ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2018, 56, S26–S31
doi: 10.1093/mmy/myx139
Review Article

Review Article

Candida vaginitis: virulence, host response and vaccine prospects

Flavia De Bernardis[1,*], Sofia Graziani[1], Flavio Tirelli[2] and Stavroula Antonopoulou[3,4]

[1]Department. of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy, [2]Università Cattolica del Sacro Cuore, Largo F. Vito, 1, 00168 Rome, Italy, [3]Departmentt. of Clinical Microbiology, G. Gennimatas General Hospital, 154 Avenue Mesogeion, 11527, Athens, Greece and [4]Department of Genetics and Biotechnology, Faculty of Biology, National Kapodistrian University of Athens, Avenue oulof Palme, Ano Ilisia 15784, Athens, Greece

*To whom correspondence should be addressed. Flavia De Bernardis, PhD, Tel/Fax: +39 06 49902809;
E-mail: flavia.debernardis@iss.it

Received 27 April 2017; Revised 20 October 2017; Editorial Decision 23 October 2017

Abstract

Vulvovaginal candidiasis is a common mucosal infection affecting a large proportion of women with some of them affected by recurrent often intractable forms of the disease. Thus, there is an increasing interest in understanding the pathogenesis of this disease. The aim of our work was to characterize, in animal models of vaginal candidiasis, the components of the host-fungus interaction at the mucosal level.

The evidence of an immune response in the vaginal compartment was very encouraging to identify the proper targets for new strategies for vaccination or immunotherapy of vaginal candidiasis. Aspartyl-proteinase (Sap2), which is an important immunodominant antigens and virulence factors of *C.albicans* acting in mucosal infections, was assembled with virosomes and a vaccine PEV7 was obtained. The results obtained in the mouse model and in the clinical trial conducted by Pevion on women have evidenced that the vaccine PEV7, intravaginally administered, has an encouraging therapeutic potential for the treatment of recurrent vulvovaginal candidiasis. This opens the way to a modality for anti-*Candida* protection at mucosal level.

Key words: *Candida* vaginitis, virulence factors, aspartyl-proteinase, immune-response, mucosal anti-*Candida* vaccine.

Introduction

The aim of this working group is to extend previous clinical and microbiological investigations and to understand host-parasite interactions in candidiasis and try to generate novel efficient therapeutic and/or immunological tools. Particularly, we will try to understand the fungal and host components involved in the pathogenesis of mucosal candidiasis and to assess the role of aspartic proteinase (Sap) in *Candida* virulence and pathogenicity and to investigate the mechanisms induced in the vagina during *Candida* infection and to identify specific *Candida* molecules potentially useful for vaccination or immunotherapy of vaginal candidiasis.

Vaginal candidiasis is one of the most frequent infections of the female genital tract of women of reproductive age.

At least 75% of women suffer once in their life from one episode of a *Candida* infection. In more than 85% of the cases, vulvovaginal candidiasis is primarily caused by *C. albicans*, followed by *C. glabrata* with 4–5%, and to a lesser extent, by *C. tropicalis* and *C. parapsilosis*.[1–5] Predisposing factors in getting an acute form of vaginal candidiasis are well-known and include immunosuppression-diseases, endocrine diseases, antibiotic therapy or diabetes.[3,4] Patients, suffering at least four episodes of *Candida* infection during 1 year, have a recurrent infection and are therefore recognized and classified as recurring vulvovaginal candidosis (RVVC) patients.[1,4] Epidemiological investigations have suggested that the prevalence of RVVC may be higher than previously estimated and can be as high as 7–8% of women who experience a first episode. This would translate into an estimated global annual incidence ranging of 1–2% of all women.[6–8] The discomfort associated with RVVC is intense; it markedly diminishes the quality of life in young women. The well-known predisposing factors seem not to play an important role in recurrent *Candida* infections. The causal reasons for getting multiple recurrences of a *Candida* infection are multivarious and still not well understood. The widespread occurrence of vaginal infections caused by *Candida*, and the development of resistance against available drugs require the need for new specific antifungal agents and the discovery of new drug-target.[9–15] It is also suggested a genetic predisposition among individual patients; thus, gene polymorphisms are to be included as a predisposing factor which puts women at risk for VVC and RVVC.[16–19] Therefore, understanding the components of the host-fungus interaction at the mucosal level can lead to a better understanding of the pathogenesis of mucosal candidiasis and result in the optimization of preventive and therapeutic antifungal strategies. Moreover, in contrast to systemic candidiasis, relatively little is known about the role of mucosal immunity in protection against *Candida*.

