[Frontiers in Bioscience 13, 6920-6937, May 1, 2008]

Anti-beta-glucan-like immunoprotective candidacidal antiidiotypic antibodies

Walter Magliani, Stefania Conti, Laura Giovati, Domenico Leonardo Maffei, Luciano Polonelli

Sezione di Microbiologia, Dipartimento di Patologia e Medicina di Laboratorio, Università degli Studi di Parma, Viale Gramsci, 14, 43100 Parma, Italy

TABLE OF CONTENTS

1. ABSTRACT

Mycoses, candidiasis in particular, are relatively common opportunistic infections still characterized by an unacceptable high mortality rate. Furthermore, they are often complicated by resistance or refractoriness to the existing antimicrobial agents. In recent years new effective therapeutic and large-scale preventative strategies have been proposed by exploiting the identification of fungal beta-glucans as target of antifungal agents such as echinocandins, yeast killer toxins and protective antibodies. Anti-beta-glucan antibodies are detectable in animal and human sera. When elicited by glucan-based vaccines they can exert a fungicidal protective activity. Beta-glucan cell wall killer toxin receptors can elicit fungicidal protective antibodies following natural and experimental infections. When used as an immunogen a killer toxin-neutralizing monoclonal antibody (beta-glucan-like) is able to elicit a significant anticandidal protection mediated by anti-idiotypic anti-beta-glucan-like candidacidal antibodies. Polyclonal, monoclonal and recombinant anti-beta-glucan-like antibodies and peptide mimotopes are able to exert an *in vitro* and/or *in vivo* microbicidal activity against eukaryotic and prokaryotic killer toxin receptor-bearing pathogenic microorganisms. Implications and perspectives for transphyletic anti-infectious control strategies, as immunoprevention and immunotherapy, are discussed.

2. INTRODUCTION

Over the last 20 years true pathogenic and opportunistic fungi have gained increasing public health concern, among the emerging pathogens responsible for severe and often life-threatening infections. This is a direct result of the tremendous medical progress and the HIV epidemic. Broad-spectrum antimicrobials, chronic immunosuppressive agents, catheters and internal prosthetic devices, aggressive chemotherapy and other medical interventions allow for an increased number of immunocompromised and otherwise debilitated individuals to survive longer. This type of patient is highly susceptible to infections caused by opportunistic organisms such as fungi, often rapidly progressive, and difficult to diagnose or treat. Strains and species resistant, refractory or intrinsically less susceptible to the available antifungals are spreading. They further complicate therapeutic approaches and medical management of the infected patients. Thus, an overall increase in mortality from mycotic infections has been observed (1-6).

Candida species remain the most common fungal pathogens, with a significant prevalence of *C. albicans* and a growing prominence of non-*albicans Candida* species, such as *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis* (7-13). Several of these species colonize skin and mucous

membranes and can have unique properties, such as the ability to produce virulence factors and biofilms, which contribute to their pathogenicity (14-19). Mucosal candidiasis often complicate HIV infection, as oropharyngeal and esophageal infections. They are also relatively common in women of childbearing age, in the form of acute and chronic, recurrent vulvovaginal candidiasis (20-25). Systemic, invasive candidiasis often ensues the breakdown of normal mucosal and skin barriers as well as immune dysfunctions, such as neutropenia (26, 27). Suitable guidelines containing disease-specific recommendations to prevent and treat opportunistic infections, especially in HIV-infected persons, have been published in the last few years (28-31). However, despite major advances in the field of antifungal therapy and efforts to control nosocomial candidiasis, its crude (from 40% to 75% in different studies) and attributable mortality still remains very high. This can represent a devastating complication of health care delivery (12, 32-35). Thus, there is a pressing need of new, more effective therapeutic and large-scale preventative strategies.

In this scenario, of great importance are studies to understand the pathogenesis of systemic and mucosal candidiasis, host-*Candida* interplay and the innate and acquired (adaptive) immune mechanisms associated with susceptibility and protection to fungal agents. Ingestion and elimination of the yeast by cells of the innate immune system (neutrophils, monocytes, and macrophages), acellular innate responses, cell-mediated (CMI) and humoral immunity, all are considered to play a different critical role in the control of mucosal and systemic candidal infections (36-42). Interestingly, adaptive immune responses differentiate between metabolically active germinating fungal cells that carry a risk for invasive disease and non-disease-causing inactive cells, being restricted to the former (43).

Besides the recognized relevant role of CMI in the host defense against candidiasis, on the basis of experimental and clinical evidences, since the 1990s, humoral immunity has begun to be recognized as deeply involved in susceptibility/protection to fungal infections (44, 45). Against the enormous number of epitopes contained in the fungal cell a plethora of different antibodies (Abs) are elicited. Among them, protective, non-protective, indifferent and even infection-enhancing Abs can interfer each other and block protection (46). Specificity, isotype and titer of different Abs can also influence Ab-mediated protection (47-49). In fungal infection or anti-fungal immunization, protective Abs would not be predominantly produced. Protective epitopes can be either scarcely represented or hidden by more external immunodominant antigens or masked by interfering non-protective Abs. Most of these inconsistencies have been recently clarified. Monoclonal (mAbs) and recombinant Abs (rAbs) to specific fungal epitopes and experimental vaccines with proper antigen formulations to elicit 'unnatural' immunity have been produced and studied. They demonstrated the possibility to confer passive or active protection against experimental systemic and/or mucosal fungal infections (50-53).

The existence of a family of fungicidal protective Abs has been suggested (54). As discussed below, our group first investigated in depth polyclonal, monoclonal, recombinant and human natural microbicidal Abs that mimic a yeast killer toxin (KT). Matthews and Burnie have produced a polyhistidine-tagged human rAb against fungal heat shock protein (hsp)90 (Mycograb), which demonstrated a direct *in vitro* inhibitory activity against a wide range of *Candida* species. Synergy between Mycograb and amphotericin B (AMB) against *Candida* spp. and *Cryptococcus neoformans in vitro* and in animal models of invasive candidiasis suggested their use in combination therapy (55-57). When tested in a multicenter trial in patients with invasive candidiasis, Mycograb in combination with AMB was well tolerated, produced an improved clinical and mycological response and reduced *Candida*-attributable mortality (58). Its use in combination with caspofungin has been reported to successfully treat a case of refractory candidiasis in a child (59). However, due to concerns over the quality and safety, such as the 'cytokine release syndrome' associated with the treatment, on November 2006, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMEA) adopted a negative opinion, recommending the refusal of the marketing authorization for Mycograb (60).

Ponton's group described a fungicidal Ab, mAb C7, which was raised against a protein epitope of a stress mannoprotein of more than 200 kDa from the cell wall of *C. albicans*. This protein is the major target of salivary immunoglobulin (Ig)A. MAb C7 exhibited different important biological activities: inhibition of yeast adhesion to a variety of surfaces and of germination, direct potent fungicidal activity against *Candida* spp., *C. neoformans*, *Aspergillus fumigatus*, and *Scedosporium prolificans* and direct tumoricidal activity. Furthermore, mAb C7 was able to passively protect mice from invasive candidiasis (61-63). The antigen recognized by mAb C7 has been recently identified as the agglutinin-like surface (Als)3 protein on the cell wall of *C. albicans*. This protein is a member of the Als protein family which has a relevant role in adhesion and virulence of the yeast (64). Kavishwar and Shukla described a mAb, of IgA isotype, that exhibited ability to bind with mannosyl moieties of proteins of *C. albicans* cell wall, direct *in vitro* candidacidal activity and protective efficacy in a murine model of vaginal candidiasis (65).

Among these Abs, of growing interest are protective Abs directed to cell wall beta-glucans (BGs), at the basis of the anti-fungal protection conferred by a glucan-conjugate vaccine recently described (66), and KT-like Abs, which have been the object of our research for many years. This review will basically focus on the role and mechanism of action of these Abs in host defense against fungal infections. Their intriguing potential as putative new tools for anti-infective therapy and prevention will be discussed, given the emerging relevant role of fungal BGs and the implications of the yeast killer phenomenon.

3. FUNGAL BETA-GLUCANS AND IMMUNITY

1,3-BGs are glucose homopolymers which are commonly found in fungi, plants and seaweeds, where they can behave as structural or storage carbohydrates. Since BGs are not found in mammals, it has been recognized for a long time that they can activate the immune system. They can act as biological response modifiers, in that they are able to boost the natural defence mechanisms of the host. When administered by different routes, BGs can prime and activate leucocytes. Thus they display an array of beneficial properties, including increase of the efficacy of antimicrobial and anticancer therapies, health promotion and disease alleviation in the context of some pathologies. Triple helix conformation and presence of hydrophilic groups on the outside surface of BGs are important for their biological activities (67-73).

In fungi, BGs form the structural skeleton of their tiered cell wall, which is actually a dynamic structure. In *C. albicans*, in particular, BGs [a backbone of beta-(1,3)-linked beta-D-glucopyranosyl units with beta-(1,6)-linked side chains] represent 50-60% by weight of the cell wall. Chitin (chains of N-acetylglucosamine; 0.6-3%) and mannoproteins (30-40%) represent other main components. BGs are composed of either alkali-soluble and -insoluble fractions. The first one consists mainly of highly branched 1,6-BGs. The second one contains 30-39% beta-1,3 and 43–53% beta-1,6 linkages in either yeast or hyphal cells, and 67% beta-1,3 and 14% beta-1,6 linkages in germ tubes. Moving outward from the plasma membrane, the cell wall is characterized by the presence of different zones of enrichment: a first one rich in mannoproteins, followed by a scaffold of BGs and a region enriched for chitin, which is covalently linked to BGs, and an outer zone composed of mannoproteins. Cell wall proteins (Cwps) are mostly glycosylphosphatidylinositol (GPI)-dependent Cwps, which are attached via 1,6-BG to 1,3-BG and, to a lesser extent, to chitin. A small proportion of Cwps is represented by Pir (Proteins with internal repeats)-related proteins directly attached to BGs (74, 75). This molecular organization is responsible for the shape of the cell and its physical strength, as well as for its ability to adhere to host tissues and crosstalk with the host. While mannoproteins mostly determine the cell surface properties, BGs play a critical structural role, whose importance is underscored also by the ability of BG synthase inhibitors, such as echinocandins, to cause cell lysis (76).

Interestingly, BGs can be released into the systemic circulation of patients with some proven or probable deep-seated mycosis. They possibly contribute to the development of fungal infection by suppressing monocyte functions directly, and T-cell function indirectly (77). Their presence in blood or other body fluids can be easily determined, by taking advantage of their ability to activate factor G of the horseshoe crab coagulation cascade (78). Thus, monitoring BG antigenemia, possibly in combination with anti-cell wall Ab level determination, has been suggested to be of diagnostic value for early diagnosis of invasive fungal infection and in predicting its therapeutic outcome, even though with some limitations (79-84).

BGs and mannans represent the major pathogen associated molecular patterns (PAMPs) recognized by the host pattern recognition receptors (PRRs) of the innate immune system. After recognition, BGs can act as potent proinflammatory molecules. BGs are predominantly buried beneath the thin but dense mannoprotein outermost coat. This masking may protect them by recognition and represents a relevant fungal virulence factor, in that, by creating an anti-inflammatory barrier, it limits and subverts the host immune response. In fact, while during budding scar glucans become accessible, hyphae, which lack a budding mechanism, escape from the recognition. This may prevent recruitment of effector cells and clearance of the fungus, promoting a more effective colonization of the host tissues and an increasing propensity for causing disease (68, 85-89).

C. albicans is recognized by the host through a very complex and multifactorial process. This involves the sequential recognition of the various layers of the fungal cell wall and the cooperation between toll-like receptors (such as TLR4 and TLR2) and lectin receptors (such as MR and dectin-1) (90). In recognition of BGs, in particular, several distinct PRRs, expressed both by immune and nonimmune cells, have been implicated. They include complement receptor 3, lactosylceramide, scavenger receptors and dectin-1. Among them, dectin-1, a member of the C-type lectin receptor family, represents the major BG receptor. Dectin-1 is mainly expressed on dendritic cells (DCs) and macrophages, mediates BG biological effects and synergizes with TLRs for the production of proinflammatory cytokines and to initiate specific responses (90-98). Anti-dectin-1 mAbs can inhibit BG-mediated anti-tumor activity in mice (99). Furthermore, BGs may interact with pituitary cells resulting in stimulation of TLR4 and CD14 gene expression and secretion of prolactin, a hormone that plays an important role in response to infection (100). Soluble or particulate BGs can activate immune cells differently and influence cytokine production, by mediating cytotoxic and phagocytic responses (75). While cell surface mannans are considered the dominant antigens, BGs are generally thought to have poor immunogenicity, as attested also by the few reports on anti-BG Abs. However, BGs can be fisiologically or experimentally unmasked by environmental conditions, drug treatment, mutation or purification as well as at the site of budding scars. Thus they can be recognized by immune cells, eliciting a specific stronger immune response. Anti-BG Abs have been detected or produced in different conditions.

