frontiers in
MICROBIOLOGY

REVIEW ARTICLE
published: 01 June 2012
doi: 10.3389/fmicb.2012.00190

Antibody peptide based antifungal immunotherapy

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Fungal infections still represent relevant human illnesses worldwide and some are accompanied by unacceptably high mortality rates. The limited current availability of effective and safe antifungal agents makes the development of new drugs and approaches of antifungal vaccination/immunotherapy every day more needed. Among them, small antibody(Ab)-derived peptides are arousing great expectations as new potential antifungal agents. In this topic, the search path from the study of the yeast killer phenomenon to the production of Ab-derived peptides characterized by *in vitro* and *in vivo* fungicidal activity will be focused. In particular, Abs that mimic the antimicrobial activity of a killer toxin ("antibiobodies") and antifungal peptides derived from antibiobodies (killer peptide) and other unrelated Abs [complementarity determining regions (CDR)-based and constant region (Fc)-based synthetic peptides] are described. Mycological implications in terms of reevaluation of the yeast killer phenomenon, roles of antibiobodies in antifungal immunity, of β-glucans as antifungal targets and vaccines, and of Abs as sources of an unlimited number of sequences potentially active as new immunotherapeutic tools against fungal agents and related mycoses, are discussed.

Keywords: antifungal antibodies, killer toxin, antibiobodies, synthetic CDRs, killer peptides, antifungal peptides

INTRODUCTION

Fungal infections still represent relevant human illnesses worldwide and often are sentinel markers of immunological primary disorders or induced suppression (Vinh, 2011). Some mucocutaneous infections can be persistent or refractory and involve much of the population, such as vulvovaginal candidiasis that may affect up to 75% of women at least once in their childbearing age (Sobel, 1997). More problematic are invasive fungal infections (IFIs) that are dramatically increasing and are often severe, difficult to treat, and accompanied by unacceptably high mortality rates. Aspergillosis, cryptococcosis, and invasive candidiasis are among the most widespread, less treatable, and life-threatening IFIs. They are emerging with increasing frequency, typically in the setting of immunocompromised patients, even those treated with new antifungal drugs (Wisplinghoff et al., 2004; Kauffman, 2006; Binder and Lass-Flörl, 2011). This poses a serious threat to public health, taking into account that the currently available antifungal agents are limited in number, and often their prolonged administration can have significant toxicity. Even newer antifungal agents have important limitations related to their spectrum of activity, pharmacokinetics, and drug–drug interactions. The increasing resistance to old and new antifungals makes the situation even more complicated and far from satisfactory. On the one hand, therefore, the development of new antifungal drugs is becoming every day more demanding (Zhai and Lin, 2011), the other, much attention has been paid in recent years to new approaches of antifungal vaccination and/or immunotherapy. In this Research Topic, the search path from the study of the yeast killer phenomenon to the production of antibodies (Abs) that mimic the antimicrobial activity of a killer toxin (KT) to Ab-derived peptides characterized by fungicidal activity will be focused.

FROM KILLER TOXIN TO "ANTIBIOBODIES"