C. albicans is capable of colonizing and persisting on mucosal surfaces of the oral cavity and of the gastrointestinal and genitourinary tracts of healthy humans where its presence stimulates mucosal responses. Odds has suggested that 40–50% of any given sample population temporarily or permanently carry this fungus in their gastrointestinal tract.[20] The transition of *C. albicans* from asymptomatic colonization to symptomatic vaginitis is primarily the consequence of a defective host cellular immune response, but of some relevance can be also the virulence of *C. albicans* strains.[21–28] Thus, there is a balanced interplay between fungus virulence and host immunity in the vaginal mucosal environment, suggesting that commensalism results from such a balance.[29,30]

More detailed information about the epidemiology, diagnosis, current treatments of the infection, and recent studies for the development of protective vaccine are included in excellent reviews already published on this subject.[3–11,31–42]

Studies that evidence the role of virulence factors of *Candida albicans* in vaginal candidiasis

The virulence factors of *C. albicans* that play a role in vaginal infections are: adherence, dimorphisms with antigenic variations, enzyme production, especially proteinase secretion and cell surface composition.[23,25,43–45]

Adherence is probably the first stage in mucocutaneous candidiasis; in fact, adherence of *Candida* cells to host cells, especially to epithelial cells, is essential for colonization, and it prevents or reduces the elimination of *Candida* by the host. Important is the role of mannoproteins for the attachment of *Candida* to epithelial cells. Several adhesins were isolated from *C.albicans*, molecules that promote *Candida*'s adhesion to host cells. Agglutinin-like sequence is a family of glycosylated proteins of *C. albicans*, homologues to *S. cerevisiae* agglutinins that are required for cell-cell recognition and contact during mating.[46–49] Hyphal wall protein is a mannoprotein of the outer layer expanding an amino-terminal part and has the carboxy-linked part covalently bound to the B-glucan of the wall.[50,51] Integrin like protein is a proteins similar to integrin which is a receptor present in important mammalian cells for binding cells with extra cellular protein matrices.[52,53] Mannosyl transferase protein is a membrane protein with mannosyl transferase activity.[54]

That these proteins have an important role as adhesin and therefore initiating the colonization of the epithelium was highlighted because genes were cloned and constructed mutants with the two alleles destroyed and it was observed that the various mutants adhere with lesser entity in vitro and are less virulent *in vivo*.[44,49,53]

Adhesins play an important role in the pathogenesis of vaginal candidiasis by facilitating adherence to vaginal tissue.[46,55–57] There is a clear evidence of the capacity of *C. albicans* to develop hyphae endowed with a multiplicity of immunoevasion mechanisms and greatly favoring implantation on the vaginal mucosa.[58–63] Tissue sections of animal vaginas show that hyphae strongly adhere to the keratinized surface of the vaginal epithelium with some hyphal tips slightly infiltrating the subepithelial layer.[64,65] There is a clear demonstration that each deletion of relevant genes affecting hyphal transition determine decrease or abolition of experimental pathogenicity.[23,58]

Strains of *C. albicans* that lack the capacity to undergo the dimorphic transition do not produce a biofilm and are typically nonpathogenic.[66–69] Naglik and collaborators showed that the two forms of growth are discriminated by activation of distinct MAP kinase pathways.[70]

Enzyme secretion in particular aspartic proteinase (Sap), a family of at least 10 enzymes, plays a role in vaginal candidiasis. In fact, mutants of *C. albicans* with Sap1-3 gene deletion do not cause vaginal infection in rats and lose the capacity of damaging, *in vitro*, reconstituted human vaginal epithelium, in both studies pathogenicity was restored using fungal strains with relevant gene reintegrated.[71,72] No such inference could be made with Sap4-6 deficient mutants, even when the triple mutant was used.[71]

