CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Epithelial Cells and Innate Antifungal Defense

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ABSTRACT

The human pathogenic fungus *Candida albicans* is the predominant cause of both superficial and invasive forms of candidiasis. Clinical observations indicate that mucocutaneous *Candida* infections are commonly associated with defective cell-mediated immune responses. The importance of the innate immune system as a first-line defense against pathogenic challenge has long been recognized. Over the last decade, many key molecules mediating innate host defense have been identified. Central to these developments is the discovery of pattern recognition receptors such as Toll-like receptors and C-type lectin-receptors that induce innate immune responses and also modulate cellular and humoral adaptive immunity during *Candida* infections. Although a large amount of information is now available in systemic infections, little is known about localized infections. We address the most relevant pattern recognition receptors and their signaling mechanisms in oral epithelial cells, to gain a better understanding of their contributions to antifungal innate immunity.

KEY WORDS: oral epithelium, innate immunity, Toll-like receptors, C-type lectin-receptors, *Candida albicans.*

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INTRODUCTION

The mucosal epithelium has immense importance in host defense and immune surveillance, since it is the primary cell layer that initially encounters the majority of micro-organisms. The most important function of the immune system is to discriminate between friend and foe, a property that is essential for maintaining immune homeostasis. This specialized interaction will result in either passive coexistence between microbe and host, as in the case of commensal microbes, or in a violation of the mucosal barrier and subsequent cell injury, as in the case of microbial pathogens. The cells that comprise the innate immune response are primarily phagocytes, including neutrophils and macrophages, and the cells that line the epithelial mucosa. Originally, it was thought that the epithelium serves only as a passive barrier against invading pathogens. Barrier function alone is usually adequate to restrain commensal microbes, but is often insufficient to protect against microbial pathogens. However, recently it has become apparent that epithelial cells are capable of triggering an immune response similar to that of cells of the myeloid lineage, thus playing a crucial role in the active recognition of microbes. Accordingly, the oral epithelium is able to secrete a variety of defense effector molecules (Diamond *et al.*, 2008) and to orchestrate an immune inflammatory response to activate myeloid cells in the submucosal layers to clear the invading pathogens (Cutler and Jotwani, 2006).

The frequency of mucosal and cutaneous fungal infections is increasing worldwide, with oral candidiasis being the most common human fungal infection, especially in early and later life (Samaranayake *et al.*, 2009). Oral candidiasis is a common opportunistic infection of the oral cavity and presents a challenge for immunologically competent and immunodeficient patients alike. Various clinical presentations are traditionally divided into acute and chronic forms. Acute pseudomembranous candidiasis (mucosal candidiasis, oral thrush) presents with stippled (later confluent) white plaque (that can be wiped off) on bright red and lightly bleeding mucosa. Chronic atrophic candidiasis (denture-related stomatitis) is associated with erythema and edema of the oral mucosa, often found on the fitting surfaces of dentures. Also belonging to the category of oral candidiasis are perlèche, candidal leukoplakia (chronic hyperplastic candidiasis), candidal cheilitis, and chronic mucocutaneous candidiasis (CMC), a rare form that is associated with immune deficiency. Life-threatening systemic infection is generally limited to severely immunocompromised patients, such as neutropenic patients, often after nosocomial infection. The number of fungal infections as a proportion of all nosocomial infections doubled during a 10-year period in the United States. In immunocompetent patients, predisposing factors are responsible for infection or even chronic recurrent mucocutaneous candidiasis. Oropharyngeal and vaginal infections are the most common manifestation; predisposing factors include antibiotic, glucocorticosteroid, and hormone therapies, as well as

diabetes mellitus and infections such as HIV and AIDS. Around 80% of all fungal infections are caused by *Candida*, typically *Candida albicans* (*C. albicans*) (Ruhnke, 2006). However, non-*albicans Candida* spp., such as *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida guilliermondii,* and *Candida krusei* are also pathogenic to humans and have emerged as important opportunistic pathogens in the oral mucosa (Li *et al.*, 2007; Samaranayake *et al.*, 2009).

C. albicans interacts with epithelial cells in terms of adherence, invasion, and induction of cell damage (Fig. 1). Virulence factors are crucial in determining the role of opportunistic pathogens in infections. Important virulence factors expressed by *C. albicans* include dimorphism (ability to grow in either yeast or mycelial form), adhesion factors, phenotypic switching, thigmotropism (ability to identify intercellular junctions at the mucosal surface by contact sensing and their targeted penetration), and secretion of hydrolytic enzymes such as lipase, phospholipase, and proteinase (reviewed in Calderone and Fonzi, 2001; Schaller *et al.*, 2005). The interaction between virulence factors of *C. albicans* and host defense mechanisms plays a central role in determining whether colonization remains harmless or leads to infection of the epithelium and possibly systemic infection. Recognition of *C. albicans* by the innate host defense system is mediated by pattern-recognition receptors (PRRs) from the Toll-like receptor (TLR), C-type lectin-receptor (CLR), and NOD-like receptor (NLR) families (Netea *et al.*, 2008a; Bryant and Fitzgerald, 2009). To date, most investigations have focused on the interaction of *C. albicans* with macrophages, and on systemic infections. At present, we understand little about how the oral mucosa regulates itself in the context of fungal infections, although recent studies have furthered our understanding of pathogen recognition and signaling mechanisms in oral epithelial cells. This review will discuss recent advances in our understanding of the role PRRs play in signaling or regulating the immune response against fungal pathogens in the oral mucosa. In addition, since PRRs form a crucial link between innate and adaptive immunity, adaptive cell responses will also be discussed.