The demonstration that the killer effect, which was previously considered to be restricted to conspecific yeasts, was extensible to taxonomically unrelated fungi opened unannounced perspectives in antifungal therapy (Polonelli and Morace, 1986). KTs are exotoxins, generally proteins or glycoproteins, that exert their antifungal activity with different mechanisms of action by means of a preliminary, basic interaction with specific cell-wall receptors (KTRs; Magliani et al., 1997b). The therapeutic effect of a wide-spectrum KT, produced by *Wickerhamomyces anomalus* (formerly *Pichia anomala* and *Hansenula anomala*; *Pa*KT), in the topical treatment of experimentally induced pityriasis versicolor-like lesions suggested the possible use of KTs as potential new antifungals (Polonelli et al., 1986). This was later ruled out because of the characteristics of *Pa*KT in terms of toxicity, as well as antigenicity and instability in the physiological milieu (Pettoello-Mantovani et al., 1995). The production and characterization of a monoclonal Ab (mAb KT4), capable of neutralizing the fungicidal activity of *Pa*KT (Polonelli and Morace, 1987), allowed its use as an immunogen in rabbit for the production of anti-idiotypic (anti-Id) Abs that competed with *Pa*KT for the binding site of mAb KT4 and, most importantly, were able to kill *in vitro* cells of *Candida albicans*, adopted as fungus model, thereby mimicking the effect of *Pa*KT (Polonelli and Morace, 1988). *Pa*KT-like anti-Id Abs allowed to visualize, by immunofluorescence, *Pa*KTRs on *C. albicans* cell wall, but not on mammalian cells (Polonelli et al., 1990). The preferential location of *Pa*KTRs in budding scars and germ tubes, where inner cell-wall components are synthesized and exposed on the surface before being buried beneath the dense mannoprotein outermost coat, confirmed other observations on the greater susceptibility to *Pa*KT of cells in their active phase of growth and then

suggested a role of inner components, such as β-glucans (BGs), as *Pa*KTR constituents (Guyard et al., 2002). Affinity chromatography purified anti-Id Abs were likewise able to visualize *Pa*KTRs also in *Pa*KT-producing cells and to kill them, which are normally resistant to the activity of their own KT. The killer activity of anti-Id Abs could be neutralized by pre-incubation with mAb KT4, thus supporting the specificity of their action. *Pa*KT-like Abs able to exert a direct fungicidal activity, without the intervention of other factors or cells of the immune system, were defined "antibiobodies" (antibiotic-like Abs; Polonelli et al., 1991).

Antibiobodies showed to compete with *Pa*KT for both the combinatorial site of the neutralizing mAb KT4 and *Pa*KTRs of susceptible microorganisms suggesting, therefore, their three-dimensional structural and functional homology. As antibiobodies could be considered to mimic in some way the fungicidal activity of *Pa*KT, the combinatorial site of mAb KT4 could be considered as a mimic of *Pa*KTR. Based on these considerations, studies on "idiotypic vaccination," using mAb KT4 as parenteral or mucosal immunogen to stimulate the production of antibiobodies in different formats and experimental conditions, were carried out. Polyclonal antibiobodies elicited in mice or rats immunized with mAb KT4 induced protection against experimental systemic or vaginal infections, respectively, caused by *Pa*KT-susceptible *C. albicans* cells. The protection was associated with rising titers of circulating or mucosal antibiobodies. MAb KT4 affinity chromatography purified antibiobodies were capable of killing *C. albicans* cells *in vitro* and were able to passively transfer the protective state to non-immunized animals (Polonelli et al., 1993, 1994). *Pa*KT-like antibiobodies were also produced in unlimited quantities of indefinitely available formats. Thus, monoclonal (mAb K10) and recombinant single-chain (scFv H6) antibiobodies were produced by immunization of rats and mice, respectively, with mAb KT4 using established hybridoma and recombinant DNA technologies. Both antibiobodies formats proved to be candidacidal *in vitro* and to compete with *Pa*KT for the specific *Pa*KTR on *C. albicans* cells. The fungicidal activity of mAb K10 and scFv H6 was neutralized by mAb KT4 and, when administered at the time of challenge or postchallenge in an experimental model of vaginal candidiasis, they proved to exert a significant therapeutic activity (Magliani et al., 1997a; Polonelli et al., 1997).