Sap proteins cooperate, and sequential expression impacts virulence at different times during *C. albicans* infection.[24,25] It has emerged that Sap2 protein plays a particularly consistent role in vaginal infection by *C. albicans*, probably as no other single virulence trait. In fact, Sap2 is the predominant enzyme in vaginal secretion of infected women and rats, and Sap2 gene was the first Sap gene that was seen to be expressed in vaginal infection.[21,22,25] Moreover, Sap2 is the member of Sap family with the broadest substrate specificity. It is capable of hydrolyzing structural host proteins, such as keratin of the skin and the lining of the surface of the vaginal epithelium. Overall, the numerous studies on proteinase activity of *C. albicans* have been established that the Saps are indeed virulence factors with an important role in the pathogenesis of vaginal candidiasis.[22,25,73,74]

Studies of immune responses at vaginal level during *Candida* infection

Mucosal colonization with *C. albicans* induces both antibody and cell mediated immunity. A successful host/*Candida* interaction that lead to a commensalism requires the coordinate actions of both innate and adaptive immune systems.

In order to obtain possible insights into the host factors involved in the defense against vaginal candidiasis, we have long been employing a rat model of vaginal infection that has similarities to human disease, including the vaginal CD4/CD8 T-cell ratio.[75] In this model, an initial self-healing infection confers a high degree of protection against subsequent reinfection by *C. albicans*.[76] The protection is associated with the presence of protective antibodies against *Candida* constituents in the vaginal fluids and increased number of activated lymphocytes in the vaginal mucosa.[77,78] Adoptive transfer of vaginal lymphocyte populations showed that distinct lymphocyte subsets participated in the adaptive anti-*Candida* immunity at vaginal level and demonstrated not only that $CD4^+$ T cells were essential for protection but also the protective role of vaginal dendritic cells.[79–81]

To further investigate the host defense mechanisms at vaginal level, we evaluated the role of Toll-like receptors (TLRs) in protection against vaginal infection in mice with different TLR knocked out genes. TLR3 and TLR9 KO mice showed the kinetic of first infection similar to that of control mice. In contrast, TLR4 and the beta-glucan receptor Dectin-1 deficient mice were more susceptible to *Candida* infection. TLR 3 KO mice were only partially protected from the second *Candida* challenge. TLR9 KO mice were protected similar to control mice. The role of TLR 9 is not important in protection against vaginal candidiasis. In contrast, TLR 4 KO mice and dectin-1 KO mice were not protected from the second infection, a finding highlighting the requirement for TLR 4 and Dectin-1 in the acquired immunity to the fungus at the vaginal level. These results suggest that TLRs have a role in the innate and adaptive immunity to *Candida* at vaginal level. Of particular interest is the finding that TLR4 and Dectin-1 are both required for the induction of acquired protective immunity to the fungus.[18,82]

Resistance and tolerance mechanisms were both activated in murine VVC, involving interleukin (IL)-22 and IL-10–producing regulatory T cells, respectively, with a major contribution by the enzyme indoleamine 2,3-dioxygenase 1. Thus, IL-22 and IDO1 are crucial in balancing resistance with tolerance to *Candida,* their deficiencies are risk factors for RVVC.[18]

Studies to identify novel approach for vaccination or immunotherapy of vaginal candidiasis

The defense mechanisms induced in the vagina during *Candida* infection evidenced in the animal model[5,77–81] could lead to new strategies for vaccination or immunotherapy of vaginal candidiasis. In particular, in the rat model, we evaluated the effect of intravaginal immunization with recombinant secretory aspartyl proteinase (r-SAP-2). The animals immunized with r-Sap2 raised local anti-Sap2 immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies and were protected from the intravaginal challenge with *C. albicans*. Protection was likely due to the specific antibodies as shown by the passive transfer of immune vaginal fluid and the protective effects of passive vaccination with anti-Sap2 IgM and IgG monoclonal antibodies. Overall, the results evidenced that this recombinant proteinase constitutes a valuable reagent for use as a vaccine candidate for prevention and /or therapy against vaginal candidiasis.[65,83]

Then, the r-Sap2 was incorporated into influenza virosomes by Pevion, a Swiss biotech company. This novel vaccine, PEV7 (r-Sap2 virosomes), conferred protection to rats experimentally challenged with *C. albicans*, as exemplified by the accelerated clearance of the fungus from the vagina and resolution of the infection at least one week before infection in controls (administration of empty virosomes).