3.1. Anti-beta-glucan antibodies

Due to the poor immunogenicity of BGs, anti-BG mAbs have been produced by conjugating BGs with proteins, such as bovine serum-albumine or keyhole limpet hemocyanin (101, 102). Anti-BG laminarin mAbs have been used as immunogens for the production of rabbit anti-idiotypic (anti-Id) Abs. These Abs recognized human monocyte receptors for yeast BG, displaying functional characteristics of soluble BG ligands (103). More importantly, Abs to solubilized C. albicans cell wall BGs have been detected in sera from normal human volunteers,

as well as in commercially available beta-globulin prepared from pooled human sera and in immune or normal murine sera, with variable individual titers (104-107). Affinity purified human and murine anti-BG Abs have shown higher reactivity to solubilized cell wall BGs derived from pathogenic fungi than to mushroom BGs. This suggests the existence of an antigenic epitope that is peculiar to the formers. Anti-BG Abs might enhance the in vitro candidacidal activity of human macrophages, suggesting their ability to influence the host defense against Candida through opsonization. Interestingly, patients with deep mycosis have a significantly lower titer of anti-BG Abs compared with normal individuals. Furthermore, anti-BG Abs titer decreases remarkably in mice, following intravenous administration of BGs. This suggests the formation of a complex between anti-BG Abs and Candida cell wall BGs, which could promote the clearance of the pathogen from the systemic circulation. A decreasing of the anti-BG Abs titer in mycosis patients can be reverse correlated to the progression of fungal infection. These and other observations have supported a protective role of these Abs (108).

In 2002, a paper from Bromuro *et al.* (48) has shed some more light upon the relative relevance of anti-BG Abs in the outcome of disseminated candidiasis. After immunization with either heat-inactivated, whole *C. albicans* cells (Y) or Y cells deprived of surface-located components by treatment with dithiothreitol and proteinase K (YDP), mice developed intense, largely cross-reactive humoral and cell-mediated immune responses. They were characterized by the presence in the sera of Abs against all major cytoplasmic and cell wall constituents, including BGs (anti-Y sera), or mostly BGs (anti-YDP sera). Only YDP-immunized animals significantly survived a lethal fungal challenge. Passive immunization with partially purified IgMs from mice immunized with YDP or with serum from Y-immunized animals, preadsorbed on Y cells, could confer protection against fungal burden both in normal and SCID mice. Protection was abolished by preadsorption of the sera with pure particulate glucan, which removes specific anti-BG Abs. During infection, antagonistic or blocking Abs, probably directed against cell surface constituents of the fungus, such as mannans, can inhibit protection conferred by anti-BG Abs. This possibly can explain the susceptibility to invasive candidiasis of individuals with elevated anti-*Candida* Abs titers.

3.2. An antifungal beta-glucan-based vaccine

On the basis of the previous observations a BG-based vaccine has been recently reported (66). This novel experimental vaccine is constituted by laminarin (Lam), a poorly immunogenic linear polymer of beta-1,3-linked glucose units with occasional beta-1,6-glucan branches of a single glucosyl residue. Lam has been purified from the brown alga *Laminaria digitata*, thus it is devoid of any contamination with other fungal antigens. To increase immunogenicity, Lam has been conjugated with a protein carrier (diphtheria toxoid CRM197), safely used in other human vaccines (Figure 1B). After parenteral immunization with Lam-CRM, mice resulted significantly protected from lethal systemic challenges by *C. albicans*, as verified in terms of median survival time, overall mortality and reduction of the fungal burden in the kidney. Moreover, mucosal (intravaginal or intranasal) immunization with the vaccine induced in rats a significant degree of protection against experimental vaginal candidiasis, characterized by an accelerated rate of *Candida* clearance from the vagina. Anti-BG Abs present in sera and vaginal fluids of immunized animals were able to transfer to nonimmunized animals a protection, that was totally abolished by pre-adsorption on *Candida* cells or insoluble BG particles. This suggested a relevant, if not exclusive, role of anti-BG Abs in protection.

To further support these observations, an anti-Lam mAb, of IgG2b isotype, had been produced. It was able to confer significant passive protection against both systemic and mucosal candidiasis. After immunofluorescence staining, anti-Lam Abs and mAb strongly bound to isolated BG fungal particles. A preferential binding was observed to germ tubes and hyphae of *C. albicans*, and particularly to hyphal tips and septa. Even more significantly, anti-Lam Abs and mAb exerted a marked direct *in vitro* candidacidal activity. A significant colony forming units reduction in *C. albicans* cultures grown overnight in their presence was observed. Thus, anti-BG Abs elicited by the Lam-CRM vaccine should be added to the growing family of candidacidal, protective Abs, which can participate in the clearance of fungal infection.

As outlined by Casadevall and Pirofski (109), this vaccine is innovative, first of all in that, being constituted by a polysaccharide antigen from algae, it stimulates protection by eliciting cross-reactive Abs that appear to be directly microbicidal. The wide availability of a stable immunogen makes the preparation of the vaccine affordable and relatively inexpensive. Importantly, this conjugate vaccine was able to confer a significant protection against systemic and mucosal fungal infections in different animal species. It demonstrated a potential usefulness for parenteral and mucosal immunizations as polyvalent vaccine, as further discussed below. In our opinion, all these observations on role and characteristics of vaccine-elicited anti-BG Abs strongly corroborate our previous findings on the occurrence of directly microbicidal protective Abs. These Abs mimic the antimicrobial activity of a yeast KT, whose receptors (KTRs) on susceptible cells are essentially constituted by BGs.

4. YEAST KILLER PHENOMENON AND BETA-GLUCANS

We have already widely revised the yeast killer phenomenon and its implications in terms of unexpectedly potential applications in Ab-mediated protective immunity, prevention and therapy (54, 110-116). Here we will briefly discuss the involvement of BGs in this phenomenon. Initially discovered in the yeast *Saccharomyces cerevisiae*, the ability to secrete protein toxins that are lethal to sensitive strains (“killer toxins”, KTs) has been described

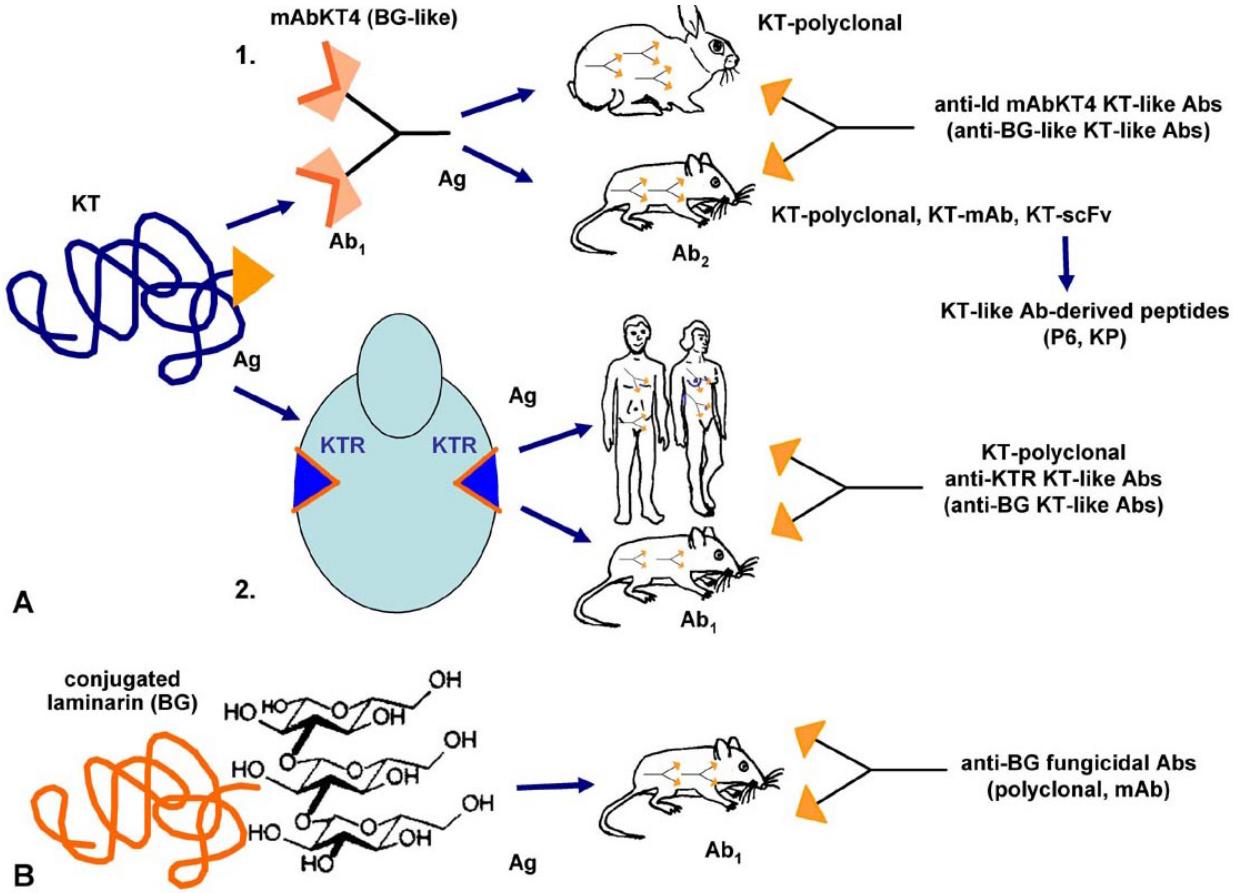


Figure 1. Experimental and natural immunizations by a BG-like mAb (A1), KTR (A2) and BG conjugated with a protein carrier (B), as antigens. A1. In animals (rabbit, mouse, rat) immunized with mAbKT4 (BG-like Ab neutralizing KT), antiidiotipic microbicidal Abs (KT-like) have been raised and produced in different formats (polyclonal, monoclonal and recombinant). Anti-Id KT-like Abs were microbicidal *in vitro*, significantly protective in different animal models of infection and could passively transfer the protective state to naive animals. A2. Following experimental and natural infections by KT-sensitive, KTR-bearing microrganisms, such as *C. albicans*, KT-like Abs were raised.B. In mice immunized with laminarin (BG) conjugated with a protein carrier, anti-BG Abs (polyclonal and monoclonal) were raised. These Abs showed antifungal activity *in vitro* and were protective against experimental systemic and vaginal candidiasis.

in many other yeast and fungal species and genera. It has been associated with different genetic determinants, such as double-stranded RNA viruses, linear dsDNA plasmids, and nuclear genes, and characterized by specific mechanisms of action (110, 117). Besides its basic biological interest, the killer phenomenon has attracted much more interest. In fact some KTs can exert an antimicrobial activity on a wide spectrum of susceptible microorganisms that are characterized by the presence of specific KTRs and lack of immunity systems. The inclusion among KT-sensitive microorganisms of species/genera of relevant clinical interest has led to a reevaluation of this sophisticated widespread process of competition in natural ecosystems. New reliable sources of safe antimicrobial agents could be available (118). In this perspective, chromosomally encoded KTs produced by killer strains belonging to species of *Pichia* and *Williopsis* have been particularly investigated. Unlike the *S. cerevisiae* K1 toxin, which targets the 1,6-BG, some of them target 1,3-BGs and can interfere with their synthesis, thus resulting in killing of sensitive strains.

