New strategies for treatment of *Candida* vaginal infections

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Summary

New strategies for treatment of vaginal candidiasis have been recently exploited, due to widespread occurrence of this disease, in particular as recurrent infections, limitations of safe and efficacious antifungals as well as the lack of reliable preventative approaches. In this review new chemotherapeutic and immunotherapeutic strategies, based on the improved understanding of the immunopathogenesis of this prevalent human infection, will be discussed. The role of killer antibodies (or their molecular derivatives), i.e. antibodies that show antibiotic activity bearing the internal image of a yeast killer toxin (KT), characterized by a wide spectrum of microbicidal activity, and of the specific cell wall KT receptor as putative new therapeutic agents and preventative or therapeutic vaccines, respectively, will be particularly outlined.

Key words Vaginal candidiasis, Anticandidal chemotherapy, Anticandidal immunotherapy, Anticandidal vaccines, Killer antibodies

Nuevas estrategias en el tratamiento de las infecciones vaginales por *Candida*

Resumen

Se están empleando nuevas estrategias para el tratamiento de la candidiasis vaginal, debido a la amplia ocurrencia de esta enfermedad, particularmente como infecciones recurrentes, la oferta limitada de antifúngicos seguros y eficaces, así como la falta de una profilaxis fiable. En esta revisión se discuten las nuevas estrategias quimioterapéuticas e inmunoterapéuticas basadas en la cada vez mejor comprensión de la inmunopatogénesis de esta prevalente infección humana. Destacamos especialmente el papel de los anticuerpos agresores (o sus derivados moleculares), p.ej. anticuerpos con actividad antibiótica por contener la imagen interna de una toxina “asesina” que se caracteriza por un amplio espectro de actividad microbicida, y del receptor de la pared celular específico para la toxina “asesina”, como posibles nuevos agentes terapéuticos o vacunas preventivas o terapéuticas, respectivamente.

Palabras clave Candidiasis vaginal, Quimioterapia anticandidiásica, Inmunoterapia anticandidiásica, Vacunas anticandidiásicas, Anticuerpos agresores

Despite the availability of several potent antifungal agents and new insights into the host-fungus interplay, *Candida* vaginitis remains a common and frequently distressing infection affecting millions of women worldwide. On the basis of the clinical symptoms and the predicted response to antifungal therapy, in 1997 a new classification of *Candida* vaginitis, into uncomplicated (vulvovaginal candidiasis - VVC -) and complicated (severe or recurrent VVC, non-*albicans Candida* species or abnormal host) disease was recommended [1].

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VVC frequently affects women of child-bearing age and postmenopausal women who have underlying risk factors, such as hormone replacement therapy and immunosuppression caused by medication or disease [1-4]. Diabetes mellitus, particularly type 1, increases the rate of vaginal colonization and infection with *Candida* species [5,6]. Fewer than 5% of women with a primary episode subsequently experience frustrating recurrent VVC (RVVC), i.e. they develop at least three-four specific episodes within one year, in part without any known predisposing factors [7,8].

C. albicans is still recognized as the most frequent aetiologic agent of VVC and RVVC, with a highly significant relationship between high estrogen levels and the occurrence of infection, probably due to the production of glycogen, an attractive substrate for the yeast, by oestrogen stimulated epithelial cells [9]. Vaginal epithelial cells from mice, nonhuman primates and healthy women inhibit the growth of *C. albicans in vitro*, which may represent an innate host resistance mechanism against *Candida*. Women affected by RVVC have reduced epithelial cell anti-*Candida* activity that may contribute to the disease [10-12].

Recent studies clearly show that an increasing percentage of vaginal infections have to be ascribed to non-*albicans* species, such as *C. glabrata* and more rarely *C. krusei*, innately less susceptible to most antifungal agents [13,14]. The increasing use of "over the counter" (OTC) antifungal treatments, as well as widespread prescription of systemic oral azoles, could have resulted in spread of azole-resistant *C. albicans*, and even more likely of non-*albicans Candida* species with intrinsic azole resistance. Even if data on correlation between OTC exposure and colonization of drug-resistant *Candida* in vaginal microbiota are conflicting, self-medication with OTC without establishing a proper diagnosis could expose women to unnecessary antifungal treatments and to the risk of missing other causes or concurrent infections, contributing to a selection pressure in favor of more resistant species [15-17].

