Introduction

Following the discovery of T helper 17 (Th17) cells in 2005, considerable research efforts identified interleukin 17 (IL-17) and Th17 responses as essential components of immunity to the commensal fungus *Candida albicans*. Much less is understood about regulatory T cells (Tregs) in candidiasis. However, emerging data point towards a surprisingly complex relationship between IL-17/Th17 and Treg responses during *C. albicans* infections, wherein Tregs both suppress and enhance immunity. This review will discuss the role of these responses during candidiasis and the consequences for disease outcome and therapy.

IL-17/Th17 Responses Are Key Mediators of *C. albicans* Antifungal Immunity

IL-17-mediated immunity is crucial for protection against *C. albicans* infections, especially mucocutaneous infections, including oral and dermal candidiasis [reviewed in [1]]. “Experiments of nature” have revealed mutations in humans that cause susceptibility to chronic mucocutaneous candidiasis (CMC), nearly all of which impact the IL-17/Th17 pathway (Table 1, reviewed in [2]). For example, individuals with mutations in *IL17RA, IL17RC, IL-17F*, or the IL-17 family-specific signaling molecule *ACT1* suffer from CMC [3,4] (Casanova and Puel, personal communication; see Acknowledgments). CMC can be defined as a heterogeneous group of disorders characterized by persistent or recurrent *Candida* infection of mucosal membranes, skin, and nails. To date, there is no animal model that fully recapitulates the complex phenotype of CMC. However, models of oral and dermal candidiasis are in agreement with human data. IL-23/IL-17 and *IL-17A*-specific memory T cells in *C. albicans* OPC [21]. Moreover, most *Candida*-specific memory T cells in humans are Th17 cells. Similarly, in models of adaptive immunity, Th17 and not Th1 cells are induced by *Candida* and are protective against oral infections [20,22].

IL-17 is produced by both conventional Th17 cells and by innate cells [23]. One recent report proposed a role for innate lymphoid cell (ILC) production of IL-17 in host defense against OPC [24]. However, IL-17 production by ILCs was not directly demonstrated. Notably, Rag1−/− mice, which lack T cells but have enriched numbers of ILCs, are highly susceptible to OPC [20,25], raising questions about the relevance of ILCs in oral candidiasis. Our recent data show that following immediate exposure to *C. albicans*, oral IL-17 is produced not by ILCs but by γδ-T cells and disseminated candidiasis, an issue that will need to be monitored, particularly considering the impending use of anti-IL-17 biologic therapy for autoimmunity [16].

IL-17 Function and Sources

IL-17 exerts protective effects principally through the recruitment and activation of neutrophils. IL-17 primarily acts upon nonhematopoietic cells by stimulating the production of cytokines and chemokines, such as granulocyte-colony stimulating factor (G-CSF), interleukin 8 (IL-8) (humans), CXCL1, and CXCL5, which serve to expand and recruit neutrophils [1]. Depletion of neutrophils renders mice susceptible to OPC [17] and disseminated candidiasis [18]. Additionally, IL-17 signaling promotes anti-*Candida* killing mechanisms such as production of antimicrobial peptides (e.g., salivary histatins, β-defensins, and S100A8/9) [5,9,19].

CD4+ T cells are traditionally considered to be the primary cellular source of IL-17 during mucosal *C. albicans* infections [5,20]. This assumption is based on the observation that patients with HIV/AIDS exhibit dramatically heightened susceptibility to OPC [21]. Moreover, most *Candida*-specific memory T cells in humans are Th17 cells. Similarly, in models of adaptive immunity, Th17 and not Th1 cells are induced by *Candida* and are protective against oral infections [20,22].