KTs produced by the yeasts *W. saturnus* var. *mrakii* IFO 0895 (HM-1) and *W. saturnus* var. *saturnus* IFO 0117 (HYI), previously known as *Hansenula mrakii* and *H. saturnus*, are strongly cytocidal small proteins (88 and 87 amino acids, respectively). They show a 87% overall homology and share the same mechanism of killing against sensitive yeasts by extracellularly inhibiting 1,3-BG synthase activity. Both affect sensitive cells primarily in the growing stage, but not yeast cells in the resting stage nor mammalian cells. The formation of pores at the distal tip of the developing buds and at the protruding conjugation tubes, where cell wall synthesis is active, is the cause of the osmotic cell lysis (119-123). A 85-kDa glycoprotein KT produced by *W. saturnus* var. *mrakii* MUCL 41968 (WmKT) shares structural similarities with yeast CWPs suspected to exhibit glucosidase activity. WmKT demonstrates a particularly wide spectrum of activity *in vitro*, by inducing rapid cell permeation and death, soon after its binding to target cells. It requires the interaction with BGs to display its killer activity and, apparently, exhibits a beta-glucosidase activity, which could explain

the disruption of the cell wall integrity of sensitive cells (124, 125). Interestingly, WmKT appears to be related to a previously decribed KT produced by *Pichia anomala* ATCC 96603 (PaKT). A neutralizing mAb produced against PaKT (mAb KT4) specifically can label the surface of both KT producing strains, suggesting that they secrete KTs bearing a common epitope (126). PaKT is a glycoprotein encoded by nuclear genes, which is heterogeneously secreted in the presence of tunicamycin, thus excluding the involvement of N-glycosylation in toxin secretion and killer activity (127-129). PaKT killer activity is mediated by direct interaction with specific cell wall KTRs, distributed mainly in budding cells and germination tubes, constituted by BGs.

Targeting BGs with KTs could be envisaged as a new potential antifungal therapeutic opportunity (130, 131). However, antigenicity, lability at physiological pH and temperature, as well as toxicity (132), limited this approach to the topical therapy in different animal species of some experimental cutaneous infections (130). We, and more recently others, have proposed a creative approach in the attempt to overcome these limitations. Anti-Id Abs which have the internal image of the active site of KTs, such as PaKT and HM-1, have been produced. Theoretically, the steric interaction between cell wall KTRs of susceptible strains and specific KTs may be comparable to the binding of the idiotype of an anti-KT Ab and its complementary anti-Id Ab (internal imagery of the yeast killer phenomenon). On the basis of the theory of the idiotypic network, Abs directed to biologically functional epitopes of KTs, which are able to neutralize their killing activity, could be used as immunogens for “idiotypic vaccination”. Thus, anti-Id KT-like (anti-Id KT-)Abs could be produced in different experimental conditions, as well as in a variety of formats.

Selvakumar *et al.* have identified and characterized the epitope involved in the potential HM-1 active site, against which a mAb (nmAb-KT) had been previously produced. NmAb-KT neutralized the yeast killing and glucan synthase inhibitory activities of HM-1. By using recombinant DNA technology and a phage display system, they produced single-chain fragment variable (scFv) anti-Id KT-Abs from the splenic lymphocytes of mice immunized with nmAb-KT. These scFvs exerted *in vitro* a strong candidacidal activity and effectively inhibited fungal BG synthase activity. They were neutralized by adsorption with nmAb-KT and specifically bound to it, as confirmed by surface plasmon resonance analysis and by their competition with HM-1 for binding to nmAb-KT (133-135).

These findings definitely confirm our previous observations on the possibility to generate what we defined as “antibiobodies”, i.e. anti-Id Abs that mimic the antimicrobial activity of a KT, thus acting as antibiotics (136). In our experimental model, a PaKT-neutralizing mAb (mAb KT4) had been used as a parenteral or mucosal Id vaccine for the production of Abs that mimic the killing activity of PaKT (anti-Id KT-Abs). Anti-Id KT-Abs were able to interact with specific cell wall KTRs constituted by BGs. In some way, a steric/functional homology between the Id of mAb KT4 and KTRs, as well as between PaKT and KT-Abs, should be envisaged. This hypothesis had been unambiguously demonstrated in different ways. For instance, repeated intravaginal inoculations in rats of PaKT-sensitive *C. albicans* cells stimulated the production in the vaginal fluid of specific Abs. When purified by affinity chromatography against mAb KT4, they functionally mimicked PaKT by directly killing *C. albicans* cells *in vitro*. Similar candidacidal KT-Abs have been consistently detected also in the vaginal fluids of symptomatic women with clinical and microbiological diagnoses of candidal vaginitis. The killing activity of KT-Abs was totally abrogated by their previous adsorption with mAb KT4. Importantly, KT-Abs were also able to confer a significant anticandidal protection in a rat vaginitis model, thus suggesting that candidacidal anti-KTR KT-Abs may be part of the Ab repertoire that follows infection or immunization with *Candida* (137). On the other side, the Id of mAb KT4 should be considered as a KTR-like, and consequently BG-like, molecule as well as anti-Id Abs as anti-BG-like Abs (Figure 1 A1 and A2).

4.1. Anti-beta-glucan-like immunoprotective candidacidal antiidiotypic antibodies

Anti-Id (anti-BG-like) candidacidal Abs (anti-Id KT-Abs) were described for the first time almost 20 years ago. By immunzing rabbits with hybridoma cells secreting mAb KT4, anti-Id KT-Abs have been raised. They were able to compete with PaKT for the binding site of mAb KT4 and to inhibit *in vitro* the growth of *C. albicans*, thereby mimicking the effect of PaKT (138). Affinity purified anti-Id KT-Abs allowed the observation by indirect immunofluorescence of cell wall KTRs on *C. albicans* cells. These KTRs are primarily localized on budding cells and germ tubes, where new cell wall is synthesized and BGs become accessible (139). The potential of mAb KT4 as an experimental parenteral or mucosal Id vaccine has been consequently verified in other animal (mouse, rat) models of systemic or vaginal candidiasis. Candidacidal anti-Id KT-Abs have been consistently raised in sera or vaginal fluids of immunized animals. They could be considered responsible for the significant protection against both candidiasis and could passively transfer the protective state to nonimmunized animals (140, 141). The steric/functional similarity between the Id of mAb KT4 and *C. albicans* cell wall KTRs had been further confirmed. Intravaginal or intragastric inoculations of PaKT-sensitive yeast cells were able to recall anti-Id KT-Abs production in the vagina of rats primarily immunized with mAb KT4. Even more significantly, as outlined before, natural KT-like Abs have been detected in human fluids. Thus, KTRs may be recognized by the host's immune system and exploited to produce microbicidal Abs (137).

The more obvious following step has been the production of anti-Id KT-Abs of absolute reproducibility and unlimited availability, such as monoclonal and recombinant KT-Abs. From mAb KT4-immunized rats or mice an anti-Id KT-mAb (mAb K10) and an anti-Id KT-scFv (scFv H6) have been produced, by the hybridoma and recombinant technologies, respectively. Both molecules

competed with PaKT for the binding to mAbKT4 and KTRs, allowing the visualization of the putative KTRs on the yeast cell wall (particularly, in the germ tubes and budding cells). Furthermore, they were able to kill PaKT-sensitive *C. albicans* cells *in vitro*, an activity neutralized by previous adsorption with mAbKT4. More importantly, they were therapeutic against experimental candidiasis (142, 143). Cloning and expression of scFv H6 in the human commensal *Streptococcus gordonii* allowed to obtain different engineered strains. They were able to stably colonize rat vaginal mucosa and to exert, by steady local production of candidacidal scFvs, a strong therapeutic effect against experimental vaginal candidiasis (144, 145). On the basis of these observations new therapeutic strategies to control candidiasis have been envisaged (111-114). Finally, sequencing of the scFv H6 gene enabled synthesis and selection of Ab-derived peptides characterized by potent antimicrobial activity. This allowed further, in some way unexpected, biotechnological and therapeutic developments.

4.2. Anti-beta-glucan-like candidacidal, therapeutic antibody-derived peptides

In the attempt to establish possible correlations between the amino acid sequence of scFv H6 and its candidacidal activity, peptides reproducing the 6 complementarity determining regions (CDRs), as well as decapeptides containing their parts, have been synthesized and tested *in vitro* for candidacidal activity. From the most active peptide, a decapeptide including part of the light chain CDR1 was obtained by alanine scanning ("killer peptide", KP, AKVTMTCSAS) (Figure 2). In time-killing studies KP demonstrated a clear, rapid candidacidal activity *in vitro* (more than 90% killing within 30 min of incubation with *C. albicans*). Furthermore, it was able to compete with mAb K10 for binding to yeast germinating cells, i.e. to the specific KTRs. This candidacidal activity was neutralized, in a dose-dependent manner, by laminarin, a soluble BG. More importantly, postchallenge mucosal or parenteral administration of KP demonstrated a remarkable therapeutic effect in animal models of vaginal and systemic candidiasis. Significantly, this effect was observed in immunocompetent as well as immunodeficient animals, even in the case of an infection caused by a fluconazole-resistant *C. albicans* strain (115, 146).

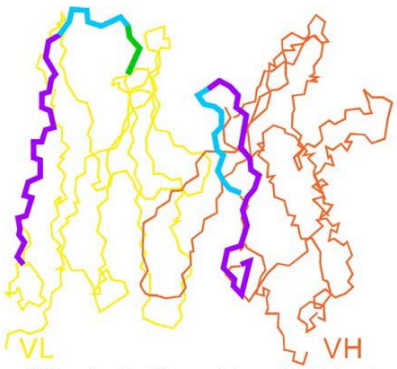
In our opinion, these are intriguing observations, in that an Ab-derived peptide retained the microbicidal activity of the whole Ab, by interacting with the same (BG)-KTRs on target microbial cells. This suggested new experimental biotechnological approaches for antifungal therapy by using candidacidal KT-Abs or, more significantly, killer peptides. These molecules can be therapeutic also in immunocompromised individuals, in that they do not require any host's immune participation (147, 148). Furthermore, KP was endowed with no toxicity and, being a small peptide, it had been easily engineered to be produced in an active form and in great amount *in planta*. Significanlty, plants expressing KP, but not the ones expressing a scramble peptide (SP), proved to be resistant to experimental infections with phytopathogenic microorganisms (149).

4.3. Wide-spectrum microbicidal antibodies and antibody-derived peptides

Immunological derivatives of PaKT in different formats (monoclonal, recombinant and small peptides) are much more stable than the toxin at different *in vitro* and *in vivo* conditions. This allowed to test them against many other eukaryotic and prokaryotic microorganisms, given the established wide spectrum of microbicidal activity of PaKT, and to demonstrate their activity and therapeutic potential. Many epidemiologically relevant pathogenic agents have been included in the list of susceptible microorganisms as new strains/species have been tested. Among them, besides *C. albicans*, other fungal agents, such as *A. fumigatus* (150), *C. neoformans* (151), *Paracoccidioides brasiliensis* (152), *Pneumocystis carinii* (153, 154), non-*albicans Candida* spp. (155), and plant pathogens, such as *Botrytis cinerea* and *Fusarium oxysporum* (149), were exquisitely susceptible *in vitro* to these molecules. They demonstrated also a significant therapeutic effect in some experimental models of infection (151, 152).

Furthermore, immunological derivatives of PaKT exhibited antibacterial and antiprotozoan activity against epidemiologically relevant agents. Multi-drug resistant *Mycobacterium tuberculosis* (156), Gram-positive cocci and Gram-negative bacteria (115, 157, 158), phytopathogenic bacteria, such as *Pseudomonas syringae* and *Erwinia carotovora* (149), protozoa, such as *Leishmania major*, *L. infantum* (159, 160) and *Acanthamoeba castellanii* (161), and others (115) were exquisitely sensitive. This antimicrobial activity proved to be irrespective of the presence of mechanisms of resistance to other conventional antimicrobial drugs. It was neutralized by either mAb KT4 or laminarin. Thus, it is mediated by the presence of a transphyletic KTR or KTR-like structures widely conserved in nature, possibly constituted by BG or BG-like molecules. The screening of a wide *S. cerevisiae* gene deletion strain collection, inclusive of mutants deleted of genes implicated in BG synthesis, characterized by enhanced resistance to conventional antifungal drugs, such as caspofungin, failed to identify genes involved in susceptibility to immunological derivatives of PaKT. Possibly, these molecules target viability-critical microbial structures conserved through natural evolution, probably not rejectable by the microorganisms (162).

On the other side, the glyco-conjugate vaccine from Cassone's group was also able to protect vaccinated mice from a lethal systemic challenge with conidia of *A. fumigatus*. Immune sera and an IgG2b anti-Lam mAb were able to bind to fungal hyphae and strongly inhibit fungal growth (66). Remarkably, similar to what we observed with KP, the anti-Lam mAb also proved to bind to the cell wall and inhibit growth of encapsulated and acapsular *C. neoformans* strains, reducing yeast capsule thickness *in vitro* and *in vivo*. It exerted protective effects in an experimental model of systemic cryptococcosis in both normal and neutropenic mice, by significantly reducing fungal burden in brain and liver (163).