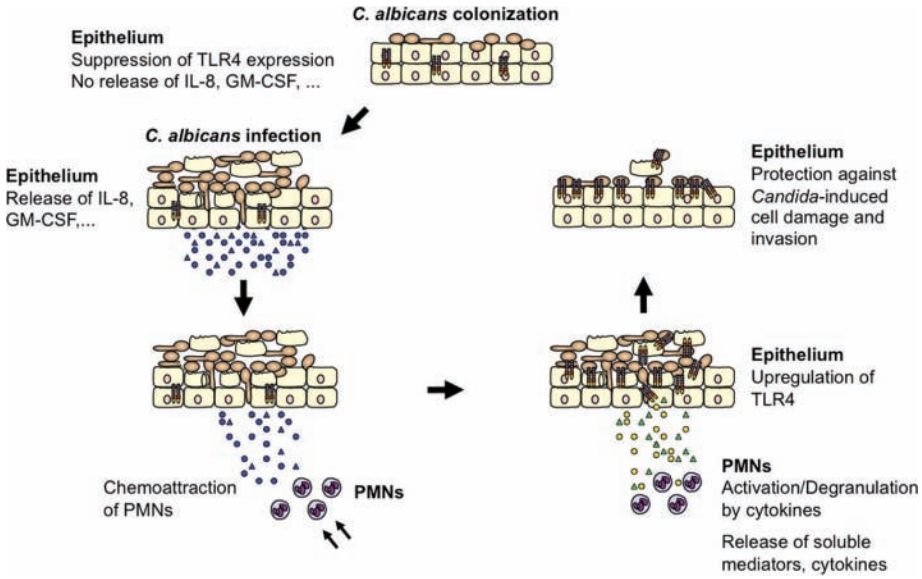

Figure 1. Model of TLR4-mediated and PMN-dependent antifungal defense by the oral epithelium. In general, experimental oral infection can be divided into 3 phases: an attachment phase, an invasion phase, and a tissue destruction phase. Although *C. albicans* normally exists as a yeast cell, adherent yeast cells rapidly form germ tubes after contact with epithelial cells, and hyphae penetrate the epithelium. Tissue damage is increased dramatically over time, when hyphae penetrate the tissue not only in the top layer, but also in deeper epithelial cell layers. In contrast, epithelial cells control fungal cell growth and invasion. During colonization of the oral epithelium, *C. albicans* suppresses TLR4 expression and does not induce cytokine production. Infection, particularly in predisposed patients, leads to increased cytokine secretion that recruits and stimulates PMNs at the site of infection. After recruitment, several cytokines, especially TNF, are directly involved in initiating the subsequent PMN-mediated up-regulation of epithelial TLR4 *via* a process that does not require PMN infiltration of the mucosal tissues. Finally, epithelial TLR4 directly protects the oral mucosa from fungal invasion and cell injury, possibly by production of antimicrobial peptides.

PATTERN-RECOGNITION RECEPTORS IN FUNGAL RECOGNITION

The innate immune system recognizes conserved pathogen-associated molecular patterns (PAMPs), which represent broad groups of microbial species rather than a single specific species, through germline-encoded proteins, such as PRRs (Janeway and Medzhitov, 2002).

Toll-like Receptors

TLRs are a family of evolutionarily conserved receptors that react to bacterial, viral, or fungal antigens or to endogenous factors released during cell injury. The capacity to recognize a variety of common microbial antigens and endogenous factors indicates that a primary function of TLRs is to act as sentinel receptors to alert the innate immune system to infection or tissue damage (Takeda *et al.*, 2003). To date, the TLR family is composed of 10 members in humans (TLR1–TLR10) and 12 in the mouse (TLR1-TLR9 and TLR11-TLR13). All TLRs are characterized as type I transmembrane receptors with an extracellular leucine-rich repeat domain and a cytoplasmic tail with high similarity to the type 1 interleukin-1 (IL-1) receptor. The leucine-rich repeat domains of TLRs bind different microbial components (PAMPs), including bacterial cell wall molecules such as lipopolysaccharide and peptidoglycan, proteins (*e.g.*, flagellin), as well as double- or single-stranded RNA of viruses or unmethylated CpG DNA. Ligation of TLRs leads to activation of a protease cascade inducing transcription factors such as nuclear factor (NF)-κB and interferon regulatory factors 3/7, followed by enhanced transcription of antimicrobial peptides,

