidalysin-dependent manner while *Il1*α was only partially dependent on Candidalysin. Consistently, the authors detected decreased numbers of innate TCRαβ$^+$ cells in IL-1R deficient mice. *Verma* at al. set to identify whether IL-1 signaling is essential for innate TCRαβ$^+$ cell activation during oral *C. albicans* infection. The authors irradiated congenically marked wild type and *Il1r1*$^{-/-}$ mice and reconstituted them with the same or reciprocal bone marrow cells. However, this effort failed to identify a clear role for IL-1 signaling in hematopoietic or non-hematopoietic cell compartments, suggesting that either the effect of IL-1 on innate TCRαβ$^+$ cells expansion was indirect or the involvement of radio-resistant cells mediating this effect.

Although assessed only *in vitro*, IL-17 production appears to further act in a positive feedback loop in synergy with Candidalysin to amplify the upregulation of several cytokines and damage-associated molecular patterns (DAMPs), including IL-1α/β by epithelial cells, which are crucial to drive proliferation of IL-17-producing innate oral TCRαβ$^+$ cell (Fig. 1). Overall, the work of *Verma* et al. suggests that Candidalysin acts as a danger signal during the invasion of hyphae through the epithelium and promotes OEC damage that triggers protective innate IL-17-mediated antifungal immunity.

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Despite establishing a role for candidalysin in alerting the innate T cell response, some important questions remain. What is the mechanism behind Candidalysin triggered initial release of cytokines and DAMPs by the epithelium? Inflammasomes are possible candidates since NLRP3 (7) and NLRC4 (10) responses are important for the control of *Candida* infection. Are innate TCR$\alpha\beta^+$ cells able to directly sense EC damage caused by Candidalysin or do they rely on help by other innate cells, such as resident phagocytes or innate lymphoid cells (ILCs)? In the present study, the authors have assessed the effect of Candidalysin using a single strain of *C. albicans*: SC5314. Yet, a recent study performed with several *C. albicans* isolates reports that the expression of Candidalysin does not always correlate with *in vivo* pathogenicity (8). Additional factors are thus likely to further contribute to the capacity of *C. albicans* to cause epithelial damage. In this regard, further studies on *C. albicans* produced toxins might shed light on how this opportunistic fungus modulates innate and adaptive immunity to influence the balance between pathogenicity and commensalism. As humans are constantly exposed to *C. albicans* and likely harbor adaptive memory to this fungus, the role of Candidalysin during a secondary oral infection remains to be explored.

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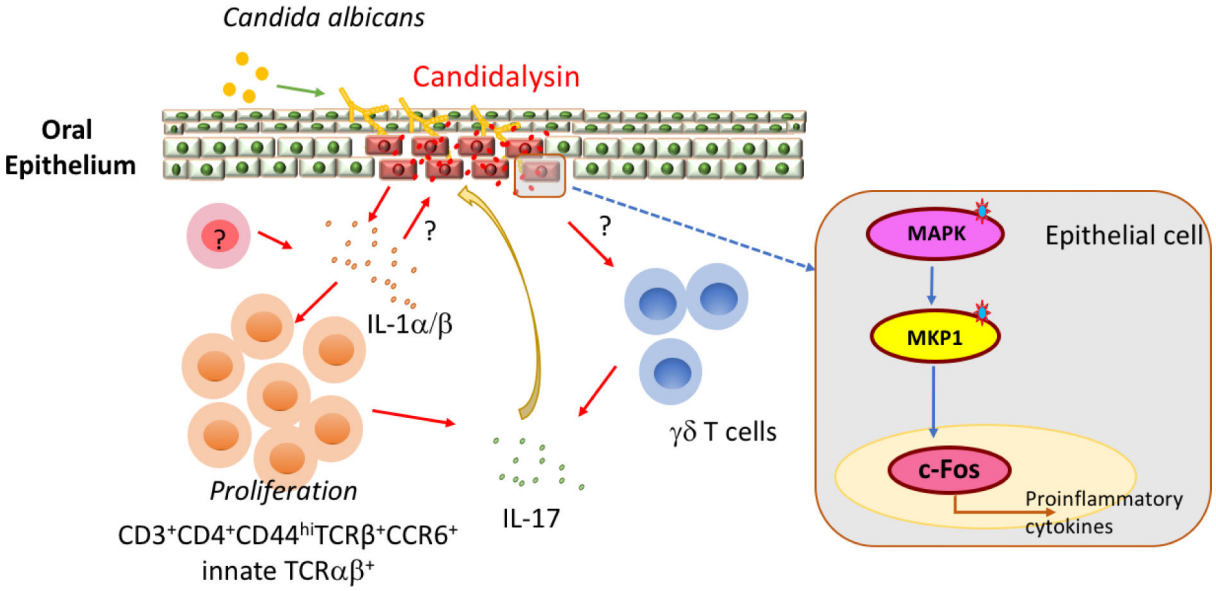

Figure 1. Candidalysin drives innate Type-17 response to oral Candidiasis.

C. albicans switches from yeast to hyphal form upon adhesion to host cells in the oral mucosa. At the invasive hyphal stage, Candidalysin is secreted by invasive hyphae and triggers epithelial cell damage through the activation of a MAPK phosphatase MKP1/c-Fos dependent danger response. Candidalysin-induced oral epithelial damage leads to the production of IL-1α/β from epithelial cells and/or other unidentified cells types. IL-1α/β further drives innate TCRαβ$^+$ cells expansion and IL-17 production, possibly by both innate TCRαβ$^+$ and γδ T. IL-17 and Candidalysin synergistically amplify the danger responses, inducing IL-1α/β and other inflammatory mediators. This positive feedback-loop, linking pro-inflammatory cytokines with fungal virulence factor, is essential for the establishment of a protective innate response to oral candidiasis.