Killer toxin-like scFv antiidiotypic antibody (KT-scFv)

Sequencing of KT-scFv (aminoacid sequence)

```
1           11          21          31          41          51
MAQVKLQESG PGLVAPSQSL SITCTVSGFS LTGYGVNWVR QPPGKGLEWL GMIWGDGSTD
61          71          81          91          101         111
YNSALKSRLS ISKDNSKSQV FLKMNSLQTD DTARYYCLYA MDYWGQGTTV TCSSGGGGSG
121         131         141         151         161         171
GGGSGGGGSD IELTQSPALM SASPGEKVTM TCSASSSVSY MYWYQQKPRS SPKPWIYLTS
181         191         201         211         221         231
NLASGVPARF SGSGSGTSYS LTISSMEAED AATYYCQQWS SNPYTFGGGT KLEIKRAAAG
```

Complementarity determining regions (CDRs) of heavy (V_H, red) and light (V_L, blue) chains of KT-scFv are indicated: from the top, V_H CDR_1, CDR_2, CDR_3, and V_L CDR_1, CDR_2, CDR_3; underlined, peptide P6 (146-155).

CDRs and two residues displaced decapeptides reproducing KT-scFv sequence have been synthesized and tested for candidacidal activity *in vitro*. Among the most candidacidal peptides, P6 has been selected for analysis by alanine scanning.

P6 E K V T M T C S A S —**alanine scanning**→ **KP** A K V T M T C S A S

Among the P6 alanine-substituted peptides, KP demonstrated significant increase in candidacidal activity, *in vitro* wide-spectrum of antimicrobial activity, therapeutic effect in different animal models of microbial infection.

Figure 2. Construction of antiidiotypic antibody-derived synthetic killer peptides.

The equivalence of KT-Abs and anti-BG Abs, or at least some of them, remains to be elucidated, while it seems to be strongly suggested by many of their functional characteristics. Thus, an analogous wide spectrum of antimicrobial activity should be expected for anti-BG Abs or peptides derived from them, given the widespread presence of BGs among microorganisms.

5. TRANSPHYLETIC ANTI-INFECTIOUS CONTROL STRATEGIES

The susceptibility of so many different microbial pathogens to the immunological derivatives of PaKT and, to a lesser extent so far, to anti-BG Abs implies the occurrence of a transphyletic (BG)-KTR or (BG)-KTR-like structures. This authorizes to envisage potential unique vaccinal and therapeutic approaches against difficult to prevent and treat microbial infections.

5.1. Transdisease immunoprevention strategies

As demonstrated by the highly immunogenic Lam-conjugate vaccine, for the first time it has been possible to immunize and protect different animal species against at least two very different types of fungal pathogens, such as *C. albicans* and *A. fumigatus*, as a result of systemic and mucosal immunizations. Thus, the proof of concept of a multivalent vaccine, possibly effective also against other pathogenic fungi, has been introduced (66). This vaccinal approach substantially confirmed what we observed in "idiotypic vaccination" with mAb KT4, i.e., the possibility to elicitate protective Abs, which exert a wide, direct antimicrobial activity whitout requiring any other effector molecule or cell. We had proposed the Id of mAb KT4, properly cloned and expressed, and purified KTRs, as polyvalent transphyletic vaccines (164). As functional internal image of the (BG)-KTR, the Id of mAb KT4 might represent the positive protein topochemical copy of a

polysaccharide, such as BG, thus qualifying as surrogate vaccine. Thus, mirroring polysaccharide epitopes such as (BG)-KTR by their anti-Ids may allow further biotechnological developments. Among them, DNA vaccination would be potentially able to induce cytotoxic T-cell responses particularly important against intracellular pathogens. Engineering of commensal bacteria and development of transgenic plants would allow to produce and evaluate live, safe antigen carrier systems and edible idiotypic vaccines, respectively (147).

By commenting on Torosantucci's BG-conjugate vaccine, Casadevall and Pirofski (109) outlined its positive novelty. It could represent a potential effective immunoprophylactic vaccine for immunocompromised patients at risk of invasive mycosis as well as for women affected by recurrent mucosal candidiasis. However, they raised some concerns for its safety and prescription. The lack of BGs in mammals would guarantee the vaccine safety regarding the potential for self-reactivity. However, such a broadly effective vaccine could induce permanent alterations of the host microbial flora with unanticipated adverse effects. As for other vaccines, the response to immunization in immunocompromised host, the most in need for it, is usually poor. Moreover it is not always possible to identify and vaccinate individuals at risk before the onset of their immunity impairment. Immunotherapeutic approaches based on anti-BG Abs, KT-Abs or Ab-derived peptide killer mimotopes appear mostly devoid of these issues.

5.2. Transdisease immunotherapeutic strategies

Anti-BG Abs, KT-Abs and Ab-derived killer peptides could represent new attractive tools to fight infectious diseases. As microbicidal molecules not requiring any host's immune competence, they could be effective also in immunocompromised hosts with various degrees of immune impairment. These Abs (in different formats, such as polyclonal, monoclonal and recombinant) and Ab-derived killer peptides, when used in passive immunization or as antimicrobial agents, were highly therapeutic against mucosal and systemic microbial infections, such as candidiasis, aspergillosis, pneumocystosis, cryptococcosis, paracoccidioidomycosis, as previously outlined. This activity attests for a sufficient *in vivo* stability of anti-BG Abs and immunological derivatives of PaKT to be therapeutic. KP, in particular, arouses major interest. Unlike Abs, which can pose questions with reference to immunogenicity, stability, and, particularly, safety (i.e., possible contamination by pathogenic viruses or other undetected aetiological agents), being a small synthetic decapeptide, KP is devoid of most of these issues. Due to its peculiar amino acidic sequence, characterized by the presence of a cysteine residue, KP can easily dimerize in non-reducing conditions by formation of disulfide bridges. This molecule still maintains unaltered antimicrobial activity over a long period of time under different temperature conditions. Specially synthesized dimeric KP exerted an even stronger candidacidal activity *in vitro* (115). Furthermore, as a small peptidic molecule, KP can be easily manipulated. For example, the use of the same amino acid residues in the D rather than L conformation, although with a little reduction of activity, may assure more *in vivo* stability against proteases (115, 152). KP expression *in planta* may allow for its easier and less expensive production (149).

Besides retaining the same antimicrobial activity of the whole KT-Ab from which it has been derived, KP exhibited additional relevant activities. Actually, it demonstrated immunomodulatory properties, especially on DCs, improving their capacity to induce lymphocyte proliferation (165). A surprisingly unexpected *in vitro* and *ex vivo* anti-HIV-1 activity has been observed, probably due to KP sequence homology with critical segments in viral gp160 and consequent down-regulation of CCR5 co-receptors and/or the physical block of the gp120-receptors interaction (166). Therefore, KP could represent a prototypal compound for the development of new drugs for simultaneous treatment of HIV-1 infection and important AIDS-related opportunistic infections by susceptible eukaryotic and prokaryotic pathogens (167).

6. CONCLUSIONS AND PERSPECTIVES

The discovery and therapeutic use of antibiotics and other antimicrobial agents had supported until recently the optimistic belief that virtually all microbial infections were treatable. The emergence of new infectious diseases and the dramatic increase in the incidence and prevalence of resistance to antimicrobial agents among epidemiologically relevant microbial pathogens have disproved it. This spurred efforts to develop new antimicrobial agents and identify novel targets in microbial cells as well as alternative approaches to infection management.

In the field of antifungal therapy, the introduction of new molecules, such as later generation azoles and echinocandins, has dramatically improved the therapeutic options, while not resolving the unacceptably high mortality rate associated with some fungal infections. The echinocandins, in particular, which target BG synthesis, are the newest addition to the antifungal armamentarium in the treatment of fungal infections. They display a broad spectrum of activity inclusive of *Candida* infections. Their concentration-dependent killing effects would further demonstrate that BGs are essential in cell wall integrity for certain fungi.

Observations on microbicidal anti-BG-like KT-like anti-Id Abs and Ab-derived killer peptides from our group and anti-BG Abs from Cassone's group open new perspectives in antimicrobial chemotherapy and immunoprevention. As recently outlined by Casadevall and Pirofski, the protection mediated by anti-BG and anti-BG-like Abs introduces a heresy into immunological dogma, in that they are not pathogen-specific microbicidal Abs (168). In fact, anti-BG Abs have shown to display fungicidal activity and protect at least against *C. albicans*, *Aspergillus* spp. and *C. neoformans*. Even more significantly, anti-BG-like Abs and Ab-derived killer peptides have shown a wider spectrum of activity, inclusive of epidemiologically

Table 1. Candidacidal and microbicidal antibodies elicited by beta-glucans, killer toxin receptors and the idiotype of killer toxin-neutralizing monoclonal antibodies (beta-glucan-like molecules) and antibody-derived killer peptide mimotopes

Antigen	Antibodies and peptide mimotopes	Sensitive microorganisms	Reference
C. albicans cells	Anti-BG Abs[1]	*Candida albicans*	48
Laminarin (BG)	Anti-BG Abs	*C. albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*	66,163
KTR (BG)[2]	Anti-KTR PaKT Abs[3]	*C. albicans, Pneumocystis carinii, Mycobacterium tuberculosis*	137,153,156
KTR(BG)-like nmAbKT[4]	Anti-KTR HM-1KT rAbs[5]	*C. albicans*	134,135
KTR(BG)-like mAbKT4[4]	Anti-BG-like PaKT Abs Anti-BG-like PaKT mAbs Anti-BG-like PaKT rAbs PaKT-rAb-derived peptide mimotopes	*C. albicans*, non-*albicans C.*, *A. fumigatus*, *C. neoformans*, *Paracoccidioides brasiliensis*, *P. carinii, Botrytis cinerea, Fusarium oxysporum*, *M. tuberculosis*, *Pseudomonas syringae, Erwinia carotovora,* Gram-positive cocci, *Leishmania major*, *L. infantum*, *Acanthamoeba castellanii*, and others	138,140-144, 146,149-161, 166

[1]Anti-BG Abs, Anti-beta-glucan antibodies; [2]KTR, killer toxin receptor; [3]PaKT-Abs, *Pichia anomala* KT-like killer Abs; [4]nmAbKT, killer toxin neutralizing monoclonal Abs; [5]HM-1KT-rAbs, *Hansenula mrakii* 1KT-like killer recombinant Abs

relevant prokaryotic and eukaryotic pathogenic agents (Table 1). BG or BG-like molecules appear to be conserved in nature among phylogenetically distant microorganisms. Their emergence as potentially "universal", "common" targets suggests innovative approaches for Ab-mediated immunity and therapy. BG-like mimotopes, such as the Id of anti-KT Abs, and protein-conjugate BGs could represent new multivalent vaccines. Furthermore, they could be properly delivered with suitable adjuvants and immunomodulators to enhance their immunogenicity especially in immunocompromised patients, frequently the most in need of them.

On the other hand, microbicidal Abs and Ab-derived killer peptides could open new perspectives for receptor/antigen-driven therapies. The mechanism by which these molecules are microbicidal and mediate protection remains to be elucidated. It could be likely based on some kind of interference with cell wall remodelling in the course of replication. Preliminary studies carried out with *C. albicans* mutants and DNA microarrays suggest a possible involvement of Na^+/H^+ pump, response to oxidative stress and ion transport within the yeast cell in the mechanism of action of KP. In KP treated yeast cells different structures seem to be affected: nucleus is fragmented, plasma membrane is collapsed, while cell wall is characterized by the appearance of a swollen, middle electron-dense region (unpublished data). These observations suggest a preferential interaction of KP, similar to KT-like Abs, with BG as KTR or, at least, as a KTR component, before the peptide can display its antimicrobial activity. Interestingly, similar to what previously described for KT-like Abs (110), KP is also able to kill the KT-producing *P. anomala* strain (ATCC 96603), that is immune to the activity of its own toxin. Thus, the self-immunity system that prevents yeast cells from suicidal tendencies is apparently bypassed by KT-like immunological derivatives.