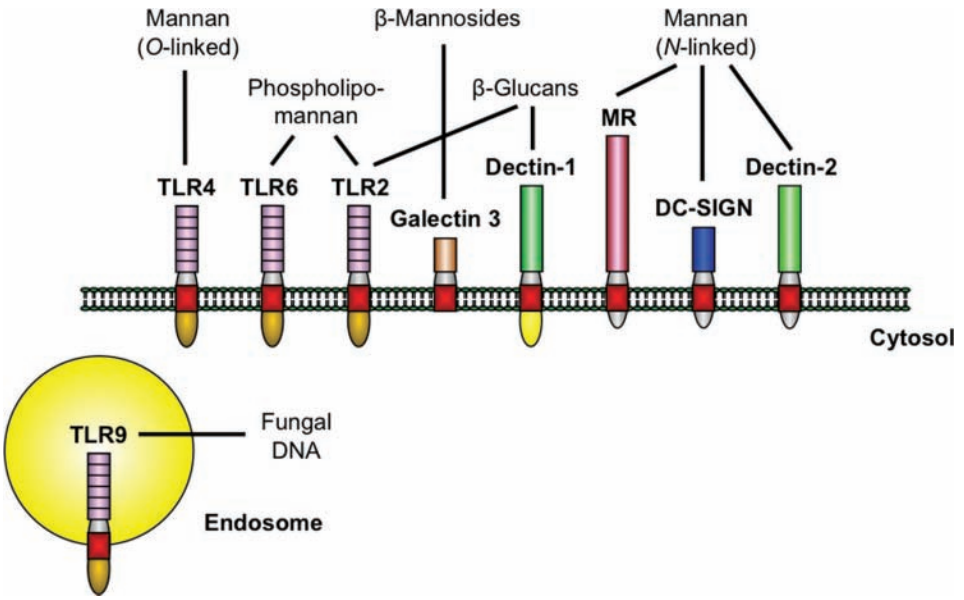

Figure 2. The major PRRs involved in the recognition of specific *C. albicans* PAMPs. Stimulation of host response by *C. albicans* at the cell membrane is mediated by a limited number of PRRs from the TLR and CLR families. Upon activation, the receptors trigger common adaptor molecules, intracellular pathways, and transcription factors (not shown). However, the specificity of the host response is maintained by the different repertoire of receptors stimulated by certain fungal PAMPs, as well as by the complex interactions between pathways. The depicted PRRs are predominantly expressed on cells of the myeloid lineage. Oral epithelial cells have been shown to express functional TLR2, TLR4, TLR6, and TLR9, emphasizing the importance of TLRs in the interaction of *C. albicans* and oral epithelium.

cytokines, chemokines, and co-stimulatory molecules. As such, TLRs function as critical mediators between innate and adaptive immune responses.

The recognition of *C. albicans* and other medically important fungi by cells of the immune system have been recently reviewed extensively (Roeder *et al.*, 2004; Netea *et al.*, 2006a); thus, only a short overview will be presented here. The major PRRs and their putative ligands derived from *C. albicans* are illustrated in Fig. 2. Most studies so far have addressed the 3 major fungal pathogens, *C. albicans, Aspergillus fumigatus*, and *Cryptococcus neoformans,* and only few reports dealt with specific fungal PAMPs and their involvement in innate immunity (Roeder *et al.*, 2004). While the major focus of antifungal innate immunity has been on systemic *Candida* infections, less is known about the function of TLRs during localized fungal infections. From the study of fungal infections in knock-out mice deficient in either TLRs or TLR-associated adaptor molecules, it appears that specific TLRs such as TLR2, TLR4 (Roeder *et al.*, 2004; Netea *et al.*, 2008a; Gil and Gozalbo, 2009), TLR6 (Netea *et al.*, 2008b), and TLR9 (Bellocchio *et al.*, 2004; van de Veerdonk *et al.*, 2008; Miyazato *et al.*, 2009) play differential roles in the activation of the various arms of the innate immune response. Deletion of the intracellular adaptor MyD88 renders mice highly susceptible to infections with fungi, although the role of the individual TLRs has not been established decisively in all cases and is often controversial. Different research groups have demonstrated divergent roles for TLR2 and TLR4, and their importance in the control of *C. albicans* and *C. neoformans* infections is still unclear.

C-type Lectin-receptor Receptors

The CLRs are a large superfamily of proteins characterized by C-type lectin-like domains (Zelensky and Gready, 2005). Several extracellular and transmembrane CLRs—including the mannose receptor (MR), Dectin-1, Dectin-2, dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), and macrophage-inducible C-type lectin (Mincle)—are involved in antifungal immunity, although their roles have not been completely understood. Importantly, these receptors mediate fungal binding, uptake, and killing and also contribute to the initiation and/or modulation of the immune response to these organisms (Netea *et al.*, 2008a; Willment and Brown, 2008).