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Treg Cells: Regulators of Infectious Disease

Tregs are a distinct subset of CD4+ T cells whose primary function is to restrict potentially pathogenic inflammatory immune responses. Tregs possess an extensive armory of suppressive mechanisms that can be cell contact dependent (acting through inhibitory receptors such as cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]) or cell contact independent (acting via inhibitory cytokines and generation of suppressive metabolites) (reviewed in [27]). This suppressive tool kit makes Tregs adept at controlling cell types from both the innate and adaptive arms of the immune system. Several Treg subsets exist, including Tregs expressing CD25 and the canonical Treg transcription factor Foxp3 that will be the focus of this review. Foxp3+ Tregs can be further divided into thymus-derived Tregs, which are fully differentiated in the thymus, and peripherally derived [p/Tregs], which differentiate from naive CD4+ T cells in the periphery following antigen stimulation. Although a detailed description of Treg biology is beyond the scope of this review, we refer the reader to several excellent reviews for further information on this subject [27,28].

It is now appreciated that Tregs contribute to immunity against infectious pathogens. Inflammatory effector responses are critical in host defense against pathogens. However, excessive inflammatory responses can be damaging and therefore must be tightly regulated. A beneficial role for Treg-mediated restraint of immunopathology has been demonstrated in several viral and parasitic infections [29,30]. In some settings, Tregs are also required for long-term maintenance of protective immunity, for parasitic infections [29,30]. In some settings, Tregs are also regulated. A beneficial role for Treg-mediated restraint of inflammatory responses can be damaging and therefore must be tightly regulated. A beneficial role for Treg-mediated restraint of immune cell recruitment to sites of infection [33]. Therefore, Tregs can have diverse impacts, depending on the infection.

IL-17/Th17 and Treg Responses Are Intricately Linked during Candidiasis

Treg responses are elevated during C. albicans infections, suggesting a functional role. An increase in the proportion of CD4+CD25+ cells and expression of Foxp3 was detected in the mesenteric lymph nodes (LNs) and stomachs of mice intragastrically inoculated with C. albicans [34,35]. Similarly, CD4+CD25+Foxp3+ cells expanded in mice systemically infected with C. albicans [36]. However, Treg-mediated responses to C. albicans, and indeed to other fungi, remain poorly understood.

Th17 and Treg subsets are reciprocally regulated during naive T cell differentiation [37]. Reciprocal regulation of such responses was observed in a model of gastrointestinal candidiasis, in which increased Treg responses were associated with reduced Th17 responses and vice versa [34,35]. Conversely, Tregs can also promote Th17 responses [37]. Accordingly, IL-17/Th17 and Treg responses are positively associated during OPC and disseminated candidiasis (Fig. 1). Treg depletion by anti-CD25 treatment results in concurrent depletion of Th17 cells during OPC. In the same model, co-transfer of CD4+CD25+ and CD4+CD25- cells into Rag1-/- mice enhanced protective Th17 responses [25]. In disseminated candidiasis, Tregs suppressed Th1 and Th2 responses while promoting Th17 responses in vitro [36]. Furthermore, Foxp3+ cell depletion in vivo was associated with reduced IL-17/Th17 responses [36]. Notably, both studies provide evidence that the mechanism of action is, at least in part, through consumption of IL-2 by Tregs through the high affinity IL-2R [25,36]. IL-2 is essential for Treg survival but limits Th17 differentiation [38]. Therefore, Treg consumption of IL-2 reduces its local concentration, favoring Th17 development [25,36,39]. Whether IL-2 consumption by Tregs is a dominant mechanism for driving IL-17 responses during candidiasis remains an open question.

Plasticity is a phenomenon whereby CD4+ T cell subsets acquire characteristics of other populations [reviewed in [40]]. For
example, in some settings Tregs can express RORγt and produce IL-17A. Indeed, pTregs and Th17 cells possess an especially high degree of phenotypic flexibility [40], which has been observed in antifungal immunity. Specifically, dendritic cell recognition of β-glucans in the Candida cell wall by dectin-1 promotes conversion of Tregs to a RORγt+IL-17A+ phenotype [41]. Moreover, CD4+CD25+Foxp3+ cells isolated from systemically infected mice expressed RORγt and produced IL-17A, with the majority also expressing pTreg markers [36]. Collectively, these studies indicate that Tregs can promote IL-17/Th17 responses and acquire characteristics of Th17 cells in response to C. albicans.