The r-Sap2 virosomal vaccine generated a persistent protection from *C. albicans* after intravaginal immunization in rats. This long-lasting protective status was associated with persistence of anti-Sap2 antibodies in the rat vagina.[84]

Subsequently, Pevion conducted the PEV7 phase I study to assess the safety and immunogenicity in 48 healthy volunteers. The results demonstrated favorable safety and the generation of specific and functional B cell memory in 100% of the vaccinated women and were very encouraging with regards to the therapeutic potential of the vaccine (PEV7 clinical trial).[85]

NovaDigm Therapeutics Inc., a company developing innovative vaccines for fungal and bacterial infections, acquired the rights to r-Sap2 from Pevion and from Istituto Superiore di Sanità in Rome (Italian National Health Institute [ISS]). The company developed a vaccine containing the Als3 antigen (NDV-3), which facilitates *Candida* adherence to and invasion of human endothelial cells.[86] The vaccine conferred protection to mice against experimental vaginal, oral, and intravenous challenge with *C. albicans* This vaccine is currently in the phase 1b/2a clinical study for the prevention of recurrent vulvovaginal candidiasis (NDV-3 clinical trial).[87] Moreover, the company has acquired the rights to a hyphally regulated protein 1 (Hyr1) and to a β-mannan conjugate.[88–90]

The aim of NovaDigm company is to produce a multivalent vaccine that can induce an immune response against multiple virulence traits of *Candida* and can enhance the probability of success against *C. albicans* mucosal infections.

The widespread occurrence of vaginal candidiasis and the development of resistance against antifungal agents has stimulated interest in understanding the pathogenesis of this disease. Sap activity has long enjoyed a putative role in *Candida* virulence. The data on *SAP* gene expression and the pathogenicity of *SAP*-deletion mutants have not only validated the previous predictions but have also greatly emphasised the general importance of this gene/enzyme family in the biology and pathogenicity of the fungus. The relationship between Saps and other putative virulence factors as hyphal and biofilm formation is an issue which needs to be addressed. Moreover, we investigate the immune response to Saps and the potential use of these responses to control *Candida* infections. Our observations with the rat vaginitis model have given evidence that the vaccine constituted of virosomal and Sap2 (PEV7) has an encouraging therapeutic potential for the treatment of recurrent vulvovaginal candidiasis. Several research teams developed anti-*Candida* vaccination.[83,84,86,91–95]

Two of these vaccines have passed phase 1 clinical trials for safety and immunogenicity, and one of them has entered a phase 2 clinical trial.[85,87] Both vaccines evidenced protection in rat and mouse models of vaginal infection by *C. albicans*, although with a slightly different mechanism of immunological protection.[31–36]

It is hoped that, in a not too distant future, a safe and protective anti-*Candida* vaccine would improve the quality of life of the high number of women affected with RVVC.

Acknowledgments

The authors are grateful to Giuseppina Mandarino for assisting in the English of the final draft and to Alfredo Caggiano for help in manuscript preparation.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Sobel JD. Pathogenesis of recurrent vulvovaginal candidiasis. *Curr Infect Dis Rep*. 2002; 4: 514–519.
2. Nyirjesy P, Sobel JD. Vuvovaginal candidiasis. *Obstet Gynecol Clin N Am*. 2003; 30: 671–684.
3. Sobel JD, Wiesenfield HC, Martens M et al. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. *N Engl J Med*. 2004; 351: 876–883.
4. Sobel JD. Management of recurrent vulvovaginal candidiasis: Unresolved issues. *Curr Infect Dis Rep*. 2006; 8: 481–486.
5. Fidel PL, Jr History and update on host defense against vaginal candidiasis. *Am J Reprod Immunol*. 2007; 57: 2–12.
6. Bauters TG, Dhont MA, Temmerman MI, Nelis HJ. Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. *Am J Obstet Gynecol*. 2002; 187: 569–574.
7. Ringdahl EN. Recurrent vulvovaginal candidiasis. *Mo Med*. 2006; 103: 165–168.
8. Foxman B, Muraglia R, Dietz JP, Sobel JD, Wagner J. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *J Low Genit Tract Dis*. 2013; 17: 340–345.
9. Cernicka J, Subik J. Resistance mechanisms in fluconazole resistant *Candida albicans* isolates from vaginal candidiasis. *Int J Antimicrob Agents*. 2006; 27: 403–408.
10. Jackson ST, Mullings AM, Rainford L, Miller A. The epidemiology of mycotic vulvovaginitis and the use of antifungal agents in suspected mycotic vulvovaginitis and its implications for clinical practice. *West Indian Med J*. 2005; 54: 192–195.
11. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. *J Clin Microbiol*. 2005; 43: 2155–2162.
12. Ventolini G, Baggish MS, Walsh PM. Vulvovaginal candidiasis from non-*albicans* species: retrospective study of recurrence rate after fluconazole therapy. *J Reprod Med*. 2006; 51: 475–478.
13. Shahid Z, Sobel JD. Reduced fluconazole susceptibility of *Candida albicans* isolates in women with recurrent vulvovaginal candidiasis: Effects of long-term fluconazole therapy. *Diagn Microbiol Infect Dis*. 2009; 64: 354–356.
14. Marchaim D, Lemanek L, Bheemreddy S, Kaye KS, Sobel JD. Fluconazole-resistant *Candida albicans* vulvovaginitis. *Obstet Gynecol*. 2012; 120: 1407–1414.
15. Kennedy MA, Sobel JD. Vulvovaginal Candidiasis Caused by Non-*albicans Candida* Species: New Insights. *Curr Infect Dis Rep*. 2010; 12: 465–470.