As an obvious corollary, natural antireceptor antibiobodies were detected in the serum or secretions of animals and humans undergoing experimental or natural infections caused by *Pa*KT-susceptible *C. albicans* cells. Rising titers of fungicidal Abs could be detected, after intravaginal or intragastric inoculations of *Pa*KTR-bearing *C. albicans* cells, in vaginal fluids of rats previously vaccinated or never immunized with mAb KT4. Antireceptor antibiobodies were also consistently found in the vaginal fluid of women afflicted with recurrent vaginal candidiasis, as well as in the serum, saliva, and/or bronchial washing of HIV positive patients with oral or lung infections caused by *Pa*KTR-bearing fungi. Similar to what previously observed, affinity chromatography purified human natural antibiobodies were capable of killing *C. albicans* cells *in vitro* and their activity was neutralized by mAb KT4. These antibiobodies were also able to passively transfer the protective state to non-immunized animals (Polonelli et al., 1996).

The natural existence of candidacidal Abs as part of the Ab response against *C. albicans* added significance to the growing evidence on the importance of Ab-mediated acquired immunity for host defense against candidiasis and other relevant fungal infections (Casadevall et al., 1998). The availability, moreover, of reproducible antibiobodies in different formats and unlimited amounts, potentially free of undesired toxic effects, suggested the feasibility of new therapeutic approaches for the immunotherapy of candidiasis (Magliani et al., 2002). Based on the wide antifungal spectrum of *Pa*KT and the potential diffusion of *Pa*KTRs (Magliani et al., 1997b), antibiobodies should display a fungicidal activity against various fungal agents. While *Pa*KT's activity was severely limited by the environmental conditions, being manifested at acidic pH (4.6) and temperatures around 28°C, antibiobodies proved to be active in physiological conditions (pH 7 and 37°C). Conversely, the idiotype of mAb KT4 or purified *Pa*KTR could be suggested as potential antifungal vaccines.

Human natural antibiobodies proved to exert *in vitro* a strong and specific inhibitory activity against rat-derived *P. carinii* organisms, in terms of attachment to cultured cells and infectivity to nude rats. This activity could be abolished by their previous incubation with mAb KT4. Immunofluorescence studies of competition with *Pa*KT showed that antibiobodies efficiently bound to specific *Pa*KTRs on the surface of *P. carinii* cells (Séguy et al., 1997). Pneumocystosis (PCP) extension was significantly reduced by aerosol administration of mAb K10 in a PCP experimental nude rat model (Séguy et al., 1998). In a murine model of allogeneic T-cell-depleted bone marrow transplantation, treatment with mAb K10 protected mice with profound neutropenia from experimental invasive pulmonary aspergillosis in terms of long-term survival and decreased pathology associated with inhibition of fungal growth and chitin content in the lungs. This finding was supported by the *in vitro* effect of mAb K10 against *Aspergillus fumigatus* swollen conidia (inhibition of the hyphal development and metabolic activity; Cenci et al., 2002). A Gram-positive generally recognized as safe bacterium, *Streptococcus gordonii*, was engineered to produce scFv H6 as molecules secreted or displayed on the bacterial surface. Recombinant bacteria were able to stably colonize vaginal mucosa, and proved to be as efficacious as fluconazole in rapidly abating the fungal burden and in curing the infection in a rat model of experimental candidiasis (Beninati et al., 2000).

FROM ANTIBIOBODIES TO KILLER PEPTIDE

Synthetic peptides derived from the sequence of scFv H6 could still display *in vitro* candidacidal activity. In particular, a decapeptide containing the first three amino acids of the light chain (L) complementarity determining region (CDR)1, with an alanine replacement of its first residue (AKVTMTCSAS), proved to exert a strong candidacidal activity *in vitro*, and was therefore designated killer peptide (KP). Significantly, KP competed with mAb K10 for binding to germinating cells of *C. albicans*. Furthermore, KP demonstrated a significant therapeutic activity against infections caused by fluconazole-susceptible or -resistant *C. albicans* strains in a rat model of vaginal candidiasis as well as against systemic candidal infections in immunocompetent or severely immunocompromised mice (Polonelli et al., 2003). Thus, KP proved to act