The MR (CD206) is a prototypical type I (group VI) transmembrane protein and is mainly expressed by macrophages, as well as by DCs (Taylor *et al.*, 2005b). Several organisms, including *C. albicans*, *C. neoformans*, *Pneumocystis carinii*, and other pathogens such as bacteria and viruses are recognized by the MR (Taylor *et al.*, 2005a; McKenzie *et al.*, 2007). The receptor binds mannose, fucose, N-acetylglucosamine, and glucose. After carbohydrate recognition, the receptor mediates internalization of pathogens by phagocytosis, thereby leading to intracellular killing. Although the MR lacks classic signaling motifs, it mediates a variety of cellular responses, including induction of NF-κB activation and the production of numerous defensive cytokines, including IL-12, IL-8, IL-1ß, IL-6, IL-17 and granulocyte-macrophage colony-stimulating factor (GM-CSF), and can also enhance MR shedding (Zhang *et al.*, 2004; Taylor *et al.*, 2005a; Netea *et al.*, 2006b; Tachado *et al.*, 2007; van de Veerdonk *et al.*, 2009b). The receptor has also been implicated in the phagocytosis of fungi, although its exact role in this process has not been fully defined (Le Cabec *et al.*, 2005; Taylor *et al.*, 2005a). Furthermore, the MR may perform an immunosuppressive function in its ability to inhibit the production of inflammatory cytokines when certain fungal pathogens are recognized (Zhang *et al.*, 2005). In MR-deficient mice, fungal infection did not result in increased susceptibility, although minor changes were observed in fungal burdens on infection with *C. albicans* (Lee *et al.*, 2003). More recently, it has been shown that the MR induces IL-17 production by *Candida* mannan in the absence of mitogenic stimulation, even more potently than Gram-negative bacteria (van de Veerdonk *et al.*, 2009b).

Dectin-1 is a type II transmembrane receptor and belongs to the natural killer cell receptor-like CLRs (Brown, 2006). The

extracellular carbohydrate recognition domain (CRD) selectively binds β-glucan polymers, a major component of yeast and mycobacterial cell walls, and mediates the phagocytosis of zymosan particles and intact yeast (Herre *et al.*, 2004; Underhill *et al.*, 2005). Dectin-1 also synergizes with TLR2 and TLR4-induced signals, inducing tumor necrosis factor (TNF), IL-10, transforming growth factor-β, and maturation of DCs (Brown *et al.*, 2003; Gantner *et al.*, 2003; Dillon *et al.*, 2006). The cell wall of *C. albicans* consists of large amounts of β-glucan covered by a layer of mannans that prevent direct exposure to dectin-1. β-Glucan is exposed and can bind dectin-1 only at the level of the budding scars of blastoconidia, where the integrity of the cell wall is disrupted. Hyphae also have a thin layer of β-glucan in their cell wall that can be detected by antibodies against β-glucan (Torosantucci *et al.*, 2009); however, this type of β-glucan does not seem to bind dectin-1 (Gantner *et al.*, 2005; Netea *et al.*, 2008a). Dectin-1$^{-/-}$ mice are more susceptible to disseminated candidiasis (Taylor *et al.*, 2007), and this observation is strengthened by higher susceptibility to disseminated candidiasis in mice deficient for caspase recruitment domain (CARD) 9, a key transducer of dectin-1 signaling. (Gross *et al.*, 2006). However, a different study could not confirm the nonredundant role of dectin-1 in *C. albicans* infection (Saijo *et al.*, 2007).

Dectin-2 is a type II transmembrane receptor with a single CRD and a stalk region, but without an intracellular signaling motif (Willment and Brown, 2008; Graham and Brown, 2009). Dectin-2 is expressed on macrophages and dendritic cells and is up-regulated when these cells are stimulated with particles containing high-mannose structures such as *C. albicans* hyphae (Taylor *et al.*, 2005c; McGreal *et al.*, 2006). Dectin-2 signaling occurs in collaboration with the Fc receptor γ chain, activates NF-κB, and can induce IL-1Ra and TNF production (Sato *et al.*, 2006).

DC-SIGN (CD209) is a type II transmembrane receptor with a single CRD and is expressed on dendritic cells and endothelium (Willment and Brown, 2008). The role of DC-SIGN in phagocytosis is questionable, whereas induction of endocytosis and uptake of pathogens has been well-documented (Taylor *et al.*, 2004). The uptake of *C. albicans* by human DCs depends on the binding of N-linked mannan to DC-SIGN and induces IL-6 production (Cambi *et al.*, 2003, 2008).

Mincle (also called Clec4e and Clecsf9) is a type II transmembrane protein with a single CRD, a short stalk region, and an intracellular region that signals by association with Fc receptor γ adaptor (Graham and Brown, 2009). Both human and mouse Mincle have been recently shown to bind *C. albicans* and contribute to cytokine stimulation (Bugarcic *et al.*, 2008), and Mincle-deficient mice were more susceptible to systemic candidiasis (Wells *et al.*, 2008).

NOD-like Receptors

NLRs are a family of intracellular immune receptors characterized by leucine-rich repeats and a nucleotide-binding domain. Like TLRs, NLRs recognize microbial products, as well as other intracellular danger signals, thus triggering host defense pathways through the activation of the NF-κB response and inflammatory caspases (Martinon *et al.*, 2009). Several members of the NLR family, including NLRP3 (also known as NALP3 and cryopyrin), form large multiprotein complexes, termed inflammasome, which in turn activate caspase-1, leading to the processing and secretion of IL-1β and IL-18 (Bryant and Fitzgerald, 2009). Recent reports link IL-1β production induced by *C. albicans* to the NLRP3 inflammasome (Gross *et al.*, 2009; Hise *et al.*, 2009; Joly *et al.*, 2009; Kumar *et al.*, 2009; van de Veerdonk *et al.*, 2009a). Noteworthy, TLR2, Dectin-1, and NLRP3 were crucial for the protection from dissemination of *C. albicans* in a murine model of oral mucosal infection (Hise *et al.*, 2009). However, at present, the extent to which oral epithelial cells contribute to the observed defense mechanism is unknown.