16. Babula O, Lazdāne G, Kroicka J, Linhares IM, Ledger WJ, Witkin SS. Frequency of Interleukin-4 (IL-4)-589 gene polymorphism and vaginal concentrations of IL-4, nitric oxide and mannose-binding lectin in women with recurrent vulvovaginal candidiasis. *Clin Infect Dis*. 2005; 40: 1258–1262.
17. Smeekens SP, van de Veerdonk FL, Kullberg BJ, Netea MG. Genetic susceptibility to *Candida* infections. *EMBO Mol Med*. 2013; 5: 805–813.
18. De Luca A, Carvalho A, Cunha C et al. IL-22 and IDO1 affect immunity and tolerance to murin and human vaginal candidiasis. *PLoS Pathogen*. 2013; 9: e1003486.
19. Jaeger M, Carvalho A, Cunha C et al. Association of a variable number tandem repeat in the NLRP3 gene in women with susceptibility to RVVC. *Eur J Clin Microbiol Infect Dis*. 2016; 35: 797–801
20. Odds FC. Chronic mucocutaneous candidosis. In *Candida and CandidosisBaltimore*, MD, USA: University Park Press, 1988: 104–110.
21. De Bernardis F, Cassone A, Sturtevant J, Calderone R. Expression of *Candida albicans* SAP1 and SAP2 in experimental vaginitis. *Infect Immun*. 1995; 63: 1887–1892.
22. De Bernardis F, Sullivan PA, Cassone A. Aspartyl proteinases of *Candida albicans* and their role in pathogenicity. *Med Mycol*. 2001; 39: 303–313.
23. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. *Trends Microbiol*. 2001; 9: 327–335.
24. Hube B. From commensal to pathogen: stage and tissue specific gene expression of *Candida albicans*. *Curr Opin Microbiol*. 2004; 7: 336–341.
25. Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev*. 2003; 67: 400–428.
26. Sobel JD. Vulvovaginal candidosis. *Lancet*. 2007; 369: 1961–1971.
27. Calderone RA, Clancy CJ. *Candida and Candidiasis*. 2nd edn. Washington, DC: ASM Press, 2012.
28. Moyes DL, Richardson JP, Naglik JR. *Candida albicans*-epithelial interactions and pathogenicity mechanisms: scratching the surface. *Virulence* 2015; 6: 338–346.
29. Drell T, Lillsaar T, Tummeleht L et al. Characterization of the vaginal micro-and microbioma in asymptomatic reproductive-age Estonian women. *Plos One*. 2013; 8: e54379.
30. Hofs S, Mogavero S, Hube B. Interaction of *Candida albicans* with host cells: virulence factors, host defense, escape strategies, and the microbiota. *J Microbiol*. 2016; 54: 149–169.
31. Cassone A. Fungal vaccines: Real progress from real challenges. *Lancet Infect Dis*. 2008; 8: 114–124.
32. Cassone A, Casadevall A. Recent progress in vaccines against fungal diseases. *Curr Opin Microbiol*. 2012; 15: 427–433.
33. Edwards JE, Jr Fungal cell wall vaccines: an update. *J Med Microbiol*. 2012; 61: 895–903.
34. Iannitti RG, Carvalho A, Romani L. From memory to antifungal vaccine design. *Trends Immunol*. 2012; 33: 467–474.
35. Cassone A. Development of vaccines for *Candida albicans*: fighting a skilled transformer. *Nat Rev Microbiol*. 2013; 11: 884–891.
36. Moragues MD, Rementeria A, Sevilla MJ, Eraso E, Quindos G. *Candida* antigens and immune responses: Implications for a vaccine. *Expert Rev Vaccines*. 2014; 13: 1–12.
37. Peters BM, Yano J, Noverr MC, Fidel PL, Jr. *Candida vaginitis*: when opportunism knocks, the host responds. *PLoS Pathog*. 2014; 10: e1003965.
38. Powell AM, Nyirjesy P. Recurrent vulvovaginitis. *Best Pract Res Clin Obstet Gynaecol*. 2014; 28: 967–976.
39. Cassone A. Vulvovaginal *Candida albicans* infections: pathogenesis, immunity and vaccine prospect. *BJOG*. 2015; 122: 785–794.
40. Cassone A, Sobel JD. Experimental models of vaginal candidiasis and their relevance to human candidiasis. *Infect Immun*. 2016; 84: 1255–1261.
41. Sobel JD. Recurrent vulvovaginal candidiasis. *Am J Obstet Ginecol*. 2016; 214: 15–21.
42. Donders GG, Sobel JD. Candida vulvovaginitis a store with a buttery and a show window. *Mycoses*. 2017; 60: 70–72.
43. Thewes S, Kretschmar M, Park H, Schaller M, Filler SG, Hube B. *In vivo* and *ex vivo* comparative transcriptional profiling of invasive and non-invasive *Candida albicans* isolates identifies genes associated with tissue invasion. *Mol Microbiol*. 2007; 63: 1606–1628.
44. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. *Virulence*. 2013; 4: 119–128.
45. Jabra-Rizk MA, Kong EF, Tsui C et al. *Candida albicans* Pathogenesis: Fitting within the Host-Microbe Damage Response Framework. *Infect Immun*. 2016; 84: 2724–2739.
46. Hoyer LL. The ALS gene family of *Candida albicans*. *Trends Microbiol*. 2001; 9: 176–180.
47. Hoyer LL, Green CB, Oh SH, Zhao X. Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family—a sticky pursuit. *Med Mycol*. 2008; 46: 1–15.
48. Roudbary M, Roudbamohammadi S, Bakhshi B, Farhadi Z. Relation of ALS1 and ALS3 genes and fluconazole resistance in *Candida albicans* isolated from vaginal candidiasis. *Inter J Mol Clin Microbiol*. 2012; 2: 170–174.
49. Hoyer LL, Cota E. *Candida albicans* agglutinin-like sequence (ALS) family vignettes: a review of ALS protein structure and function. *Front Microbiol*. 2016; 7: 280.
50. Staab JF, Bradway SD, Fidel PL, Sundstrom P. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science*. 1999; 283: 1535–1538.
51. Staab JF, Datta K, Rhee P. Niche-specific requirement for hyphal wall protein 1 in virulence of *Candida albicans*. *PLoS One*. 2013; 8: e80842
52. Gale CA, Bendel CM, McClellan M et al. Linkage of adhesion, filamentous growth, and virulence in *Candida albicans* to a single gene, INT1. *Science* 1998; 279: 1355–1358.
53. Hostetter MK. The iC3b receptor of *Candida albicans* and its roles in pathogenesis. *Vaccine*. 2008; 26: I108–I112.
54. Mora-Montes HM, Bates S, Netea MG et al. A multifunctional mannosyltransferase family in *Candida albicans* determines cell wall mannan structure and host-fungus interactions. *J Biol Chem*. 2010; 285: 12087–12095.
55. Sundstrom P. Adhesion in *Candida* spp. *Cell Microbiol*. 2002; 4: 461–469.
56. Spellberg BJ, Ibrahim AS, Avanesian V. *et al.* Efficacy of the anti-*Candida* rAls3p-N or Als1p-N vaccines against disseminated and mucosal candidiasis. *J Infect Dis*. 2006, 194: 256–260.
57. Zordan R, Cormack B. Adhesins on opportunistic fungal pathogens. In Calderone RA, Clancy CJ, eds. *Candida and Candidiasis*. Washington, DC: ASM Press, 2012: 243–259.
58. Kumamoto CA, Vinces MD. Contribution of hyphae and hypha-coregulated genes to *Candida albicans* virulence. *Cell Microbiol*. 2005; 7: 1546–1554.
59. Saville SP, Lazell AL, Monteagudo C, Lopez-Ribot JL. Engineered control of cell morphology *in vivo* reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryot Cell*. 2003; 2: 1053–1060.
60. Sudbery P, Gow N, Berman J. The distinct morphogenic states of *Candida albicans*. *Trends Microbiol*. 2004; 12: 317–324.
61. Sandini S, La Valle R, De Bernardis F, Macri C, Cassone A. The 65 kDa mannoprotein gene of *Candida albicans* encodes a putative beta-glucanase adhesin required for hyphal morphogenesis and experimental pathogenicity. *Cell Microbiol*. 2007; 9: 1223–1238.
62. Sudbery PE. Growth of *Candida albicans* hyphae. *Nat Rev Microbiol*. 2011; 9: 737–748.
63. Jacobsen ID, Wilson D, Wächtler B, Brunke S, Naglik JR, Hube B. *Candida albicans* dimorphism as a therapeutic target. *Expert Rev Anti Infect Ther*. 2012; 10: 85–93.
64. De Bernardis F, Molinari A, Boccanera M et al. Modulation of cell surface-associated mannoprotein antigen expression in experimental candidal vaginitis. *Infect Immun*. 1994; 62: 509–519.
65. De Bernardis F, Liu H, O'Mahony R et al. Human domain antibodies against virulence traits of *Candida albicans* inhibit fungus adherence to vaginal epithelium and protect against experimental vaginal candidiasis. *J Infect Dis*. 2007; 195: 149–157.
66. Lo H, Köhler JR, Di Domenico B, Loebenberg D, Cacciapuoti A, Fink GR. Nonfilamentous *C. albicans* mutants are avirulent. *Cell*. 1997; 90: 939–949.
67. Peters BM, Palmer GE, Nash AK, Lilly EA, Fidel PL, Jr, Noverr MC. Fungal morphogenetic pathways are required for the hallmark