as functional mimotope of *Pa*KT. KP demonstrated a broad antifungal spectrum without any detectable toxicity (Magliani et al., 2004a). Rapid candidacidal activity of KP was confirmed in time-killing studies and proved to be inhibited, in a dose-dependent fashion, by laminarin, a soluble 1,3-BG (Magliani et al., 2004b). KP was able to kill, *in vitro*, both capsular and acapsular *Cryptococcus neoformans* cells and impaired the production of specific virulence factors, such as the capsule, rendering the fungus more susceptible to natural effector cells. More importantly, KP reduced significantly the fungal burden in immunosuppressed mice with cryptococcosis and protected most of them from an otherwise lethal experimental infection (Cenci et al., 2004). KP demonstrated a significant activity against *Paracoccidioides brasiliensis* and experimental paracoccidioidomycosis being fungicidal *in vitro*, even in its D-isomeric form, and therapeutic *in vivo* by markedly reducing the fungal load in target organs (liver, lung, spleen) of infected animals (Travassos et al., 2004).

Killer peptide exerted a strong dose-dependent candidacidal activity against a large number of candidal strains isolated from saliva of adult diabetic and non-diabetic subjects, regardless of their species and pattern of resistance to conventional antifungal drugs (Manfredi et al., 2005). KP showed killing activity on *C. albicans* cells even adhered to sanded acrylic resin disks, a major condition in which candidal biofilms are formed (Manfredi et al., 2007).

The spectrum of KP activity was subsequently extended to phytopathogenic fungal agents, such as *Botrytis cinerea* and *Fusarium oxysporum*. KP was expressed in an active form in plants (*Nicotiana benthamiana*) by using a Potato virus X-derived vector. KP-expressing plants showed enhanced resistance to an experimental bacterial challenge with *Pseudomonas. syringae* pv. *tabaci* (Donini et al., 2005).

Killer peptide, moreover, was able to bind selectively to murine dendritic cells (DCs) and, to a lesser extent, to macrophages, possibly through major histocompatibility complex (MHC) class II, CD16/32, and cellular molecules recognized by anti-specific intercellular adhesion molecule-grabbing non-integrin R1 Abs. The peptide proved to modulate the multiple functions of DCs, improving their capacity to induce better immune antimicrobial response (Cenci et al., 2006).

The fungicidal activity of KP was apparently based on a new mechanism of action as no resistant mutant was found by testing a wide *Saccharomyces cerevisiae* non-essential gene deletion strain library that included isolates resistant to conventional antifungal drugs such as caspofungin and fluconazole (Conti et al., 2008).

Even though the precise molecular mechanism of action has still to be clarified, KP caused in *C. albicans* the appearance of significant internal alterations, such as cell-wall swelling, plasma membrane collapse, and condensation and fragmentation of nuclear material, similar to those observed by treatment of the yeast cells with classical apoptotic agents (Magliani et al., 2008b). KP proved to be very stable in its lyophilized form and, when solubilized in non-reducing conditions, due to the presence of a cysteine residue, it could easily dimerize by formation of disulfide bridges. KP dimer turned out to be the functional unit as confirmed by the instant and total candidacidal effect showed by the dimeric molecule synthesized *ad hoc*. After dimerization, KP revealed its ability to spontaneously and reversibly self-assemble in an organized network of fibril-like structures that resembled physical hydrogels. This process was catalyzed by the addition of 1,3-BG, as soluble laminarin or *C. albicans* cells exposing BGs on their surface, that caused an immediate conformational conversion of the peptide from random coil to antiparallel β-sheet. This self-assembled state was concentration- and temperature-dependent and could provide protection against proteases and assure a release of the active form over time. KP was proposed as paradigmatic of a new class of autodelivering therapeutic peptides (Pertinhez et al., 2009).