EXPRESSION OF PRRs IN ORAL EPITHELIAL CELLS

Most TLRs are expressed constitutively in oral epithelial cells, healthy epithelial tissue (Mahanonda and Pichyangkul, 2007; Beklen *et al.*, 2008), and oral mucosa biopsies from patients with oral candidiasis (Ali *et al.*, 2008). Previously, using a model of oral reconstituted human epithelium (RHE), we studied several different aspects of host-*Candida* interactions (Schaller *et al.*, 1998, 1999, 2002, 2004, 2006; Weindl *et al.*, 2007; Schaller and Weindl, 2009). Although the model consists of transformed cells (cell line TR146 derived from a carcinoma of the oral epithelium) (Rupniak *et al.,* 1985), all natural major markers of the epithelial basement membrane and of epithelial differentiation are expressed. More important, despite the artificiality of the model, it behaves like human *in vivo* epithelium when treated with pathogens and pharmacologically active agents, respectively (Schaller and Weindl, 2009), and it mimics the clinical setting of *C. albicans* infections in the oral cavity (Wilson *et al.*, 2009). Analysis of the oral RHE by real-time RT-PCR demonstrated a high degree of similarity in TLR expression profiles between the oral RHE and buccal epithelial samples isolated from healthy individuals (Weindl *et al.*, 2007). In the oral RHE model, all TLRs except TLR7 at a low level are constitutively expressed. Similarly, in samples from healthy individuals, all TLRs except TLR5 and TLR7 are detected. The most commonly expressed TLR genes *in vivo* are TLR1, TLR2, TLR4, and TLR8, with TLR1 being the most highly expressed gene. Increased expression of TLR2 and TLR4 has previously been observed in inflamed gingival epithelial tissues (Sugawara *et al.*, 2006). The immunohistochemical expression of 9 classes of TLRs (TLR1 to TLR9) was demonstrated in a series of sections from chronic hyperplastic candidiasis, leukoplakia, and healthy tissue (Ali *et al.*, 2008).

In contrast, much less is known about the expression of CLRs in the oral cavity. Currently, there are no data published on the role of MR in localized *Candida* infections. The receptor is expressed in keratinocytes (Szolnoky *et al.*, 2001) and oral epithelial cells (Wagener *et al.* and Weig *et al*., unpublished observations). However, gene expression analysis in the oral RHE model showed no significant differences upon infection with *C. albicans* (Weig *et al.,* unpublished observations). In oral epithelial cells, MR blocking did not alter cytokine secretion levels of IL-6, IL-8, and GM-CSF upon stimulation with *Candida* cell wall components (Wagener *et al*., unpublished observations).

The function of dectin-1 in mucosal candidiasis has not yet been established, but several facts suggest that dectin-1 might play a crucial role in the mucosal immunity against *C. albicans*, at least in the intestine. Myeloid lineage cells in the intestinal tract express dectin-1, and the outgrowth of *C. albicans* in the digestive tract from dectin-$1^{-/-}$ mice was disproportionately high, leading to occlusion and contributing to increased mortality (Reid *et al.*, 2004; Taylor *et al.*, 2002, 2007). Furthermore, dectin-1 is paramount for IL-17 induction by *C. albicans* (Leibundgut-Landmann *et al.*, 2007; Osorio *et al.*, 2008), and patients with impaired IL-17 production caused by STAT3 (signal transducer and activator of transcription 3) mutations (hyper-IgE syndrome) and CMC have recurrent *Candida* infections (Eyerich *et al.*, 2008; Ma *et al.*, 2008; Milner *et al.*, 2008). Previous studies have failed to demonstrate Dectin-1 expression in epithelial cells from the gastrointestinal tract (Rice *et al.*, 2005), lung (Evans *et al.*, 2005; Lee *et al.*, 2009b), and gingiva (Laube *et al.*, 2008). However, epidermal keratinocytes appear to express functional Dectin-1 (Lee *et al.*, 2009a), and its expression can also be induced by mycobacteria in airway epithelial cells (Lee *et al.*, 2009b). We observed that Dectin-1 is expressed in the oral RHE, but gene expression is not inducible by *C. albicans,* and Dectin-1 ligands did not stimulate cytokine secretion (Wagener *et al.* and Weig *et al.,* unpublished observations). This suggests that Dectin-1 plays only a minor role in oral epithelial cells, and that other PRRs might contribute to the interaction between the epithelial cells and *C. albicans* PAMPs. As for Dectin-2, DC-SIGN, and Mincle, these receptors seem not to be expressed in oral epithelial cells (Weindl and Schaller, unpublished observations).