inflammatory response during *Candida albicans* vaginitis. *Infect Immun*. 2014; 82: 532–543.
68. Muzny CA, Schwebke JR. Biofilms: An underappreciated mechanism of treatment failure and recurrence in vaginal infections. *Clin Infect Dis*. 2015; 61: 601–606.
69. Sobel JD. Editorial commentary. Vaginal biofilm: much ado about nothing, or a new therapeutic challenge? *Clin Infect Dis*. 2015; 61: 607–608.
70. Moyes DL, Murciano C, Runglall M, Islam A, Thavaraj S, Naglik JR. *Candida albicans* yeast and hyphae are discriminated by MAPK signaling in vaginal epithelial cells. *PLoS One*. 2011; 6: e26580.
71. De Bernardis F, Arancia S, Morelli L et al. Evidence that members of the secretory aspartyl proteinases gene family (SAP), in particular SAP2, are virulence factors for *Candida vaginitis*. *J Infect Dis*. 1999; 179: 201–208.
72. Schaller M, Borelli C, Korting H.C, Hube B. Hydrolytic enzymes as virulence factors of *Candida albicans*. *Mycoses*. 2005; 48: 365–377.
73. Pericolini E, Gabrielli E, Amacker M et al. Secretory aspartyl proteinases cause vaginitis and can mediate vaginitis caused by *Candida albicans* in mice. *mBIO*. 2015; 6: e00724–15.
74. Cassone A, Vecchiarelli A, Hube B. Aspartyl proteinase of eukaryotic microbial pathogens: from eating to heating. *PLoS Pathog*. 2016; 12: e1005992.
75. Elitsur Y, Jackman S, Neace C et al. Human vaginal mucosal immune system: characterization and function. *Gen Diagn Pathol*. 1998; 143: 271–277.
76. Cassone A, Boccanera M, Adriani D, Santoni G, De Bernardis F. Rat clearing a vagina infection by *Candida albicans* acquired specific antibody-mediated resistance to vaginal reinfection. *Infect Immun*. 1995; 63: 2619–2625.
77. De Bernardis F, Boccanera M, Adriani D, Spreghini E, Santoni G, Cassone A. Protective role of antimannan and anti-aspartyl proteinase antibodies in an experimental model of *Candida albicans* vaginitis in rats. *Infect Immun*. 1997; 65: 3399–3405.
78. De Bernardis F, Santoni G, Boccanera M et al. Local anticandidal immune responses in a rat model of vaginal infection by and protection against *Candida albicans*. *Infect Immun*. 2000; 68: 3297–3304.
79. Santoni G, Boccanera M, Adriani D et al. Immune cell-mediated protection against vaginal candidiasis: Evidence for a major role of vaginal $CD4^+$ T cells and possible participation of other local lymphocyte effectors. *Infect Immun*. 2002; 70: 4791–4797.
80. De Bernardis F, Lucciarini R, Boccanera M et al. Phenotypic and functional characterization of vaginal dendritic cells in a rat model of *Candida albicans* vaginitis. *Infect Immun*. 2006; 74: 4282–4294.
81. De Bernardis F, Santoni G, Boccanera M et al. Protection against rat vaginal Candidiasis by adoptive transfer of vaginal B lymphocytes. *FEMS Yeast Res*. 2010; 10: 432–440.