Innate as well as acquired humoral and cell-mediated immune (CMI) responses are involved in the defense against *Candida* infection. In particular, T-cell-dependent CMI is considered to play a key role for prevention of mucocutaneous infections [18,19]. Furthermore, cytokines produced by monocytes during the innate response may modify T cell-mediated immune response and different *Candida* species can induce differential production of immunoregulatory cytokines during infection [20,21].

The role of antibodies in this scenario is still controversial, probably because the antigenic complexity of *Candida* is responsible for the production of a plethora of different antibodies, including protective antibodies directed against a limited number of epitopes that are probably not produced at sufficient levels during natural infections. Protection against *Candida* infection probably requires a deep interaction of all the above mentioned host factors [22]. To date, observations coming from studies in women with RVVC, in HIV-infected women, and in animal models of experimental vaginitis, demonstrate that local immunity is more important than that in the systemic circulation for host defense against vaginitis, suggesting an immunological independence or compartmentalization of the vaginal mucosa [23-28]. Gamma/delta T cells have been suggested to play an immunoregulatory role in *Candida* vaginitis [29].

T helper (Th) 1, such as gamma interferon (IFN-γ) and interleukin-12 (IL-12), and Th 2 cytokines, such as IL-10, are produced at the vaginal level, with little to no modulation in response to infection [24]. Vaginal epithelial cells produce constitutively chemokines and proinflammatory cytokines, such as IL-1α, tumor necrosis factor α (TNF-α) and others, that, if produced *in vivo*, could be responsible for the inflammatory condition (edema and erythema) associated to VVC [30]. In experimental murine infections, transforming growth factor ß1 (TGF-ß1) was found in the vaginal fluids at high levels, and it could be responsible for a downregulatory or tolerance role [28]. Local production of chemokines, such as MCP-1, plays some role in reducing the fungal burden during experimental vaginal candidiasis in a manner independent of cellular chemotactic activity [31]. Recent evidences indicate an important role for dendritic cells (DC), particularly abundant at the skin and mucosal surfaces, in discriminating between the two forms of *C. albicans* (filamentous and budding yeast) in terms of elicited immune responses. By producing IL-4 and IL-12 in response to the virulent and nonvirulent forms of the fungus, DC have been demonstrated to be capable of Th priming, initiating T cell immunity [32].

Finally, *C. albicans* in some situations can act as a potent allergen and it has been suggested that local hypersensitivity to the yeast can be a factor in the prolongation of the disease [33]. RVVC would be correlated to a still undefined local immune deficiency or dysfunction [34].

The complexity of the immunopathology, the wide spectrum of clinical VVC and the pharmacotherapeutic failures in the treatment of *Candida* vaginitis require the implementation of all known measures to fight the pathogen and call for new therapeutic tools and strategies. Two areas have been recently deeply investigated and will be outlined: antimycotic chemotherapy and immunotherapy.

New chemotherapeutic strategies for treatment of *Candida* vaginal infections

Despite the availability of antifungals for oral and intra-vaginal treatment of VVC, unmet needs still exist for new therapeutic agents or schedules. As outlined by Sobel [35], perhaps the most important advance in this field in the last decade has been the recognition that not all forms of *Candida* vaginitis are equal and that their treatment requires individualization. Clinical evaluation of recurrent episodes as well as microbiological approaches to identify the aetiologic agent are essential to address the optimal therapeutic approach.

Most of the women with uncomplicated *Candida* vaginitis respond well to short-course therapy with fluconazole, the oral route being the preferred route of administration [36-39]. No differences have been observed between the relative effectiveness of oral and intra-vaginal antifungal treatment of *Candida* vaginitis; duration of therapy seems to be the most critical factor [40]. Although antifungal therapy is highly effective for individual attacks, persistence of vaginal colonization and recurrences after therapy are quite common, at least in part due to the fungistatic activity of azoles and caused by persistent yeast in the vagina, rather than by re-infections [41]. Women with recurrences of mild or moderate severity respond well to a single dose of fluconazole in the short term [42]; in contrast, women with complicated *Candida* vaginitis could require antimycotic therapies of longer duration to achieve clinical cure and mycologic eradication and to break the pattern of recurrence. After the acute episode has been treated, subsequent prophylaxis (maintenance therapy) could be important to prevent future frequent relapses, and long-term therapy may be warranted because of recurrences once prophylaxis is discontinued [43-45]. Treatment with sequential doses of fluconazole enhances clinical cure and mycologic eradication rates in patients infected with *C. albicans* [42].