Even though the presence of the Fc region can contribute to the therapeutic efficacy of microbicidal anti-BG and anti-BG-like Abs, their direct *in vitro* microbicidal activity definitely do not rely on complement or effector cells activation and therefore on that region. This could be at least one of the reasons why they are protective even in immunocompromised hosts and why Ab-derived peptides can still exert their microbicidal activity. Furthermore, as evidence indicates that BGs are major inflammatory components in yeast and fungal cell walls (169), these microbicidal Abs could also prevent, *in vivo*, the development of inflammation. The recent demonstration that BGs are relevant components of microbial biofilms, such as in *Candida* spp. (170, 171) and *P. aeruginosa* (172), possibly involved in antimicrobial resistance, would suggest a positive impact of their targeting by Abs and peptides on drug susceptibility and therapeutic outcome.

The availability of molecules, such as microbicidal Abs and peptides, which can be easily engineered, could allow to experiment with new therapeutic approaches. Their conjugation with antimicrobial drugs (173) or radionuclides, similar to what experienced with radiopharmaceuticals for tumor diagnosis and therapy, could be envisaged (174, 175). This antigen/receptor-driven therapy would possibly result in an increase in specificity with lower side effects. Finally, the use of Ab-derived killer peptides would avoid the formation of potentially detrimental immune complexes and unwanted Ab responses to Fc regions in the course of antimicrobial therapy.

Many questions on the molecular mechanism of these molecules, the impact of their wide-spectrum activity on the endogenous microbial flora and possible selection of resistant mutants, their pharmacokinetics and safety, still await experimental answers. KP, in particular, exhibited a sufficient *in vivo* stability to be therapeutic in different animal models of systemic and mucosal microbial infections. Besides its lack of immunogenicity and any detectable cytotoxicity (148, 166), this would suggest the possibility to attain KP therapeutic concentrations also for treatment of human infections. Pharmacokinetic studies currently in progress should elucidate this aspect. However, in our opinion, the most attractive prospect could be the availability of a prototypal compound for the development of new wide-spectrum antimicrobial drugs. In the same way, the potential of multivalent vaccines still requires active investigation. The emergence of BGs and BG-like molecules as potential cross reactive targets for fungi and other pathogenic microorganisms should underline once again the importance of anti-BG Ab active and passive immunity. They represent critical aspects of both host defense and therapy

against a variety of pathogens. This would furtherly produce evidence in support of what has been called from Casadevall "the third age of antimicrobial therapy", i.e. the widespread introduction of immunotherapy in combination with conventional antimicrobials as adjunctive therapy (176).

7. ACKNOWLEDGMENTS

This work was supported by grants from the Istituto Superiore di Sanità in the frame of the Research Project "Coinfezioni, infezioni opportunistiche e tumori associati all'AIDS".

8. REFERENCES

1. McNeil, M. M., S. L. Nash, R. A. Hajjeh, M. A. Phelan, L. A. Conn, B. D. Plikaytis and D. W. Warnock: Trends in mortality due to invasive mycotic diseases in the United States, 1980-1997. *Clin Infect Dis* 33, 641-647 (2001)
2. Marr, K. A., R. A. Carter, F. Crippa, A. Wald and L. Corey: Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 34, 909-917 (2002)
3. Fleming, R. V., T. J. Walsh and E. J. Anaissie: Emerging and less common fungal pathogens. *Infect Dis Clin North Am* 16, 915-933 (2002)
4. Bouza, E., P. Munoz and J. Guinea: Mucormycosis: an emerging disease? *Clin Microbiol Infect* 12 (s7), 7-23 (2006)
5. Pagano, L., M. Caira, A. Candoni, M. Offidani, L. Fianchi, B. Martino, D. Pastore, M. Picardi, A. Bonini, A. Chierichini, R. Fanci, C. Caramatti, R. Invernizzi, D. Mattei, M. E. Mitra, L. Melillo, F. Aversa, M. T. Van Lint, P. Falcucci, C. G. Valentini, C. Girmenia and A. Nosari: The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. *Haematologica* 91, 1068-1075 (2006)
6. Pagano, L., M. Caira, M. Picardi, A. Candoni, L. Melillo, L. Fianchi, M. Offidani and A. Nosari: Invasive Aspergillosis in patients with acute leukemia: update on morbidity and mortality-SEIFEM-C Report. *Clin Infect Dis* 44, 1524-1525 (2007)
7. Eggimann, P., J. Garbino and D. Pittet: Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis* 3, 685-702 (2003)
8. Fridkin, S. K.: The changing face of fungal infections in health care settings. *Clin Infect Dis* 41, 1455-1460 (2005)
9. Fridkin, S. K., D. Kaufman, J. R. Edwards, S. Shetty and T. Horan: Changing incidence of Candida bloodstream infections among NICU patients in the United States: 1995-2004. *Pediatrics* 117, 1680-1687 (2006)
10. Morgan, J.: Global trends in candidemia: review of reports from 1995-2005. *Curr Infect Dis Rep* 7, 429-439 (2005)
11. Pappas, P. G.: Invasive Candidiasis. *Infect Dis Clin North Am* 20, 485-506 (2006)
12. Pfaller, M. A. and D. J. Diekema: Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 20, 133-163 (2007)
13. Richardson, M. D.: Changing patterns and trends in systemic fungal infections. *J Antimicrob Chemother* 56 Suppl 1, i5-i11 (2005)
14. Dan, M., R. Segal, V. Marder and A. Leibovitz: Candida colonization of the vagina in elderly residents of a long-term-care hospital. *Eur J Clin Microbiol Infect Dis* 25, 394-396 (2006)
15. Farmaki, E., J. Evdoridou, T. Pouliou, E. Bibashi, P. Panagopoulou, J. Filioti, A. Benos, D. Sofianou, G. Kremenopoulos and E. Roilides: Fungal colonization in the neonatal intensive care unit: risk factors, drug susceptibility, and association with invasive fungal infections. *Am J Perinatol* 24, 127-135 (2007)
16. Mukherjee, P. K., G. Zhou, R. Munyon and M. A. Ghannoum: Candida biofilm: a well-designed protected environment. *Med Mycol* 43, 191-208 (2005)
17. Ramage, G., J. P. Martinez and J. L. Lopez-Ribot: Candida biofilms on implanted biomaterials: a clinically significant problem. *FEMS Yeast Res* 6, 979-986 (2006)
18. Shin, J. H., S. J. Kee, M. G. Shin, S. H. Kim, D. H. Shin, S. K. Lee, S. P. Suh and D. W. Ryang: Biofilm production by isolates of Candida species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. *J Clin Microbiol* 40, 1244-1248 (2002)
19. Westbrook, S. D., W. R. Kirkpatrick, C. O. Freytes, J. J. Toro, S. Bernardo, T. F. Patterson, S. W. Redding and S. A. Lee: Candida krusei sepsis secondary to oral colonization in a hemopoietic stem cell transplant recipient. *Med Mycol* 45, 187-190 (2007)
20. de Repentigny, L., D. Lewandowski and P. Jolicoeur: Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency virus infection. *Clin Microbiol Rev* 17, 729-759 (2004)
21. Eckert, L. O.: Clinical practice. Acute vulvovaginitis. *N Engl J Med* 355, 1244-1252 (2006)
22. Loh, K. Y. and N. Sivalingam: Recurrent vaginal candidiasis. *Med J Malaysia* 58, 788-792 (2003)
23. Nyirjesy, P.: Chronic vulvovaginal candidiasis. *Am Fam Physician* 63, 697-702 (2001)
24. Pankhurst, C. L.: Candidiasis (oropharyngeal). *Clin Evid* 15, 1849-1863 (2006)
25. Sobel, J. D.: Vulvovaginal candidiasis: a comparison of HIV-positive and -negative women. *Int J STD AIDS* 13, 358-362 (2002)
26. Blumberg, H. M., W. R. Jarvis, J. M. Soucie, J. E. Edwards, J. E. Patterson, M. A. Pfaller, M. S. Rangel-Frausto, M. G. Rinaldi, L. Saiman, R. T. Wiblin, R. P. Wenzel and National Epidemiology of Mycoses Survey (NEMIS) Study Group: Risk factors for candidal bloodstream infections in surgical intensive care unit patients: The NEMIS Prospective Multicenter Study. *Clin Infect Dis* 33, 177-186 (2001)
27. Saiman, L., E. Ludington, M. Pfaller, S. Rangel-Frausto, R. T. Wiblin, J. Dawson, H. M. Blumberg, J.

E. Patterson, M. Rinaldi, J. E. Edwards, R. P. Wenzel and W. Jarvis: Risk factors for candidemia in neonatal intensive care unit patients. *Pediatr Infect Dis J* 19, 319-324 (2000)
28. Benson, C. A., J. E. Kaplan, H. Masur, A. Pau, K. K. Holmes, CDC, National Institutes of Health and Infectious Diseases Society of America: Treating opportunistic infections among HIV-exposed and infected children: recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. *MMWR Recomm Rep* 53 (RR-15), 1-112 (2004)
29. Masur, H., J. E. Kaplan, K. K. Holmes, U.S. Public Health Service and Infectious Diseases Society of America: Guidelines for preventing opportunistic infections among HIV-infected persons - 2002. Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *Ann Intern Med* 137, 435-478 (2002)
30. Mofenson, L. M., J. Oleske, L. Serchuck, R. Van Dyke and C. Wilfert: Treating opportunistic infections among HIV-exposed and infected children: recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. *Clin Infect Dis* 40 Suppl 1, S1-84 (2005)
31. Pienaar, E. D., T. Young and H. Holmes: Interventions for the prevention and management of oropharyngeal candidiasis associated with HIV infection in adults and children. *Cochrane Database Syst Rev* 3, CD003940 (2006)
32. Gudlaugsson, O., S. Gillespie, K. Lee, J. Vande Berg, J. Hu, S. Messer, L. Herwaldt, M. Pfaller and D. Diekema: Attributable mortality of nosocomial candidemia, revisited. *Clin Infect Dis* 37, 1172-1177 (2003)
33. Magill, S. S., S. M. Swoboda, E. A. Johnson, W. G. Merz, R. K. Pelz, P. A. Lipsett and C. W. Hendrix: The association between anatomic site of Candida colonization, invasive candidiasis, and mortality in critically ill surgical patients. *Diagn Microbiol Infect Dis* 55, 293-301 (2006)
34. Morgan, J., M. I. Meltzer, B. D. Plikaytis, A. N. Sofair, S. Huie-White, S. Wilcox, L. H. Harrison, E. C. Seaberg, R. A. Hajjeh and S. M. Teutsch: Excess mortality, hospital stay, and cost due to candidemia: a case-control study using data from population-based candidemia surveillance. *Infect Control Hosp Epidemiol* 26, 540-547 (2005)
35. Segal, B. H., N. G. Almyroudis, M. Battiwalla, R. Herbrecht, J. R. Perfect, T. J. Walsh and J. R. Wingard: Prevention and early treatment of invasive fungal infection in patients with cancer and neutropenia and in stem cell transplant recipients in the era of newer broad-spectrum antifungal agents and diagnostic adjuncts. *Clin Infect Dis* 44, 402-409 (2007)
36. Ashman, R. B., C. S. Farah, S. Wanasaengsakul, Y. Hu, G. Pang and R. L. Clancy: Innate versus adaptive immunity in Candida albicans infection. *Immunol Cell Biol* 82, 196-204 (2004)
37. Cassone, A., F. De Bernardis and A. Torososantucci: An outline of the role of anti-Candida antibodies within the context of passive immunization and protection from candidiasis. *Curr Mol Med* 5, 377-382 (2005)
38. Fidel, P. L. Jr.: Candida-host interactions in HIV disease: relationships in oropharyngeal candidiasis. *Adv Dent Res* 19, 80-84 (2006)
39. Fidel, P. L. Jr.: History and update on host defense against vaginal candidiasis. *Am J Reprod Immunol* 57, 2-12 (2007)
40. Shoham, S. and S. M. Levitz: The immune response to fungal infections. *Br J Haematol* 129, 569-582 (2005)
41. Vonk, A. G., M. G. Netea, J. W. van der Meer and B. J. Kullberg: Host defence against disseminated Candida albicans infection and implications for antifungal immunotherapy. *Expert Opin Biol Ther* 6, 891-903 (2006)
42. Zelante, T., C. Montagnoli, S. Bozza, R. Gaziano, S. Bellocchio, P. Bonifazi, S. Moretti, F. Fallarino, P. Puccetti and L. Romani: Receptors and pathways in innate antifungal immunity: the implication for tolerance and immunity to fungi. *Adv Exp Med Biol* 590, 209-221 (2007)
43. Rivera, A., H. L. Van Epps, T. M. Hohl, G. Rizzuto and E. G. Pamer: Distinct CD4+-T-cell responses to live and heat-inactivated Aspergillus fumigatus conidia. *Infect Immun* 73, 7170-7179 (2005)
44. Casadevall, A., A. Cassone, F. Bistoni, J. E. Cutler, W. Magliani, J. W. Murphy, L. Polonelli and L. Romani: Antibody and/or cell-mediated immunity, protective mechanisms in fungal disease: an ongoing dilemma or an unnecessary dispute? Med Mycol 36 Suppl 1, 95-105 (1998)
45. Polonelli, L., A. Casadevall, Y. Han, F. De Bernardis, T. N. Kirkland, R. C. Matthews, D. Adriani, M. Boccanera, J. P. Burnie, A. Cassone, S. Conti, J. E. Cutler, R. Frazzi, C. Gregory, S. Hodgetts, C. Illidge, W. Magliani, G. Rigg and G. Santoni: The efficacy of acquired humoral and cellular immunity in the prevention and therapy of experimental fungal infections. Med Mycol 38 Suppl 1, 281-292 (2000)
46. Casadevall, A.: Antibody immunity and invasive fungal infections. Infect Immun 63, 4211-4218 (1995)
47. Bromuro, C., R. La Valle, S. Sandini, F. Urbani, C. M. Ausiello, L. Morelli, C. Fe D'Ostiani, L. Romani and A. Cassone: A 70-kilodalton recombinant heat shock protein of *Candida albicans* is highly immunogenic and enhances systemic murine candidiasis. *Infect Immun* 66, 2154-2162 (1998)
48. Bromuro, C., A. Torosantucci, P. Chiani, S. Conti, L. Polonelli and A. Cassone: Interplay between protective and inhibitory antibodies dictates the outcome of experimentally disseminated candidiasis in recipients of a Candida albicans vaccine. *Infect Immun* 70, 5462-5470 (2002)
49. Han, Y., T. Kanbe, R. Cherniak and J. E. Cutler: Biochemical characterization of *Candida albicans* epitopes that can elicit protective and nonprotective antibodies. *Infect Immun* 65, 4100-4107 (1997)
50. De Bernardis, F., M. Boccanera, D. Adriani, E. Spreghini, G. Santoni and A. Cassone: Protective role