FROM KILLER PEPTIDE TO Ab-DERIVED ANTIFUNGAL PEPTIDES

All the peptides reproducing the six CDRs of scFv H6 showed candidacidal activity *in vitro*, even if to a lesser extent compared to KP (Polonelli et al., 2003). Other Abs have been reported meanwhile as characterized by direct antifungal activity: a human anti-heat shock protein 90 recombinant Ab (Mycograb; Matthews et al., 2003); a mAb (C7), directed to a protein epitope of a *C. albicans* cell-wall stress mannoprotein, that, besides its candidacidal activity, proved to exert inhibition of both adhesion and filamentation as well as blockage of the reductive iron uptake pathway of the yeast (Moragues et al., 2003; Brena et al., 2011); a scFv and a scFv-derived peptide able to mimic the fungicidal activity of the *H. anomala* HM-1 KT (Selvakumar et al., 2006; Kabir et al., 2011). The existence of a family of antifungal Abs, from which new innovative wide-spectrum fungicidal tools could be properly derived, was suggested (Magliani et al., 2005).

As seen in available databases, the sequence of P6, the peptide from which KP was derived, was present within the V regions of many unrelated Abs. On this basis, it was speculated that CDR-related peptides may display antifungal activity regardless of their specificities. Synthetic peptides with sequences identical to the CDRs of mAb C7 were proved for candidacidal activity in comparison to the CDRs of two unrelated Abs, whose variable region sequences were deposited and available. A murine IgM (mAb pc42), directed to a synthetic peptide containing the surface antigen of hepatitis B virus and the T-helper-cell epitope from the circumsporozoite protein of *Plasmodium falciparum*, was selected because it shared CDR H1 and H2 with mAb C7. A human IgM (mAb HuA), specific for difucosyl human blood group A substance, was selected because not sharing any sequence homology with either mAb C7 or mAb pc42 CDRs and because representing an Ab widely diffused in normal population. When tested in *in vitro* and *in vivo* experimental models against *C. albicans*, some CDR peptides showed differential fungicidal and therapeutic activities. Alanine substituted derivatives of candidacidal CDR peptides showed further differential increased, unaltered, or decreased candidacidal activity. Thus, short synthetic CDR-related peptides may display fungicidal activity irrespective of Ab specificity for a given antigen, conceivably involving different mechanisms of action. Alanine substitution can be used to increase variability of CDR peptides' fungicidal activity (Polonelli et al., 2008). A synthetic peptide representative of CDR H3 of a murine mAb (MoA) conspecific with HuA and representing

the different ways by which the same epitope can be recognized by different immune systems though presenting unrelated primary sequences, showed no candidacidal activity *in vitro*. MoA H3, however, was able to induce a significant increased production of proinflammatory cytokines, IL-6, and TNF-α, in murine splenocytes and peritoneal macrophages (PMs), but not in peritoneal neutrophils. Further characterization of MoA H3 allowed to visualize its binding and uptaking by PMs. This activated the Akt pathway in correlation to an increased production of TNF-α, and significantly up-regulated TLR-4 gene and protein expression. The state of PM activation could explain the therapeutic effect observed by treatment with MoA H3 in the mouse experimental model of systemic candidiasis in terms of survival and impressive decrease of candidal recovery from kidneys (Gabrielli et al., 2009).

MYCOLOGICAL IMPLICATIONS

These studies contributed to the advancement of knowledge on various aspects of treatment and control of fungal diseases. In particular, they suggested unusual considerations and perspectives on potential therapeutic and prophylactic approaches based on the yeast killer phenomenon, idiotypic vaccination, antibiobodies, and Ab-derived peptides.

REEVALUATION OF THE YEAST KILLER PHENOMENON

Given the impossibility of directly using KTs as antifungal therapeutic agents, their fungicidal properties have been harnessed by generating Ab derivatives. The production of antibiobodies and Ab-derived antimicrobial peptides (Magliani et al., 1997b, 2008b; Selvakumar et al., 2006; Kabir et al., 2011) suggests that very similar approaches can be applied with other KTs. Different antifungal molecules could be obtained, thereby taking advantage of the mimic of a widely spread natural phenomenon. Unraveling their mechanisms of action could result in the discovery of new potential targets for antifungal agents and/or immunoprevention, such as 1,3-BG, the suggested target of antibiobodies and Ab-derived antimicrobial peptides.