Oral epithelial cells express the members of the NLR family NLRC1 (NOD1) and NLRC2 (NOD2), and stimulation with synthetic ligands strongly increased the expression of antimicrobial molecules, while pro-inflammatory cytokines were not induced (Uehara *et al.*, 2005, 2007; Sugawara *et al.*, 2006; Uehara and Takada, 2008). Although *C. albicans* is not recognized by NLRC1 and NLRC2 (van der Graaf *et al.*, 2006), another NLR member, NLRP3, might have an important function in the host defense against mucosal *Candida* infections (Hise *et al.*, 2009). Interestingly, NLRP3 is strongly expressed by keratinocytes in non-keratinizing epithelia such as the oral cavity and esophagus (Kummer *et al.*, 2007). The potential role of NLRP3 in oral epithelial cells is further supported by studies showing increased IL-1β and IL-18 levels upon stimulation with *C. albicans* (Rouabhia *et al.*, 2002; Mostefaoui *et al.*, 2004; Schaller *et al.*, 2004; Tardif *et al.*, 2004; Weindl *et al.*, 2007).

REGULATION OF INNATE IMMUNE PATHWAYS BY *C. ALBICANS*

C. albicans is a harmless colonizer of mucosal surfaces in healthy individuals. During the period of colonization, extensive fungal growth is limited through the release of antimicrobial peptides from epithelial cells, or due to the existence of other bacteria of the microbial flora. In this stage of colonization, without clinical symptoms and signs of inflammation, neither the facultative pathogen nor the host might induce a (TLR-mediated) inflammatory cytokine response. However, when these conditions become unbalanced—for instance, due to antibiotic therapy or immunosuppression—superficial or even systemic infections may occur.

Although oral epithelial cells express TLRs, no studies have yet demonstrated TLR up-regulation upon stimulation with *C. albicans*. Heat-killed *C. albicans* cells failed to modulate epithelial TLR expression (Pivarcsi *et al.*, 2003). Similarly, in an infection model of oral candidiasis, both heat-killed and viable *C. albicans* cells were unable to up-regulate epithelial TLR expression, despite the fungus causing clear signs of mucosal damage (Weindl *et al.*, 2007). However, *C. famata*, which accounts for 1-3% of candidiasis, has been shown to induce slight TLR mRNA expression in human gingival epithelial cells (Bahri *et al.*, 2010). Specifically, the expression of TLR2, 4, and 6 was up-regulated, although only minimally. With regard to commensal organisms, it has been suggested that rapid responsiveness by epithelial TLRs may create the danger of an immune overreaction (Strober, 2004). Thus, one possible explanation for the lack of direct TLR up-regulation by *C. albicans* may be because the fungus is usually a harmless colonizer of oral mucosal surfaces in approximately 40% of healthy individuals (Arendorf and Walker, 1979) and may even actively down-regulate epithelial responses by unknown mechanisms (Weindl and Schaller, unpublished data). In addition, during the carrier state, it would serve little purpose for the host to activate a TLR-mediated inflammatory response when it is not required. However, during oral infection with *Candida*, many cytokines are secreted by oral epithelial cells, which maintain a central role in the protection against fungal organisms (Dongari-Bagtzoglou and Fidel, 2005). In general, pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8, TNF, GM-CSF, and others) regulate leukocyte trafficking (Eversole *et al.*, 1997) and/or activate a strong antifungal response by these cells (Dongari-Bagtzoglou *et al.*, 2005). In addition, oral epithelial cells are capable of inducing antimicrobial peptides, such as defensins, cathelicidins, and histatins (reviewed in Diamond *et al.*, 2008), which control *C. albicans* growth and infection. Among these peptides, human β-defensin-2 (hBD-2), hBD-3 (Joly *et al.*, 2004; Feng *et al.*, 2005; Schneider *et al.*, 2005), LL-37 (Turner *et al.*, 1998), and histatin-5 (Oppenheim *et al.*, 1988) exhibit potent anti-candidal properties.

The addition of PMNs to an *in vitro* model of oral candidiasis enhanced a T-helper (Th)1-type immune response (interferon-γ, TNF), down-regulated the expression of the Th2-type cytokine IL-10, and was associated with protection against *Candida*-induced tissue damage (Schaller *et al.*, 2004). PMNs could protect the epithelium from *C. albicans*–induced cell injury *via* a process that was independent of phagocytosis, PMN transmigration, or physical PMN-epithelial cell contact. Interestingly, the immunological cross-talk between *C. albicans*-infected oral epithelium and PMNs causes PMN-mediated up-regulation of epithelial TLR4 (Weindl *et al.*, 2007). Furthermore, epithelial TLR4 is directly responsible for protecting the mucosal surface from fungal invasion and cell injury. Cytokines seem not to have an essential function in direct host defense against invading fungi, even in the presence of PMNs. Rather, cytokines are crucial for

the activation of PMNs and/or are released from the PMNs, which, in turn, results in up-regulation of epithelial TLR4 and protection from fungal invasion (see Fig. 1). Among the cytokines, TNF showed the most potent effect, which confirms the important role of this cytokine in host defense against opportunistic fungal infections (Filler *et al.*, 2005). Absence of this cytokine strongly impairs neutrophil recruitment and effective phagocytosis of *C. albicans* (Netea *et al.*, 1999). In an *in vitro* model of esophageal candidiasis, co-incubation of PMNs with *C. albicans* leads to a significant up-regulation of hBD-2 and hBD-3 in esophageal cells compared with effects of PMNs or *C. albicans* alone (Steubesand *et al.*, 2009). Thus, increased PMN-dependent production of antimicrobial peptides by epithelial cells could contribute to the protective effect and further underlines the important role for PMNs in clearance of experimental oral candidiasis.