Little evidence exists that antimicrobial resistance could be involved in RVVC, but the spread of non-*albicans Candida* species with intrinsic azole resistance may be an emerging concern, adversely influencing outcome of therapy [46]. Combined topical flucytosine and amphotericin B [47], terconazole cream [48] and others have been proposed. Maintenance therapy with topical boric acid has been experienced, but its efficacy in the cure of vaginitis and in the prevention of relapses ends with the suspension of the therapy [49].

There is a need of new antifungal drugs with greater potency and a broader spectrum of activity. Some of them, such as sordarins and caspofungin, characterized by mechanisms of action escaping consolidate acquired resistance, are promising and under study [50-53]. Problems inherent to antifungal chemotherapy and the attempt to preserve effective drugs by sparing their use have prompted recent interest and investigations of non-drug alternative or integrative strategies for candidiasis, such as immunotherapy [54-57].

New immunotherapeutic strategies for treatment of *Candida* vaginal infections

As outlined before, better understanding of the immunopathogenesis of VVC and RVVC and identification and characterization of immunodominant candidal antigens would be crucial to address optimal immunotherapeutic approaches to human candidiasis. Preventive or therapeutic vaccination and antibody-mediated immunotherapy have been recently proposed as new potential approaches.

Members of the secretory aspartyl proteinase (Sap) gene family, in particular Sap2 [58-60], a 65kDa mannoprotein (MP65) [61], surface mannan [62], a mannoprotein extract (MP) [63], and other constituents of *Candida* cell, have been proposed as immunodominant candidal antigens able to stimulate a potentially protective immune response against either systemic and/or mucosal candidiasis, and have been considered as potential vaccine candidates. Natural and monoclonal antibodies generated against some of those antigens proved to be protective when given before infection and therapeutic when given after infection [62].

Intravaginal and intranasal (i.n.) immunizations with MP or secreted Sap of *C. albicans*, with an appropriate adjuvant, have been demostrated to be equally effective in inducing vaginal antibodies and in conferring protection against vaginal candidiasis [63].

Protection from systemic fungus challenges has been demonstrated by i.n. delivery of a vaccine consisting of whole inactivated *Candida* cells and a cholera-toxin-like mucosal adjuvant [64] as well as by injection of DC pulsed with *Candida* yeasts or yeast RNA, even in hematopoietic transplantation [65].

Over the past years, a new idiotypic (Id) vaccination and anti-Id therapy approaches against systemic and mucosal *Candida* infections have been described, on the basis of the theory of the Id network [66] and the yeast killer phenomenon [67]. A murine monoclonal antibody (mAb KT4) that neutralized the wide-spectrum antimicrobial activity of a killer toxin (KT) from a selected strain of the yeast *Pichia anomala* (*Pa*KT) [68] was used as a parenteral or mucosal Id vaccine in animal (rabbit, rat, mouse) experimental models, eliciting the production of protective serum or secretory anti-Id antibodies. These antibodies carryed the internal image of *Pa*KT, in that they were able to functionally mimic it, and were designated as killer antibodies (KAbs) [69-71]. Mab KT4-affinity chromatography-purified serum or secretory KAbs exerted a direct significant *in vitro* killer activity against *Pa*KT-susceptible *C. albicans* cells by interacting with a specific not yet identified putative cell wall KT receptor (KTR), mainly expressed in budding cells and germe tubes, and were able to confer passive immunoprotection to unvaccinated experimentally infected animals. Particularly, intravaginal immunization with mAb KT4 resulted in effective protection in a well established rat model of candidal vaginitis [72].

The functional equivalence of KAbs with *Pa*KT suggested a similar homology between the Id of mAb KT4 and KTR. Such an hypothesis was demonstrated in that KAbs and *Pa*KT competed for the binding site of mAb KT4, intravaginal or intragastric inoculations of *Pa*KT-sensitive *C. albicans* cells were able to recall mucosal KAbs production in rats primarily immunized intravaginally with mAb KT4 and to elicit by themselves antibodies that functionally mimicked *Pa*KT. More interestingly, the latter observation was confirmed also in women experiencing RVVC, who had obviously never been exposed to the Id vaccine; from their vaginal fluid natural anti-KTR KAbs functionally similar to those previously described were significantly revealed and purified by affinity-chromatography. These purified anti-KTR human natural KAbs proved to confer immunoprotection by passive transfer to rats in the experimental model of vaginal candidiasis [73]. These data demonstrate that KAbs should be part of the Ab repertoire elicited by experimental and natural *Candida* infections, even though their clinical role in human disease remains to be elucitated. As described in experimental infections with *C. neoformans* [74], natural KAbs might be produced at non-protective levels, due to not constitutive expression of KTR on the *Candida* cell wall, and their activity might be interferred or even inhibited by the plethora of antibodies directed against other surface strongly antigenic constituents of the fungus cell wall. Once characterized, purified KTR and mAb KT4 idiotype could deserve great consideration as putative new anti-*Candida* vaccines.