ANTIBIOBODIES AND HUMORAL ANTIFUNGAL IMMUNITY

The relative importance of cell-mediated (CMI) and humoral immunity against fungal infections has been longly debated. While CMI continues to be rightly considered the primary mechanism for antifungal defense, Ab response has been increasingly taken into consideration (Polonelli et al., 2000). In particular, antibiobodies were shown to occur in the Ab repertoire mounted during fungal infections caused by *Pa*KT-sensitive fungi. Their clinical relevance, however, still needs to be determined. They may represent only a minor part of the plethora of Abs produced during experimental or natural infections by *Pa*KTR-bearing fungal organisms and they could be very scarcely produced *in vivo* being unable to reach protective titers. The occurrence of interfering Abs of different specificities and isotype, moreover, could explain the negative results often achieved in active and passive immunoprotection experiments based on humoral immunity. The interplay between protective and interfering Abs could dictate the outcome of fungal infections and may also help to explain why subjects with elevated anti-*Candida* Ab titers could remain nonetheless susceptible to candidiasis (Bromuro et al., 2002). The observations made in the past decade, showing that Abs can function as direct effector molecules against fungi, suggest the need for new conceptual approaches in the understanding of humoral immunity to fungal infections (Casadevall and Pirofski, 2011).

β-GLUCANS AS CRITICAL VIABILITY MOLECULES, ANTIFUNGAL TARGETS, AND VACCINES

BGs, 1,3-BG in particular, have emerged as viability-critical inner components of many fungal cell walls and were reasonably suggested as *Pa*KTRs constituents. While 1,3-BGs are biosynthesized by a wide range of fungal species, they are not produced by mammalian cells (Magliani et al., 2008a). In the fungal cell wall, 1,3-BGs are usually masked beneath the dense mannoprotein outermost layer and this may protect them by recognition of Abs. When exposed on the surface, mainly during the active phase of growth, such as in budding cells and germ tubes in *C. albicans* (Iorio et al., 2008), BGs can represent a relevant fungal virulence factor being recognized as major pathogen associated molecular pattern able to act as potent proinflammatory molecules. Their critical structural role was underscored by the discovery of a new class of antifungals, the echinocandins, that are fungicidal by inhibiting the 1,3-BG synthesis (Denning, 2003) and by reports on antibiobodies and Ab-derived peptides. An innovative antifungal vaccine composed of laminarin, a soluble poorly immunogenic linear polymer of 1,3-BG purified from the brown alga *Laminaria digitata*, conjugated with diphtheria toxoid CRM197, was developed. In animal models, the elicited Abs proved to protect against ascomycetous and basidiomycetous fungal agents, such as *A. fumigatus*, *C. albicans*, and *C. neoformans* (Torosantucci et al., 2005; Rachini et al., 2007; Bromuro et al., 2010). As outlined by Casadevall and Pirofski, these observations introduced a fungal heresy into the immunological dogma that effective immune responses should be pathogen specific and that Abs to "common," "universal," or "cross-reactive" antigens may not be protective. In the case of 1,3-BG, over all derived from a non-fungal source, a single vaccine induced protection against three major fungal pathogens. Furthermore, this provides a vulnerable Achilles heel for Ab-mediated antifungal protection, suggesting the possibility to develop Abs for passive therapy of the diseases caused by each of these fungal pathogens (Casadevall and Pirofski, 2007). Recently, radiolabeled Abs to BG and other common fungal antigens as well as plant-derived recombinant Abs to BG have been described and proposed as universal tools for fungal disease (Capodicasa et al., 2011; Bryan et al., 2012). 1,3-BG-conjugated vaccines can be seen as "universal" vaccines that could be administered to patients who share risk factors (e.g., neutropenia) to immunize them, before they become debilitated and immunocompromised, against all of the main opportunistic fungal agents (Cassone and Rappuoli, 2010). Further studies will hopefully clarify all the different aspects in this field, including the role that anti-BG Abs, that are ubiquitous at low levels in human sera, may play in determining susceptibility or resistance to fungal infections (Chiani et al., 2009).

