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Candida albicans is a crafty microbe that deceives its host by using complement regulators and proteases

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The fungus *Candida albicans* can be considered as part of our normal flora and is found in up to 80 % of all individuals. It colonizes the oral cavity, gastrointestinal tract, vagina, and intertriginous folds such as in the inguinal area. Infections or overgrowth with *C. albicans* are commonly seen. Most of these episodes are fortunately benign; for example, oral candidiasis, "thrush" in newborns. However, *C. albicans* is the fourth most common species isolated from patients with nosocomial sepsis after coagulase-negative staphylococci, *Staphylococcus aureus* and enterococci [1]. Patients with urinary catheters and on broad-spectrum antibiotics as well as immunocompromised hosts are more susceptible to infections caused by *C. albicans*. The latter group is increasing and includes transplanted patients on immunosuppressive treatment and neutropenic cancer patients [2]. Moreover, patients with T cell deficiencies and defects in killing by phagocytes, such as patients with AIDS and CGD (chronic granolumatous disease), respectively, are at risk for infections with *C. albicans*. Another troublesome clinical condition is CMC (chronic mucocutaneous candidiasis) secondary to various underlying medical diseases, including impaired T cell function related to dysfunction in interleukin 17 immunity [3]. Thus, there is an urgent need for us to learn more about *C. albicans* colonization and disease in order effectively to keep these organisms in check.

Our barrier against the world around us consists, in part, of sophisticated humoral defense systems found in the epidermal and mucosal tissues. Antimicrobial peptides, immunoglobulins and the complement system are all very important, in addition to the cellular immune system. The complement system is part of the innate immune system which recognizes molecular patterns displayed by different microbes. It is activated via three different pathways, and the well known classical pathway of complement activation plays a major role since it is initiated by C1q bound to antibodies targeted against specific microbes [4]. In addition, the lectin pathway is initiated by mannan-binding lectins (MBL), and both of these pathways lead to activation of C3 resulting in the C3 convertase. In parallel, the alternative pathway is triggered by spontaneous deposition of the C3b molecule at the microbial surface leading to C3 convertase. All three pathways merge via C5 convertase and are finalized in the terminal pathway of complement activation resulting in the terminal complement complex (TCC), also designated the membrane attack complex (MAC), consisting of C5b to C9, which subsequently will drill holes in the microbial cell membrane.

It is extraordinarily important for the human host to keep the complement system tightly controlled in order to avoid tissue damage. In addition to cellular receptors (e.g., CD46 and CD55), this control is achieved for the classical and lectin pathways by a series of fluid phase inhibitors, including C4BP (C4b binding protein) and C1INH (C1 inhibitor) (4). Factor H (FH), FHL1 (FH-like protein 1), and properdin are inhibitors keeping track of the alternative pathway, whereas CFHR1 (complement factor H related protein 1), clusterin and vitronectin regulate the formation of the MAC and are, thus, important inhibitors of the terminal pathway.

In recent years, several microbial proteins, both from the outer membrane and cytoplasm, have been shown to bind host factors such as complement regulators FH and C4BP [4,5]. In fact, it can be anticipated that the vast majority of microbes have more or less specific receptors for numerous complement regulators. A particular pathogen can also present several receptors attracting the same ligand, but with different affinities. This phenomenon is exemplified by the respiratory pathogen *Haemophilus influenzae* that binds vitronectin with both Hsf (*Haemophilus* surface fibrils) and protein E [6,7]. Intriguingly, *H. influenzae* protein E also simultaneously binds laminin and vitronectin at different regions of the protein [8]. The adhesin *Moraxella catarrhalis* Usp A1 (ubiquitous surface protein) belongs to another well examined family of multifunctional outer membrane proteins interacting with the host and its immune system [9]. UspA1 and A2 have binding sites for C4BP and vitronectin in addition to fibronectin, laminin, antichymotrypsin and CAECAM1. Furthermore, UspA2 neutralizes C3d, resulting in a highly serum resistant species. *M. catarrhalis* armored with the UspAs, thus, survives as a commensal in the mucosa. This interesting way of inhibiting the alternative pathway also plays a role in the interaction between *M. catarrhalis* and other microbes. We found that the relatively serum-susceptible *H. influenzae* is protected by OMVs (outer membrane vesicles; nanoparticles) derived from *M. catarrhalis*. OMVs neutralize C3d and, hence, inhibit the complement activation at a distance, like a cluster bomb launched from the bacteria, protecting both bacterial species from the deleterious complement killing [10]. Another strategy to overcome the innate immunity is to degrade complement directly. This degradation is performed by bacteria dwelling in the oral cavity. For example, *Tanerella forsythia* has a very powerful metalloproteinase (kariolysin) directly degrading several complement proteins [11].