A rat monoclonal KAb (mKAb) [75] and a mouse recombinant KAb in the single-chain format (rKAb) [76] have been produced, by hybridoma and phage display technologies from the spleen lymphocytes of animals immunized by idiotypic vaccination with mAb KT4. Both the molecules were able to kill *C. albicans in vitro*, bind to specific KTR on the yeast cells, and exert a strong therapeutic effect in the experimental model of rat vaginal candidiasis.

Cloning of the rKAb gene in a suitable vector in the human commensal *Streptococcus gordonii* allowed its expression as a secreted or surface-displayed molecule. The engineered streptococcal clones demonstrated to be highly efficacious in killing *C. albicans in vitro* and in significantly accelerating the clearance of high fungus burdens in the rat model of *Candida* vaginitis, persistently colonizing the vaginal mucosa. Stable colonization with the secreting strain, in particular, was as therapeutic as a full course of fluconazole [77].

On the basis of the previous observations and sequencing of the rKAb gene, two different experimental immunotherapeutic developments are under study.

Recent studies have characterized KTR as a component of the glucan fraction of the inner cell wall of *C. albicans*. Immunization of mice with fungal cells deprived of cell-surface constituents, such as mannoproteins, thus exposing the underlying ß-glucan-rich components, resulted in a significant protection against systemic, lethal infections by *C. albicans*, at least in part mediated by antibodies, with anti-ß-glucan IgM playing a relevant role [78]. These results and those reported by others, previously mentioned, stimulate to further study the preventative or therapeutic use of different putative vaccine candidates against vaginal candidiasis.

On the other side, a decapeptide synthesized and optimized from the sequence of the rKAb gene was demonstrated to maintain the strong *in vitro* candidacidal activity of rKAb and, when used to treat experimental vaginal and systemic candidiasis, it exerted a significant therapeutic activity, similar to that of fluconazole, beeing fully active also against a fluconazole-resistant strain of *C. albicans* (manuscript in preparation). Thus, synthetic killer mimotopes could represent conceptually new candidacidal therapeutic agents.

A further major interest of these approaches could rely on their potential wide antimicrobial spectrum. As the original KT, its anti-Id polyclonal, monoclonal and recombinant derivatives have been demonstrated to be microbicidal against a wide spectrum of epidemiologically important prokaryotic and eukaryotic pathogens

such as azole-resistant *Candida, Pneumocystis carinii, Aspergillus fumigatus, Cryptococcus neoformans, Mycobacterium tuberculosis*, methicillin and vancomycin resistant cocci, and others [79-83].

The existence of a putative tranphyletic KTR and its use as a multivalent vaccine could open new perspectives in the investigation of its ability to protect against several mucosal and systemic diseases caused by infectious *Pa*KT-sensitive microorganisms presenting KTR on their surface and of the wide-spectrum therapeutic activity of recombinant and synthetic derivatives of KAbs, that could represent conceptually new antimicrobial agents also deliverable at the mucosal sites by engineered commensal bacteria.

These approaches could be of wider potential in that specific toxin receptors of microbial pathogens and the Id of antibodies specifically neutralizing the biological activity of those toxins could be used to produce microbicidal antibodies and their molecular derivatives representing the internal images of antimicrobial toxins, thus mimicking the evolution of the biological competition occurring among microorganisms in natural habitats [84].

Finally, need still exists for further molecular, biochemical and immunological studies to validate and strengthen these experimental evidences and to determine the immune correlates at the basis of disease, protection and successfull therapy. The concerted use of drugs, antibodies or their derivatives and cytokines and the development of reliable and safe vaccines will probably be in the next future the most potentially powerful approaches for the treatment or the prevention of mucosal as well as systemic candidal infections.

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