Final Outcome: Location, Location, Location

Although IL-17/Th17 and Treg responses can act cooperatively during candidiasis, disease outcome is strikingly different depending on infection site. In OPC, Th17 enhancement by Tregs increased resistance to infection [25]. In contrast, Treg enhancement of Th17 responses in disseminated candidiasis was associated with reduced resistance [36]. These studies suggest that inflammatory Th17 and Treg responses are protective at mucosal surfaces but pathogenic in systemic candidiasis. Consistent with this idea, humans with defective Th17 and Treg responses are susceptible to CMC but not to disseminated candidiasis [42–45]. However, the concept that elevated Th17 and Treg responses are harmful in disseminated candidiasis seemingly contrasts with the apparent protective role of IL-17 in mice [12–14,36]. One explanation is that these studies use knockout animals with complete genetic ablation of IL-17 components and therefore do not address the requirement of balanced immune responses. In support of a pathogenic role for unbalanced Th17 and Treg responses during candidiasis, cytokines associated with Th17 responses positively correlate with increasing disease severity in disseminated candidiasis [46]. Similarly, overzealous Th17 responses are associated with immunopathology in gastrointestinal candidiasis [47]. Furthermore, depletion of Tregs during disseminated candidiasis increases resistance to disease [48]. Ultimately, the balance between protective versus pathogenic immunity is crucial in determining disease outcome.

How immune responses are shaped depends on factors in the microenvironment. Commensal microbes ferment dietary fibers to short chain fatty acids (SCFAs) that favor tolerogenic Tregs [49]. Additionally, transforming growth factor beta (TGFβ) and retinoic acid, which are enriched at the intestinal mucosa, promote Tregs...
over Th17 responses [50]. Since C. albicans is a commensal of human mucosa, it is likely that these tissues have evolved tolerogenic mechanisms to live in harmony with this fungus. In contrast, internal organs are shielded from the external environment and typically lack high levels of SCFAs and retinoic acid. Therefore, an immune response induced during C. albicans infection is more likely to go unchecked compared to mucosal surfaces, resulting in collateral tissue damage. Overall, site-specific factors are pivotal in dictating the balance between protective and pathogenic Th17 and Treg responses.

Concluding Remarks

It is clear that IL-17/Th17 and Treg cells have a complex relationship, exemplified during infections with C. albicans. Although Th17 and Treg responses appear to be reciprocally regulated in certain situations (e.g., gastrointestinal candidiasis), Tregs promote Th17 responses and even acquire phenotypic characteristics of Th17 cells in other settings (e.g., oral and disseminated candidiasis). Notably, the impact of Th17 and Treg responses on disease outcome is distinct in different forms of candidiasis, highlighting the importance of microenvironment in shaping overall immunity. Elucidating the factors that determine the balance between protective versus pathogenic Th17 and Treg responses during candidiasis will be an important future avenue of research. Ultimately, it may be possible to exploit this information in order to help tune appropriate responses in the context of candidiasis.

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References

1. Hernández-Santos N, Gaffen SL (2012) Th17 cells in immunity to Candida albicans. Cell Host Microbe 11: 425–435.
2. Hupper AR, Bishu S, Gaffen SL (2012) Mucocutaneous candidiasis: the IL-17 pathway and implications for targeted immunotherapy. Arthritis Res Ther 14: 217.
3. Paul A, Cypowyj S, Bastamaante J, Wright JP, Liu L, et al. (2011) Chlamydia muridarum mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immune system. Science 332: 65–68.
4. Boisson B, Wang C, Pedergnana V, Wu L, Cypowyj S, et al. (2013; An ACT1 mutation selectively abolishes interleukin-17 responses in humans with chronic mucocutaneous candidiasis. Immunity 39: 676–689.
5. Coni HR, Shen F, Nayar N, Scocum E, Sun JN, et al. (2009) Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against candidiasis. J Exp Med 206: 299–311.
6. Ho AW, Shen F, Coni HR, Patel N, Childs EE, et al. (2010) IL-17RC is required for immune signaling via an extended SEF/IL-17R signaling domain in the cytoplasmic tail. J Immunol 185: 1063–1070.
7. Ferreira MC, Whibley N, Mamo AJ, Siebenlist U, Chan YR, et al. (2014) Dectin-2 required for immune signaling via an extended SEF/IL-17R signaling domain in the cytoplasmic tail. J Immunol 185: 5453–5462.
8. Yano J, Kolls JK, Happel KL, Wormley F, Wozniak KL, et al. (2012). The acute neutrophil response mediated by S100 alarmins during vaginal candidal infection is independent of the Th17 pathway. PLoS ONE 7: e4911.
9. Pietrella D, Rachini A, Pines M, Pandey N, Mosci P, et al. (2011) Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against Candida albicans. J Immunol 185: 1063–1070.
10. Sciot-Browne JP, Shafiani S, Tucker-Heard G, Ishida-Tsubota K, Fontenot JD, et al. (2007) Expansion and function of Foxp3-expressing T regulatory cells during parasitic helminthic infections. J Immunol 179: 1357–1366.
11. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL (2002) CD4+ CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 420: 502–507.
12. Neufeld ZS, Lu LF, Rudensky AY (2012) Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 30: 587–620.
13. Bonifazi P, Zelante T, D’Angelo C, De Luca A, Moretti S, et al. (2009) Oral-resident ‘natural’ Th17 cells and Th17 responses on disease outcome is distinct in different forms of candidiasis.