Plasminogen (Plg) is found in the blood stream as a zymogen and is, upon activation, converted to the protease plasmin by interactions with tPA (tissue Plg activator) and urokinase uPa (Plg activator) [12]. The main target of plasmin is fibrin, resulting in fibrinolysis that keeps hemostasis at balance. Plg is tightly controlled by inhibitors, such as PAI (Plg activator inhibitor) 1 and 2, among others [8]. Microbes have numerous immune evasion strategies, and some have the capability to degrade PAI using specific proteases, in addition to the ability to attract Plg directly and, thus, convert it to plasmin. All of these different mechanisms lead to destruction of the extracellular matrix (including laminin), allowing subsequent invasion. Several different pathogens utilize Plg and plasmin for their survival in the host, and examples of common bacteria are the Gram-positive *Staphylococcus aureus* and *Streptococcus pneumoniae*, and the Gram-negative *H. influenzae* and *Borrelia burgdorferi*.

C. albicans also has an array of different strategies to survive in the host [13]. The species is equipped with a hardy cell wall that partly resists attacks from the immune system. However, its PAMPs (pathogen-associated molecular patterns) are recognized by several TLRs (Toll like receptors) and CLRs (C-type lectin receptors). *C. albicans* β-glucans activate immune cells via the cell receptor dectin-1, and mannosyl residues are targets for efficient opsonization and phagocytosis. Interestingly, *C. albicans* also releases aspartic protease that disarms C3b preventing opsonization [14].

In the article by Luo and collaborators in this issue of *The Journal*, FH-coupled Sepharose was used to isolate glycerol-3-phosphate dehydrogenase 2 (Gpd2), a novel FH-binding protein of *C. albicans* [15]. Interestingly, Gpd2 also attracts FHL and plasminogen, and mediates binding to non-phagocytic cells, i.e., endothelial and epithelial cells. In general, bacteria and fungi use numerous back-up mechanisms for optimal interaction(s) with the host innate immune system, both humoral and cellular arms, and epithelial cell barriers. *C. albicans* is not an exception; the fungus binds FH with three other proteins; Gpm1/CRASP1 (phosphoglycerate mutase 1) [5], pH-regulated antigen (Pra1/CRASP2), and finally Hgt1p [16]. The two former proteins have been shown to also interact with FHL-1 and plasminogen. In addition, one of these outer membrane proteins, Pra1/CRASP2, binds C4b binding protein (C4BP) and C3 [17]. Gpd2 is expressed both on the *C. albicans* surface and hyphae [15]. The target sequence was shown to be within SCR 7 (short consensus repeat) in FH and FHL, a SCR that is shared with *Streptococcus pyogenes* [18] and partly by *H. iInfluenzae,* among others [19]. In

contrast, *S. pneumoniae* binds both to SCR 8-11 and SCR 12-15 [5]. Despite Gpd2-dependent binding to FH and FHL-1, the two complement regulatory proteins were able to cleave C3b. In parallel, Gpd2-bound plasminogen was accessible for uPA resulting in plasmin generation and, consequently, fibrinogen degradation.

It is intriguing how pathogens can utilize a cytoplasmic protein for several purposes. *C. albicans* Gpd2 is a NAD-dependent enzyme that plays a role in the glycerol metabolism in the cytoplasm, but also attracts complement regulators at the surface. An interesting parallel is the cytoplasmically located protein Ef-Tuf (elongation factor) from *P. aeruginosa* that binds both FH and plasminogen at the bacterial surface [20].

A clinically important example highlighting newly gained knowledge regarding complement regulators is the recently developed vaccine against meningococcus group B (4CMenB), which includes the highly immunogenic fHbp (*N. meningitidis* FH binding protein) [21]. From a vaccine point of view, it is useful to target microbial proteins that bind complement regulators such as FH. However, since most pathogens also have back up mechanisms for combating the host complement system, we cannot just rely on one single protein, but need multicomponent vaccines. Since we consider *C. albicans* as a part of our normal flora, we should most likely not immunize against this species. However, proteins attracting complement regulators and plasminogen such as *C. albicans* Gpd2 might be useful targets for intervention. Since the incidence of fungal infections is steadily increasing, more research is required in defining efficient future therapies, and the article by Luo *et al.* [15] is an interesting step in that direction.

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