of antimannan and anti-aspartyl proteinase antibodies in an experimental model of Candida albicans vaginitis in rats. *Infect Immun* 65, 3399-3405 (1997)
51. Fidel, P. L. Jr.: Immunity to Candida. *Oral Dis* 8 Suppl. 2, 69-75 (2002)
52. Han, Y. M., R. P. Morrison and J. E. Cutler: A vaccine and monoclonal antibodies that enhance mouse resistance to Candida albicans vaginal infection. *Infect Immun* 66, 5771-5776 (1998)
53. Matthews, R. and J. Burnie: Antifungal antibodies: a new approach to the treatment of systemic candidiasis. *Curr Opin Invest Drugs* 2, 472-476 (2001)
54. Magliani, W., S. Conti, S. Arseni, A. Salati, L. Ravanetti, D. L. Maffei, L. Giovati and L. Polonelli: Antibody-mediated protective immunity in fungal infections. *New Microbiol* 28, 299-309 (2005)
55. Matthews, R. C., G. Rigg, S. Hodgetts, T. Carter, C. Chapman, C. Gregory, C. Illidge and J. P. Burnie: Preclinical assessment of the efficacy of Mycograb, a human recombinant antibody against fungal HSP90. *Antimicrob Agents Chemother* 47, 2208-2216 (2003)
56. Matthews, R. C. and J. P. Burnie: Human recombinant antibody to HSP90: a natural partner in combination therapy. *Curr Mol Med* 5, 403-411 (2005)
57. Nooney, L., R. C. Matthews and J. P. Burnie: Evaluation of Mycograb, amphotericin B, caspofungin, and fluconazole in combination against *Cryptococcus neoformans* by checkerboard and time-kill methodologies. *Diagn Microbiol Infect Dis* 51, 19-29 (2005)
58. Pachl, J., P. Svoboda, F. Jacobs, K. Vandewoude, B. van der Hoven, P. Spronk, G. Masterson, M. Malbrain, M. Aoun, J. Garbino, J. Takala, L. Drgona, J. Burnie, R. Matthews and Mycograb Invasive Candidiasis Study Group: A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. *Clin Infect Dis* 42, 1404-1413 (2006)
59. Rowlands, H. E., K. Morris and C. Graham: Human recombinant antibody against *Candida*. *Pediatr Infect Dis J* 25, 959-960 (2006)
60. Doc. Ref. EMEA/454649/2006, London, 16 November (2006) www.emea.europa.eu/pdfs/human/opinion/45464906en.pdf
61. Moragues, M. D., M. J. Omaetxebarria, N. Elguezabal, M. J. Sevilla, S. Conti, L. Polonelli and J. Ponton: A monoclonal antibody directed against a *Candida albicans* cell wall mannoprotein exerts three anti-*C. albicans* activities. *Infect Immun* 71, 5273-5279 (2003)
62. Omaetxebarria, M. J., M. D. Moragues, N. Elguezabal, A. Rodríguez-Alejandre, S. Brena, J. Schneider, L. Polonelli and J. Ponton: Antifungal and antitumor ativities of a monoclonal antibody directed against a stress mannoprotein of *Candida albicans*. *Curr Mol Med* 5, 393-401 (2005)
63. Sevilla, M. J., B. Robledo, A. Rementería, M. D. Moragues and J. Ponton: A fungicidal monoclonal antibody protects against murine invasive candidiasis. *Infect Immun* 74, 3042-3045 (2006)
64. Brena, S., M. J. Omaetxebarria, N. Elguezabal, J. Cabezas, M. D. Moragues and J. Ponton: The fungicidal monoclonal antibody C7 binds to Candida albicans Als3. *Infect Immun* 75, 3680-3682 (2007)
65. Kavishwar, A. and P. K. Shukla: Candidacidal activity of a monoclonal antibody that binds with glycosyl moieties of proteins of Candida albicans. *Med Mycol* 44, 159-167 (2006)
66. Torosantucci, A., C. Bromuro, P. Chiani, F. De Bernardis, F. Berti, C. Galli, F. Norelli, C. Bellucci, L. Polonelli, P. Costantino, R. Rappuoli and A. Cassone: A novel glyco-conjugate vaccine against fungal pathogens. *J Exp Med* 202, 597-606 (2005)
67. Bedirli, A., M. Kerem, H. Pasaoglu, N. Akyurek, T. Tezcaner, S. Elbeg, L. Memis and O. Sakrak: Beta-glucan attenuates inflammatory cytokine release and prevents acute lung injury in an experimental model of sepsis. *Shock* 27, 397-401 (2007)
68. Brown, G. D. and S. Gordon: Immune recognition of fungal beta-glucans. *Cell Microbiol* 7, 471-479 (2005)
69. Demir, G., H. O. Klein, N. Mandel-Molinas and N. Tuzuner: Beta glucan induces proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer. *Int Immunopharmacol* 7, 113-116 (2007)
70. Descroix, K., V. Ferrieres, F. Jamois, J. C. Yvin and D. Plusquellec: Recent progress in the field of beta-(1,3)-glucans and new applications. *Mini Rev Med Chem* 6, 1341-1349 (2006)
71. Sandvik, A., Y. Y. Wang, H. C. Morton, A. O. Aasen, J. E. Wang and F. E. Johansen: Oral and systemic administration of beta-glucan protects against lipopolysaccharide-induced shock and organ injury in rats. *Clin Exp Immunol* 148, 168-177 (2007)
72. Yan, J., D. J. Allendorf and B. Brandley: Yeast whole glucan particle (WGP) beta-glucan in conjunction with antitumour monoclonal antibodies to treat cancer. *Expert Opin Biol Ther* 5, 691-702 (2005)
73. Zekovic, D. B., S. Kwiatkowski, M. M. Vrvic, D. Jakovljevic and C. A. Moran: Natural and modified (1-->3)-beta-D-glucans in health promotion and disease alleviation. *Crit Rev Biotechnol* 25, 205-230 (2005)
74. Kapteyn, J. C., L. L. Hoyer, J. E. Hecht, W. H. Muller, A. Andel, A. J. Verkleij, M. Makarow, H. Van Den Ende and F. M. Klis: The cell wall architecture of *Candida albicans* wild-type cells and cell wall-defective mutants. *Mol Microbiol* 35, 601-611 (2000)
75. Masuoka, J.: Surface glycans of *Candida albicans* and other pathogenic fungi: physiological roles, clinical uses, and experimental challenges. *Clin Microbiol Rev* 17, 281-310 (2004)
76. Denning, D. W.: Echinocandin antifungal drugs. *Lancet* 362, 1142-1151 (2003)
77. Nakagawa, Y., N. Ohno and T. Murai: Suppression by *Candida albicans* beta-glucan of cytokine release from activated human monocytes

and from T cells in the presence of monocytes. *J Infect Dis* 187, 710-713 (2003)
78. Obayashi, T., M. Yoshida, T. Mori, H. Goto, A. Yasuoka, H. Iwasaki, H. Teshima, S. Kohno, A. Horiuchi and A. Ito: Plasma (1,3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 345, 17-20 (1995)
79. Kedzierska, A.: (1,3)-beta-D-glucan-a new marker for the early serodiagnosis of deep-seated fungal infections in humans. *Pol J Microbiol* 56, 3-9 (2007)
80. Kondori, N., L. Edebo and I. Mattsby-Baltzer: Circulating beta (1-3) glucan and immunoglobulin G subclass antibodies to *Candida albicans* cell wall antigens in patients with systemic candidiasis. *Clin Diagn Lab Immun* 11, 344-350 (2004)
81. Marty, F. M., S. Koo, J. Bryar and L. R. Baden: (1->3)beta-D-glucan assay positivity in patients with *Pneumocystis* (*carinii*) *jiroveci* pneumonia. *Ann Intern Med* 147, 70-72 (2007)
82. Ostrosky-Zeichner, L., B. D. Alexander, D. H. Kett, J. Vazquez, P. G. Pappas, F. Saeki, P. A. Ketchum, J. Wingard, R. Schiff, H. Tamura, M. A. Finkelman and J. H. Rex: Multicenter clinical evaluation of (1,3)-beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin Infect Dis* 41, 654-659 (2005)
83. Pazos, C., M. D. Moragues, G. Quindos, J. Ponton and A. del Palacio: Diagnostic potential of (1,3)-beta-D-glucan and anti-*Candida albicans* germ tube antibodies for the diagnosis and therapeutic monitoring of invasive candidiasis in neutropenic adult patients. *Rev Iberoam Micol* 23, 209-215 (2006)
84. Yoshida, M.: Usefulness of determination of beta-D-glucan in the diagnosis of deep mycosis - experience in Japan. *Med Mycol* 44 Suppl, 185-189 (2006)
85. Gantner, B. N., R. M. Simmons and D. M. Underhill: Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *EMBO J* 24, 1277-1286 (2005);
86. Poulain, D. and T. Jouault: *Candida albicans* cell wall glycans, host receptors and responses: Elements for a decisive crosstalk. *Curr Opin Microbiol* 7, 342-349 (2004)
87. Romani, L.: Immunity to fungal infections. *Nat Rev Immunol* 4, 1-23 (2004)
88. Ruiz-Herrera, J., M. V. Elorza, E. Valentín and R. Sentandreu: Molecular organization of the cell wall of *Candida albicans* and its relation to pathogenicity. *FEMS Yeast Res* 6, 14-29 (2006)
89. Wheeler, R. T. and G. R. Fink: A drug-sensitive genetic network masks fungi from the immune system. *PLoS Pathog* 2, e35 (2006)
90. Netea, M. G., N. A. R. Gow, C. A. Munro, S. Bates, C. Collins, G. Ferwerda, R. P. Hobson, G. Bertram, H. B. Hughes, T. Jansen, L. Jacobs, E. T. Buurman, K. Gijzen, D. L. Williams, R. Torensma, A. McKinnon, D. M. MacCallum, F. C. Odds, J. W. M. Van der Meer, A. J. P. Brown, and B. J. Kullberg: Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J Clin Invest* 116, 1642-1650 (2006)
91. Adachi, Y., T. Ishii, Y. Ikeda, A. Hoshino, H. Tamura, J. Aketagawa, S. Tanaka and N. Ohno: Characterization of beta-glucan recognition site on C-type lectin, dectin 1. *Infect Immun* 72, 4159-4171 (2004)
92. Brown, J., C. A. O'Callaghan, A. S. Marshall, R. J. Gilbert, C. Siebold, S. Gordon, G. D. Brown and E. Y. Jones: Structure of the fungal beta-glucan-binding immune receptor dectin-1: Implications for function. *Protein Sci* 16, 1042-1052 (2007)
93. Dennehy, K. M. and G. D. Brown: The role of the {beta}-glucan receptor Dectin-1 in control of fungal infection. *J Leukoc Biol* 82, 253-258 (2007)
94. Gantner, B. N., R. M. Simmons, S. J. Canavera, S. Akira and D. M. Underhill: Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* 197, 1107-1117 (2003)
95. Goodridge, H. S., R. M. Simmons and D. M. Underhill: Dectin-1 stimulation by *Candida albicans* yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. *J Immunol* 178, 3107-3115 (2007)
96. Herre, J., S. Gordon and G. D. Brown: Dectin-1 and its role in the recognition of beta-glucans by macrophages. *Mol Immunol* 40, 869-876 (2004)
97. Taylor, P. R., S. V. Tsoni, J. A. Willment, K. M. Dennehy, M. Rosas, H. Findon, K. Haynes, C. Steele, M. Botto, S. Gordon and G. D. Brown: Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nat Immunol* 8, 31-38 (2007)
98. Willment, J. A., A. S. Marshall, D. M. Reid, D. L. Williams, S. Y. Wong, S. Gordon and G. D. Brown: The human b-glucan receptor is widely expressed and functionally equivalent to murine Dectin-1 on primary cells. *Eur J Immunol* 35, 1539-1547 (2005)
99. Ikeda, Y., Y. Adachi, T. Ishii, H. Tamura, J. Aketagawa, S. Tanaka and N. Ohno: Blocking effect of anti-Dectin-1 antibodies on the anti-tumor activity of 1,3-beta-glucan and the binding of Dectin-1 to 1,3-beta-glucan. *Biol Pharm Bull* 30, 1384-1389 (2007)
100. Breuel, K. F., P. Kougias, P. J. Rice, D. Wei, K. De Ponti, J. Wang, J. J. Laffan, C. Li, J. Kalbfleisch and D. L. Williams: Anterior pituitary cells express pattern recognition receptors for fungal glucans: implications for neuroendocrine immune involvement in response to fungal infections. *Neuroimmunomodulation* 11, 1-9 (2004)
101. Adachi, Y., N. Ohno and T. Yadomae: Preparation and antigen specificity of an anti-(1-3)-beta-D-glucan antibody. *Biol Pharm Bull* 17, 1508-1512 (1994)
102. Hirata, A., Y. Adachi, W. Itoh, M. Komoda, K. Tabata and I. Sugawara: Monoclonal antibody to proteoglycan derived from *Grifola frondosa* (Maitake). *Biol Pharm Bull* 17, 539-542 (1994)
103. Czop, J. K., M. F. Gurish and J. L. Kadish: Production and isolation of rabbit anti-idiotypic antibodies directed against the human monocyte