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43. Kekalainen E, Tuovinen H, Joensuu J, Gylling M, Franssila R, et al. (2007) A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Immunol 178: 1208–1215.

44. Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, et al. (2011) Defective IL-10 signaling in hyper-IgE syndrome results in impaired generation of tolerogenic dendritic cells and induced regulatory T cells. J Exp Med 208: 233–249.

45. Sharfi N, Dadi HK, Shahar M, Roifman CM (1997) Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. Proc Natl Acad Sci U S A 94: 3160–3171.

46. MacCallum DM, Castillo L, Brown AJ, Gow NA, Odds FC (2009) Early-expressed chemokines predict kidney immunopathology in experimental disseminated Candida albicans infections. PLoS ONE 4: e6420.

47. Zelante T, De Luca A, Bonifazi P, Montagnoli C, Bozza S, et al. (2007) IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. Eur J Immunol 37: 2695–2706.

48. Netea MG, Smits R, Hermann C, Van der Graaf CA, Van der Meer JW, et al. (2004) Toll-like receptor 2 suppresses immunity against Candida albicans through induction of IL-10 and regulatory T cells. J Immunol 172: 3712–3718.

49. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, et al. (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341: 569–573.

50. Mucida D, Lee Y, Kim G, Turovskaya O, Scott I, et al. (2007) Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 317: 256–260.

51. Ferwerda B, Ferwerda G, Plantinga TS, Willhurt JA, van Spijlen AB, et al. (2009) Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med 361: 1760–1767.

52. Ma CS, Chew GY, Simpson N, Priyadarshi A, Wong M, et al. (2008) Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. J Exp Med 205: 1531–1537.

53. Mihm JD, Breachley JM, Laurence A, Freeman AF, Hill BJ, et al. (2006) Impaired Th17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 452: 773–776.

54. Minegishi Y, Saito M, Morio T, Watanabe K, Agematsu K, et al. (2006) Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity 25: 743–755.

55. Engelhardt KR, McGhee S, Winkler S, Sassi A, Woellner C, et al. (2009) Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J Allergy Clin Immunol 124: 1289–1302.e1284.

56. Liu L, Okada S, Kong X, Krems C, Cypowyj S, et al. (2011) Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. J Exp Med 208: 1633–1648.

57. van de Veerdonk FL, Plantinga TS, Bloemen H, Smeekens SP, Joosten LA, et al. (2011) STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N Engl J Med 365: 34–61.

58. de Beaucoudrey L, Samarina A, Hostamante J, Cobo A, Boisson-Dupuis S, et al. (2010) Revisiting human IL-12Rbeta1 deficiency: a survey of 141 patients from 30 countries. Medicine (Baltimore) 89: 381–402.

59. Kisand K, Bror Wolf A, Podkrajsek KT, Tserel L, Link M, et al. (2010) Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 207: 299–308.

60. Puel A, Doffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, et al. (2010) Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 207: 291–297.