82. De Bernardis F, Arancia S, Sandini S, Graziani S. Studies of immune responses in *Candida* vaginitis. *Embo workshop on AIDS-related Mycoses Cape Town, South Africa. July 3–5*, 2013; abstract page 33.
83. Sandini S, La Valle R, Deaglio S, Malavasi F, Cassone A, De Bernardis F. A highly immunogenic recombinant and truncated protein of the secreted aspartic proteases family (rSap2t) of *Candida albicans* as a mucosal anticandidal vaccine. *FEMS Immunol Med Microbiol*. 2011; 62: 215–224.
84. De Bernardis F, Amacker M, Arancia S et al. A virosomal vaccine against candidal vaginitis: immunogenicity, efficacy and safety profile in animal models. *Vaccine*. 2012; 30: 4490–4498.
85. Safety and immunogenicity study of a virosomal vaccine against recurrent vulvovaginal *Candida* infection. PEV7 Clinical Trial. http://www.clinicaltrials.gov/ct2/show/NCT01067131.
86. Segal E. Testing antifungal vaccines in an animal model of invasive candidiasis and in human mucosal candidiasis. *Methods Mol Biol*. 2017; 1625: 343–353.
87. Safety, tolerability, immunogenicity and efficacy of NDV-3A vaccine in preventing recurrent vulvovaginal candidiasis. ClinicalTrials.gov Identifier: NCT01926028. https://clinicaltrials.gov/ct2/show/ NCT01926028.
88. Luo G, Ibrahim AS, Spellberg B, Nobile CJ, Mitchell AP, Fu Y. *Candida albicans* Hyr1p confers resistance to neutrophil killing and is a potential vaccine target. *J Infect Dis*. 2010; 201: 1718–1728.
89. Luo G, Ibrahim AS, French SW, Edwards JE, Jr, Fu Y. Active and passive immunization with rHyr1p-N protects mice against hematogenously disseminated candidiasis. *PLoS One*. 2011; 6: e25909.
90. Nitz M, Ling C, Otter A, Cutler JE, Bundle DR. The unique solution structure and immunochemistry of the *Candida albicans* β-1,2-Mannopyranan cell wall antigens. *J Biol Chem*. 2002; 277: 3440–3446.
91. Cutler JE, Deepe GS, Klein D. Advances in combating fungal diseases: vaccines on the threshold. *Nat Rev Microbiol*. 2007; 5: 13–28.
92. Xin H, Dziadek S, Bundle DR, Cutler JE, Synthetic glycopeptide vaccines combining beta-mannan and peptide epitopes induce protection against candidiasis. *Proc Natl Acad Sci U S A*. 2008; 105: 13526–13531.
93. Ostrowski-Zeichner L, Casadevall A, Galgiani JN, Odds FC, Rex JH. An insight into the antifungal pipeline: selected new molecules and beyond. *Nat Rev Drug Discov*. 2010; 9: 719–727.
94. Bromuro C, Romano M, Chiani P et al. Beta-glucan-CRM197 conjugates as candidates antifungal vaccines. *Vaccine*. 2010; 28: 2615–2623.
95. Schmidt CS, White CJ, Ibrahim AS et al. NDV-3, a recombinant alum-adjuvanted vaccine for *Candida* and *Staphylococcus aureus*, is safe and immunogenic in healthy adults. *Vaccine*. 2012; 30: 7594–7600.