receptor for yeast beta-glucans. *J Immunol* 145, 995-1001 (1990)
104. Harada, T., N. N. Miura, Y. Adachi, M. Nakajima, T. Yadomae and N. Ohno: Antibody to soluble 1,3/1,6-beta-Dglucan, SCG in sera of naive DBA/2 mice. *Biol Pharm Bull* 26, 1225-1228 (2003)
105. Masuzawa, S., M. Yoshida, K. Ishibsahi, N. Saito, M. Akashi, N. Yoshikawa, T. Suzuki, S. Nameda, N. N. Miura, Y. Adachi and N. Ohno: Solubilized *Candida* cell wall b-glucan, CSBG, is epitope of natural human antibody. *Drug Dev Res* 58, 179-189 (2003)
106. Miura, T., N. Ohno, N. N. Miura, Y. Adachi, S. Shimada and T. Yadomae: Antigen-specific response of murine immune system toward a yeast beta-glucan preparation, zymosan. *FEMS Immunol Med Microbiol* 24, 131-139 (1999)
107. Uchiyama, M., N. Ohno, N. N. Miura, Y. Adachi, H. Tamura, S. Tanaka and T. Yadomae: Solubilized cell wall beta-glucan, CSBG, is an epitope of *Candida* immune mice. *Biol Pharm Bull* 23, 672-676 (2000)
108. Ishibashi, K., M. Yoshida, I. Nakabayashi, H. Shinohara, N. N. Miura, Y. Adachi and N. Ohno: Role of anti-beta-glucan antibody in host defense against fungi. *FEMS Immunol Med Microbiol* 44, 99-109 (2005)
109. Casadevall, A. and L. A. Pirofski: Polysaccharide-containing conjugate vaccines for fungal diseases. *Trends Mol Med* 12, 6-9 (2006)
110. Magliani, W., S. Conti, M. Gerloni, D. Bertolotti and L. Polonelli: Yeast killer systems. *Clin Microbiol Rev* 10, 369-400 (1997)
111. Magliani, W., S. Conti, S. Arseni, R. Frazzi, A. Salati and L. Polonelli: Killer anti-idiotypes in the control of fungal infections. *Curr Opin Invest Drugs* 2, 477–479 (2001)
112. Magliani, W., S. Conti, A. Cassone, F. De Bernardis and L. Polonelli: New immunotherapeutic strategies to control vaginal candidiasis. *Trends Mol Med* 8, 121-126 (2002)
113. Magliani, W., S. Conti, A. Salati, S. Arseni, R. Frazzi, L. Ravanetti and L. Polonelli: New strategies for treatment of Candida vaginal infections. *Rev Iberoam Micol* 19, 144-148 (2002)
114. Magliani, W., S. Conti, R. Frazzi, G. Pozzi, M. Oggioni and L. Polonelli: Engineered commensal bacteria as delivery systems of anti-infective mucosal protectants. *Biotechnol Genet Eng Rev* 19, 139-156 (2002)
115. Magliani, W., S. Conti, A. Salati, S. Vaccari, L. Ravanetti, D. L. Maffei and L. Polonelli: Therapeutic potential of yeast killer toxin-like antibodies and mimotopes. *FEMS Yeast Res* 5, 11-18 (2004)
116. Magliani, W., S. Conti, R. Frazzi, L. Ravanetti, D. L. Maffei and L. Polonelli: Protective antifungal yeast killer toxin-like antibodies. *Curr Mol Med* 5, 443-452 (2005)
117. Schmitt, M. J. and F. Breinig. Yeast viral killer toxins: lethality and self-protection. *Nat Rev Microbiol* 4, 212-221 (2006)
118. Polonelli, L., S. Conti, M. Gerloni, W. Magliani, C. Chezzi and G. Morace: Interfaces of the yeast killer phenomenon. *Crit Rev Microbiol* 18, 47-87 (1991)
119. Kimura, T., N. Kitamoto, K. Matsuoka, K. Nakamura, Y. Iimura and Y. Kito: Isolation and nucleotide sequences of the genes encoding killer toxins from *Hansenula mrakii* and *H. saturnus*. *Gene* 137, 265-270 (1993)
120. Komiyama, T., T. Ohta, Y. Furuichi, Y. Ohta and Y. Tsukada: Structure and activity of HYI killer toxin from *Hansenula saturnus*. *Biol Pharm Bull* 18, 1057-1059 (1995)
121. Komiyama, T., T. Shirai, T. Ohta, H. Urakami, Y. Furuichi, Y. Ohta and Y. Tsukada: Action properties of HYI killer toxin from *Williopsis saturnus* var. *saturnus*, and antibiotics, aculeacin A and papulacandin B. *Biol Pharm Bull* 21, 1013-1019 (1998)
122. Komiyama, T., T. Ohta, H. Urakami, Y. Shiratori, T. Takasuka, M. Satoh, T. Watanabe and Y. Furuichi: Pore formation on proliferating yeast *Saccharomyces cerevisiae* cell buds by HM-1 killer toxin. *J Biochem* 119, 731-736 (1996)
123. Takasuka, T., T. Komiyama, Y. Furuichi and T. Watanabe: Cell wall synthesis specific cytocidal effect of *Hansenula mrakii* toxin-1 on *Saccharomyces cerevisiae*. *Cell Mol Biol Res* 41, 575-581 (1995)
124. Guyard, C., E. Dehecq, J. P. Tissier, L. Polonelli, E. Dei-Cas, J. C. Cailliez and F. D. Menozzi: Involvement of [beta]-glucans in the wide-spectrum antimicrobial activity of *Williopsis saturnus* var. *mrakii* MUCL 41968 killer toxin. *Mol Med* 8, 686-694 (2002)
125. Guyard, C., N. Séguy, J. C. Cailliez, H. Drobecq, L. Polonelli, E. Dei-Cas, A. Mercenier and F. D. Menozzi: Characterization of a *Williopsis saturnus* var. *mrakii* high molecular weight secreted killer toxin with broad-spectrum antimicrobial activity. *J Antimicrob Chemother* 49, 961-971 (2002)
126. Guyard, C., P. Evrard, A. M. Corbisier-Colson, H. Louvart, E. Dei-Cas, F. D. Menozzi, L. Polonelli and J. C. Cailliez: Immuno-crossreactivity of an anti-*Pichia anomala* killer toxin monoclonal antibody with a *Williopsis saturnus* var. *mrakii* killer toxin. *Med Mycol* 39, 395-400 (2001)
127. Cailliez, J. C., M. Gerloni, G. Morace, S. Conti, C. Cantelli and L. Polonelli: Ultrastructural immunodetection of a *Pichia anomala* killer toxin: a preliminary study. *Biol Cell* 75, 19-23 (1992)
128. Cailliez, J. C., C. Cantelli, S. Conti, M. Gerloni, W. Magliani, G. Morace and L. Polonelli: *Pichia anomala* killer toxin secretion in the presence of tunicamycin. *J Med Vet Mycol* 31, 337-342 (1993)
129. Cailliez, J. C., C. Cantelli, N. Séguy, S. Conti, M. Gerloni, G. Morace and L. Polonelli: Killer toxin secretion through the cell wall of the yeast *Pichia anomala*. *Mycopathologia* 126, 173-177 (1994)
130. Polonelli, L., R. Lorenzini, F. De Bernardis and G. Morace: Potential therapeutic effect of yeast killer toxin. *Mycopathologia* 96, 103–107 (1986)