At present, it is unknown which fungal cell wall structures are recognized in oral epithelial cells. Both TLR2 and TLR4 have been implicated in the recognition of *C. albicans* by immune cells (Roeder *et al.*, 2004). Recognition of surface N-linked mannosyl residues is mediated by mannose receptor and O-linked mannosyl residues by TLR4, phospholipomannan is detected by TLR2, and β-glucan structures are recognized by TLR2 in collaboration with Dectin-1 (Gantner *et al.*, 2003; Jouault *et al.*, 2003; Netea *et al.*, 2006b). In epidermal keratinocytes, *C. albicans*-native phospholipomannan may contribute to the inflammatory responses of cutaneous candidiasis *via* TLR2-NF-κB and p38 mitogen-activated protein kinase signaling pathways (Li *et al.*, 2009). However, studies on oral epithelial cells are needed to address the recognition of fungal cell wall structures by PRRs.

Similarly, epithelial cytokine production or expression of antimicrobial peptides, induced by *C. albicans,* has not yet been associated with specific PRRs. Various *Candida* strains strongly induce GM-CSF in human oral epithelial cells and three-dimensional models (Dongari-Bagtzoglou and Kashleva, 2003; Weindl *et al.*, 2007; Li and Dongari-Bagtzoglou, 2009). GM-CSF is a potent cytokine involved in the enhancement of proliferation, activation, and fungicidal activity of immune cells. Analysis of the data indicates that TLR4 is not involved in *Candida*-induced GM-CSF and IL-8 production in epithelial cells (Weindl *et al.*, 2007; Li and Dongari-Bagtzoglou, 2009). In this regard, the adhesion receptor CDw17 (lactosylceramide) might be responsible, at least partially, for GM-CSF activation mediated by NF-κB (Li and Dongari-Bagtzoglou, 2009). Studies indicate that CDw17 may function as an alternate β-glucan receptor in *Pneumocystis carinii*; however, it has been suggested that secretion of GM-CSF is independent of the cell wall component ß-glucan in *C. glabrata*.

Recently, specific human gene mutations and polymorphisms have been linked to signal pathways resulting in susceptibility for *C. albicans*. STAT3 (signal transducer and activator of transcription 3) mutations identified in patients with hyper-IgE syndrome have been attributed to a defective IL-17 production and a diminished Th17 response, resulting in recurrent mucosal *Candida* infections (Eyerich *et al.*, 2008; Ma *et al.*, 2008; Milner *et al.*, 2008). Similarly, deficiency in Dectin-1 signaling pathways has been linked to mucocutaneous candidiasis (Ferwerda *et al.*, 2009; Glocker *et al.*, 2009).

A recent study reported a Dutch family with impaired *in vitro* responses to β-glucan, leading to infections of nails and mucosa (Ferwerda *et al.*, 2009). The study demonstrated that the production of IL-6 and IL-17 was impaired only in response to the dectin-1 ligand, β-glucan. The specific stop codon the authors identified in dectin-1 is remarkably common in some parts of Africa and Europe (allele frequency, 3 to 7%), suggesting that unknown factors maintain it in populations. Another study identified an extended Iranian family with predominantly mucocutaneous, but also fatal, candidiasis of the central nervous system, caused by mutations in the adaptor protein CARD9, impairing both dectin-1 signaling and Th17 production (Glocker *et al.*, 2009). CARD9 also directs a variety of other cell-surface and intracellular signals, including the p38 mitogen-activated protein kinase and Jun N-terminal kinase pathways, possibly accounting for its greater clinical severity as compared with isolated dectin-1 deficiency (Ferwerda *et al.*, 2009). The CARD9 mutation appears to be rare, and its rarity is commensurate with its severity. Both studies, however, need to be interpreted with caution, since both patient groups show mucocutaneous manifestations, whereas the functional studies were performed on leukocytes. It remains to be proven whether the key mechanisms in these cases of severe candidiasis consist of impaired dectin-1 signaling at the epithelial level or impaired leukocyte activation, mediated through cytokines such as IL-17.