131. Yamamoto, T., K. Uchida and T. Hiratani: *In vitro* activity of the killer toxin from yeast *Hansenula mrakii* against yeasts and molds. *J Antibiot* 41, 398-403 (1988)
132. Pettoello-Mantovani, M., A. Nocerino, L. Polonelli, G. Morace, S. Conti, L. Di Martino, G. De Ritis, M. Iafusco and S. Guandalini. *Hansenula anomala* killer toxin induces secretion, and severe acute injury in the rat intestine. *Gastroenterology* 109, 1900-1906 (1995)
133. Selvakumar, D., Q. Z. Zhang, M. Miyamoto, Y. Furuichi and T. Komiyama: Identification and characterization of a neutralizing monoclonal antibody for the epitope on HM-1 killer toxin. *J Biochem (Tokyo)* 139, 399-406 (2006)
134. Selvakumar, D., N. Karim, M. Miyamoto, Y. Furuichi and T. Komiyama: Recombinant single-chain anti-idiotypic antibody: an effective fungal beta-1,3-glucan synthase inhibitor. *Biol Pharm Bull* 29, 1848-1853 (2006)
135. Selvakumar, D., M. Miyamoto, Y. Furuichi and T. Komiyama: Inhibition of fungal beta-1,3-glucan synthase and cell growth by HM-1 killer toxin single-chain anti-idiotypic antibodies. *Antimicrob Agents Chemother* 50, 3090-3097 (2006)
136. Polonelli, L., S. Conti, M. Gerloni, W. Magliani, M. Castagnola, G. Morace and C. Chezzi: "Antibiobodies": antibiotic-like anti-idiotypic antibodies. *J Med Vet Mycol* 29, 235-242 (1991)
137. Polonelli, L., F. De Bernardis, S. Conti, M. Boccanera, W. Magliani, M. Gerloni, C. Cantelli and A. Cassone. Human natural yeast killer toxin-like candidacidal antibodies. *J Immunol* 156, 1880-1885 (1996)
138. Polonelli, L., and G. Morace: Yeast killer toxin-like anti-idiotypic antibodies. *J Clin Microbiol* 26, 602-604 (1988)
139. Polonelli, L., F. Fanti, S. Conti, L. Campani, M. Gerloni, M. Castagnola, G. Morace and C. Chezzi: Detection by immunofluorescent anti-idiotypic antibodies of yeast killer toxin cell wall receptors of *Candida albicans*. *J Immunol Methods* 132, 205-209 (1990)
140. Polonelli, L., R. Lorenzini, F. De Bernardis, M. Gerloni, S. Conti, G. Morace, W. Magliani and C. Chezzi: Idiotypic vaccination: immunoprotection mediated by anti-idiotypic antibodies with antibiotic activity. *Scand J Immunol* 37, 105-110 (1993)
141. Polonelli, L., F. De Bernardis, S. Conti, M. Boccanera, M. Gerloni, G. Morace, W. Magliani, C. Chezzi and A. Cassone: Idiotypic intravaginal vaccination to protect against candidal vaginitis by secretory, yeast killer toxin-like anti-idiotypic antibodies. *J Immunol* 152, 3175-3182 (1994)
142. Magliani, W., S. Conti, F. De Bernardis, M. Gerloni, D. Bertolotti, P. Mozzoni, A. Cassone and L. Polonelli: Therapeutic potential of antiidiotypic single chain antibodies with yeast killer toxin activity. *Nat Biotechnol* 15, 155-158 (1997)
143. Polonelli, L., N. Seguy, S. Conti, M. Gerloni, D. Bertolotti, C. Cantelli, W. Magliani and J. C. Cailliez: Monoclonal yeast killer toxin-like candidacidal anti-idiotypic antibodies. *Clin Diagn Lab Immunol* 4, 142-146 (1997)
144. Beninati, C., M. R. Oggioni, M. Boccanera, M. R. Spinosa, T. Maggi, S. Conti, W. Magliani, F. De Bernardis, G. Teti, A. Cassone, G. Pozzi and L. Polonelli: Therapy of mucosal candidiasis by expression of an anti-idiotype in human commensal bacteria. *Nat Biotechnol* 18, 1060-1064 (2000)
145. Oggioni, M. R., C. Beninati, M. Boccanera, D. Medaglini, M. R. Spinosa, T. Maggi, S. Conti, W. Magliani, F. De Bernardis, G. Teti, A. Cassone, G. Pozzi and L. Polonelli: Recombinant *Streptococcus gordonii* for mucosal delivery of a scFv microbicidal antibody. *Int Rev Immunol* 20, 275-287 (2001)
146. Polonelli, L., W. Magliani, S. Conti, L. Bracci, L. Lozzi, P. Neri, D. Adriani, F. De Bernardis and A. Cassone: Therapeutic activity of an engineered synthetic killer antiidiotypic antibody fragment against experimental mucosal and systemic candidiasis. *Infect Immun* 71, 6205-6212 (2003)
147. Magliani, W., S. Conti, A. Salati, S. Arseni, L. Ravanetti, R. Frazzi and L. Polonelli: Biotechnological approaches to the production of idiotypic vaccines and antiidiotypic antibiotics. *Curr Pharm Biotechnol* 4, 91-97 (2003)
148. Magliani, W., S. Conti, A. Salati, S. Arseni, L. Ravanetti, R. Frazzi and L. Polonelli: Engineered killer mimotopes: new synthetic peptides for antimicrobial therapy. *Curr Med Chem* 11, 1793-1800 (2004)
149. Donini, M., C. Lico, S. Banchieri, S. Conti, W. Magliani, L. Polonelli and E. Benvenuto: Production of an engineered killer peptide in Nicotiana benthamiana by using a potato virus X expression system. *Appl Environ Microbiol* 71, 6360-6367 (2005)
150. Cenci, E., A. Mencacci, A. Spreca, C. Montagnoli, A. Bacci, K. Perruccio, A. Velardi, W. Magliani, S. Conti, L. Polonelli and L. Romani: Protection of killer antiidiotypic antibodies against early invasive aspergillosis in a murine model of allogeneic T-cell-depleted bone marrow transplantation. *Infect Immun* 70, 2375-2382 (2002)
151. Cenci, E., F. Bistoni, A. Mencacci, S. Perito, W. Magliani, S. Conti, L. Polonelli and Vecchiarelli A. A synthetic peptide as a novel anticryptococcal agent. *Cell Microbiol* 6, 953-961 (2004)
152. Travassos, L. R., L. S. Silva , E. G. Rodrigues, S. Conti, A. Salati, W. Magliani and L. Polonelli: Therapeutic activity of a killer peptide against experimental paracoccidioidomycosis. *J Antimicrob Chemother* 54, 956-958 (2004)
153. Séguy, N., J. C. Cailliez, P. Delcourt, S. Conti, D. Camus, E. Dei-Cas and L. Polonelli: Inhibitory effect of human natural yeast killer toxin-like candidacidal antibodies on *Pneumocystis carinii*. *Mol Med* 3, 544-552 (1997)
154. Séguy, N., L. Polonelli, E. Dei-Cas and J. C. Cailliez: Monoclonal killer toxin-like antiidiotypic antibodies to control rat-pneumocystosis. *J Eukaryot Microbiol* 44, 37S (1997)

155. Manfredi, M., M. J. McCullough, S. Conti, L. Polonelli, P. Vescovi, Z. M. Al-karaawi and S. R. Porter: *In vitro* activity of a monoclonal killer antiidiotypic antibody and a synthetic killer peptide against oral isolates of *Candida* spp. differently susceptible to conventional antifungals. *Oral Microbiol Immunol* 20, 226-232 (2005)
156. Conti, S., F. Fanti, W. Magliani, M. Gerloni, D. Bertolotti, A. Salati, A. Cassone and L. Polonelli: Mycobactericidal activity of human natural, monoclonal, and recombinant yeast killer toxin-like antibodies. *J Infect Dis* 177, 807-811 (1998)
157. Conti, S., W. Magliani, S. Arseni, E. Dieci, R. Frazzi, A. Salati, P. E. Varaldo & L. Polonelli: *In vitro* activity of monoclonal and recombinant yeast killer toxin-like antibodies against antibiotic-resistant Gram-positive cocci. *Mol Med* 6/7, 613-619 (2000)
158. Conti, S., W. Magliani, S. Arseni, R. Frazzi, A. Salati, L. Ravanetti and L. Polonelli: Inhibition by yeast killer toxin-like antibodies of oral streptococci adhesion to tooth surfaces in an ex vivo model. *Mol Med* 8, 313-317 (2002)
159. Savoia, D., C. Avanzini, S. Conti, W. Magliani, R. Frazzi and L. Polonelli: In vitro leishmanicidal activity of a monoclonal antibody mimicking a yeast killer toxin. *J Eukaryot Microbiol* 49, 319-323 (2002)
160. Savoia, D., S. Scutera, S. Raimondo, S. Conti, W. Magliani and L. Polonelli: Activity of an engineered synthetic killer peptide on *Leishmania major* and *Leishmania infantum* promastigotes. *Exp Parasitol* 113, 186-192 (2006)
161. Fiori, P. L., A. Mattana, D. Dessì, S. Conti, W. Magliani and L. Polonelli: *In vitro* acanthamoebicidal activity of a killer monoclonal antibody and a synthetic peptide. *J Antimicrob Chemother* 57, 891-898 (2006)
162. Conti, S., W. Magliani, L. Giovati, I. Libri, D. L. Maffei, A. Salati and L. Polonelli: Screening of a *Saccharomyces cerevisiae* nonessential gene deletion collection for altered susceptibility to a killer peptide. *New Microbiol* 31, 157-159 (2008)
163. Rachini, A., D. Pietrella, P. Lupo, A. Torosantucci, P. Chiani, C. Bromuro, C. Proietti, F. Bistoni, A. Cassone and A. Vecchiarelli: An anti-beta glucan monoclonal antibody inhibits growth and capsule formation of *Cryptococcus neoformans in vitro* and exerts therapeutic, anti-cryptococcal activity in vivo. *Infect Immun* 75, 5085-5094 (2007)
164. Conti, C., W. Magliani, M. Gerloni, A. Salati, E. Dieci, S. Arseni, P. Fisicaro and L. Polonelli: A transphyletic anti-infectious control strategy based on the killer phenomenon. *FEMS Immunol Med Microbiol* 22, 151-61 (1998)
165. Cenci, E., E. Pericolini, A. Mencacci, S. Conti, W. Magliani, F. Bistoni, L. Polonelli and A. Vecchiarelli: Modulation of phenotype and function of dendritic cells by a therapeutic synthetic killer peptide. *J Leukoc Biol* 79, 40-45 (2006)
166. Casoli, C., E. Pilotti, C. F. Perno, E. Balestra, E. Polverini, A. Cassone, S. Conti, W. Magliani and L. Polonelli: A killer mimotope with therapeutic activity against AIDS-related opportunistic microorganisms inhibits *ex vivo* HIV-1 replication. *AIDS* 20, 975-980 (2006)
167. Magliani, W., S. Conti, D. L. Maffei, L. Ravanetti and L. Polonelli: Antiidiotype-derived killer peptides as new potential tools to combat HIV-1 and AIDS-related opportunistic pathogens. *Anti-Infective Agents Med Chem* 6, 263-272 (2007)
168. Casadevall, A. and L.-A. Pirofski: Antibody-mediated protection through cross-reactivity introduces a fungal heresy into immunological dogma. *Infect Immun* 75, 5074-5078 (2007)
169. Young, S. H., G. R. Ostroff, P. C. Zeidler-Erdely, J. R. Roberts, J. M. Antonini and V. Castranova: A comparison of the pulmonary inflammatory potential of different components of yeast cell wall. *J Toxicol Environ Health A* 70, 1116-1124 (2007)
170. Al-Fattani, M. A. and L. J. Douglas. Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance. *J Med Microbiol* 55, 999-1008 (2006)
171. Nett, J., L. Lincoln, K. Marchillo, R. Massey, K. Holoyda, B. Hoff, M. VanHandel and D. Andes: Putative role of beta-1,3 glucans in *Candida albicans* biofilm resistance. *Antimicrob Agents Chemother* 51, 510-520 (2007)
172. Mah, T. F., B. Pitts, B. Pellock, G. C. Walker, P. S. Stewart and G. A. O'Toole: A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. *Nature* 426, 306-310 (2003)
173. Singla, M.: Antibody based target tailored antimicrobial chemotherapy – A new approach. *Med Hypotheses* 68, 957-959 (2007)
174. Dadachova, E., R. A. Bryan, C. Apostolidis, A. Morgenstern, T. Zhang, T. Moadel, M. Torres, X. Huang, E. Revskaya and A. Casadevall: Interaction of radiolabeled antibodies with fungal cells and components of the immune system in vitro and during radioimmunotherapy for experimental fungal infection. *J Infect Dis* 193, 1427-1436 (2006)
175. Dijkgraaf, I., O. C. Boerman, W. J. Oyen, F. H. Corstens and M. Gotthardt: Development and application of peptide-based radiopharmaceuticals. *Anticancer Agents Med Chem* 7, 543-551 (2007)
176. Casadevall, A.: The third age of antimicrobial therapy. *Clin Infect Dis* 42, 1414-1416 (2006)

Abbreviations: Ab: antibody, Ag: antigen, Als: agglutinin-like surface, AMB: amphotericin B, anti-Id: anti-idiotypic, BG: beta-glucan, CDR: complementarity determining region, CMI: cell-mediated immunity, Cwp: Cell wall protein, DC: dendritic cell, hsp: heat shock protein, HM-1: *Hansenula mrakii* KT-1, Ig: Immunoglobulin, KP: killer peptide, KT: killer toxin, KT-Abs: Abs that mimic the killing activity of a KT, KTR: KT receptor, Lam: laminarin, mAb: monoclonal Ab, nmAb: neutralizing monoclonal Ab, PaKT: *Pichia anomala* KT, PRR: pattern recognition receptor, rAb: recombinant Ab, scFv: single-chain fragment variable (rAb), SP: scramble peptide, TLR: toll-like receptor, WmKT: *Williopsis mrakii* KT

Key Words: Beta-Glucans, Anti-Beta-Glucan Antibodies, Anti-Beta-Glucan-Like Antibodies, Candidacidal Antibodies, Microbicidal Antibodies, Candidacidal Peptides, Microbicidal Peptides, Killer Peptides, Review

Send correspondence to: Walter Magliani, Sezione Microbiologia, Dipartimento di Patologia e Medicina di Laboratorio, Viale Gramsci, 14, 43100 Parma, Italy, Tel: 39 0521988877, Fax: 39 0521993620, E-mail: magliwal@unipr.it

http://www.bioscience.org/current/vol13.htm