ORAL MUCOSAL T-CELL RESPONSES TO *C. ALBICANS*

Activation of the innate immune system by *C. albicans* induces the secretion of a variety of pro-inflammatory cytokines and the expression of co-stimulatory molecules. It is generally accepted that induction of a Th1-type cellular response is crucial for the defense against *C. albicans* (Fidel *et al.*, 1997; Romani, 1999; Schaller *et al.*, 2004). In contrast, a Th2 cellular response is considered non-protective, since it induces class-switch to non-opsonizing antibody subclasses and IgE (Savolainen *et al.*, 1996; Clemons and Stevens, 2001). Investigation of the role of Th17 in mediating the immune response has shown that Th17 memory cells are induced by *Candida* hyphae (Acosta-Rodriguez *et al.*, 2007; Zhou *et al.*, 2008), and in a murine model, IL-17AR knock-out mice had an increased susceptibility to systemic (Huang *et al.*, 2004) and oropharyngeal candidiasis (OPC) (Conti *et al.*, 2009). In contrast, deleterious effects of IL-17 inflammatory activities have also been demonstrated (De Luca *et al.*, 2007; Zelante *et al.*, 2007; Bozza *et al.*, 2008). Patients with impaired IL-17 production suffer from mucosal *C. albicans* infections in hyper-IgE syndrome and CMC (Eyerich *et al.*, 2008; Ma *et al.*, 2008; Milner *et al.*, 2008). In contrast to Th cells, regulatory T-cells suppress inflammatory responses in disseminated *C. albicans*, resulting in higher susceptibility in mice (Netea *et al.*, 2004; Sutmuller *et al.*, 2006). However, the tolerance-inducing effects of regulatory T-cells seem to be beneficial at mucosal sites (De Luca *et al.*, 2007; Vignali *et al.*, 2008).

Th17 cells are a distinct lineage from Th1 and Th2 cells, characterized by the release of IL-17A and IL-17F, IL-22 and IL-26. Receptors for IL-17A and IL-17F (Il-17Ra and IL-17Rc)

are present in several cell types, including antigen-presenting cells and epithelial cells (Xie *et al.*, 2000; Gaffen, 2009). In contrast, receptors for IL-22 and IL-26 appear to be localized to the epithelium (Xie *et al.*, 2000; Sheikh *et al.*, 2004; Wolk *et al.*, 2004). Very little is known about the role of IL-26 during mucosal infection, because rodents do not express this cytokine. During colonization of the oral cavity with *C. albicans,* IL-17 receptor signaling is essential for defense (Conti *et al.*, 2009). Interestingly, Th17-deficient (IL-23p19$^{-/-}$) and IL-17R-deficient (IL-17RA$^{-/-}$) mice experienced severe OPC, whereas Th1-deficient (IL-12p35$^{-/-}$) mice showed low fungal burdens and no apparent sign of disease. Furthermore, neutrophil recruitment was impaired in IL-23p19$^{-/-}$ and IL-17RA$^{-/-}$, but not IL-12$^{-/-}$, mice, and T-cell receptor αβ cells were more important than γδ cells. In contrast, mice deficient in the Th17 cytokine IL-22 were only mildly susceptible to OPC, indicating that IL-17 rather than IL-22 is crucial in defense against oral candidiasis. Gene profiling of oral mucosal tissue showed strong induction of Th17 signature genes, including beta defensin-3 and CXC chemokines. hBD-3 has candidacidal activity *in vitro* (Vylkova *et al.*, 2006), and saliva from wild-type mice, but not IL-17Ra$^{-/-}$ mice, has candidacidal activity, indicating that IL-17 also controls *C. albicans* proliferation by promoting secretion of antimicrobial peptides (Conti *et al.*, 2009). However, more work is needed to understand whether Th17 responses also govern the response to oral candidiasis in humans, because it is unclear whether mouse and human diseases have the same etiology. In humans, OPC has diverse etiologies ranging from antimicrobial use, to immune dysregulation associated with advanced HIV infection, to mutations in autoimmune regulator genes. Patients with CMC show differences in cytokine production, including IL-23, depending on the etiology of the disorder (Ryan *et al.*, 2008). Although HIV infection and host genetics are likely to be important variables in Th17 expression, environmental factors, particularly those that affect the microbiota, could also influence Th cell polarization. Although microbe-specific motifs could induce Th polarization, common microbial PAMPs that induce Th1 or Th17 may also be present. Thus, a diverse group of microbial ligands could induce different responses, and immunity toward pathogens could be less specific than previously thought.

Analysis of the data together indicates optimal protection against (chronic) mucosal *Candida* infections by Th1, Th17, and regulatory T-cells. An effective Th1 and antibody (humoral) response are crucial for defense against disseminated *Candida* infections. In the case of localized *Candida* infections, however, more work is needed to decipher their relative contributions.

CONCLUSIONS

The above-mentioned studies clearly demonstrate that PRRs contribute to the signal transduction induced by *C. albicans*, to the induction of inflammation, and to the activation of adaptive immunity. The simultaneous activation of multiple PRRs by one fungal pathogen endows the immune system with a broad range of possibilities for a specific and effective immune response. However, the contribution of PRRs in mucosal tissues has only recently been studied. Among PRRs, TLRs play a crucial role in detecting danger signals and triggering innate immune responses that prevent pathogens from invading the host and spreading systemically. Other innate immune receptors expressed at the mucosal surfaces are likely to participate in such defense mechanisms as shown for dectin-1. PRRs are also involved in mediating epithelial homeostatic functions facilitating mucosal tissue repair and remodeling following inflammation. How pathogens and commensals stimulate distinct mucosal responses while expressing similar molecular patterns is only now beginning to be understood. On other tissues, such as the intestine, it has been shown that commensals are able to modulate pro-inflammatory responses by interfering with TLR signaling cascades. Pathogens use additional virulence factors that participate in the onset of pro-inflammatory responses. Thus, the wide variety of responses to micro-organisms may be explained by the diversity of co-receptors that can be engaged to induce functional innate immune signaling.

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