Ab-DERIVED PEPTIDES AS NEW IMMUNOTHERAPEUTIC TOOLS

The concept that short synthetic peptides corresponding to segments of variable region of immunoglobulins (Igs), CDRs

particularly, may display antifungal activities regardless of the specificity of the belonging Ab was claimed. This opened new perspectives in the field of antifungal therapy and encouraged to continue research on Abs as source of fungicidal peptides. Peptides encompassing sequences of the constant region of mammalian Abs (Fc-peptides) belonging to different isotypes (IgG, IgM, IgA), putatively released *in vivo* by proteolysis of Igs, were synthesized. Selected Fc-peptides proved to exert a fungicidal activity *in vitro* against pathogenic yeasts, such as *C. albicans*, *C. glabrata*, *C. neoformans*, and *Malassezia furfur*, including caspofungin and triazole resistant strains, without any hemolytic, cytotoxic, and genotoxic effect. An Fc-peptide (N10K), included in all human IgGs and selected as a proof-of-concept, displayed a therapeutic activity when administered in consolidated mouse models of systemic and vaginal candidiasis. N10K proved to spontaneously aggregate in a rich β-sheet structure and this possibly contributed to its *in vivo* therapeutic activity. The decapeptide bound to the surface of *Candida* cells, without causing major lysis. However, gross alterations in the morphology of yeast cells, with disruption of internal organelles, were seen (Polonelli et al., 2012). N10K, moreover, was able to induce in human monocytes, *in vitro*, IL-6 secretion, pIkB-α activation and up-regulation of Dectin-1 expression, leading to an increased activation of BG-induced pSyk, CARD9, and pIkB-α, and an increase in the production of proinflammatory cytokines, such as IL-6, IL-12, IL-1β, and TNF-α (manuscript submitted for publication). These findings may be of great interest from an immunological point of view. While significant amounts of specific fragments from the Ab variable regions, such as CDRs, are unlikely to be released *in vivo*, Fc-peptides could potentially occur *in vivo* and influence the antifungal immune response in a way reminiscent of molecules of innate immunity. Ongoing studies using mass spectrometry-based approaches are aimed to search for the presence of Fc-peptides in human sera from individuals in various clinical conditions. Positive results would shed new light on the role that Ab fragments could exert in the antifungal homeostasis. Furthermore, the reported high frequency of Ab-derived fungicidal peptides suggests that Abs, irrespective of their isotype and specificity for a given antigen, may be the source of potentially active and therapeutically exploitable molecules for devising new immunotherapeutic tools against pathogenic fungi (Magliani et al., 2009).

CONCLUSION

From the study of the interesting, but apparently therapeutically impracticable, yeast killer phenomenon, fungicidal antibiobodies, antibiobody-derived peptides, and Ab-derived CDR – as well as Fc-peptides were produced. Like many other proteins, such as bactericidal proteins (D'Alessio, 2011), hemoglobin (Catiau et al., 2011), *Helicobacter pylori* ribosomal protein L1 (Park and Hahm, 2012), human lactoferrin (Brouwer et al., 2011), human milk lysozyme (Ibrahim et al., 2011), human salivary protein (Gorr et al., 2011), and thrombin (Kasetty et al., 2011), longicin (Galay et al., 2012), thymic stromal lymphopoietin and kininogen (Sonesson et al., 2011a,b), ubiquitin (Pasikowski et al., 2011), among the most recently reported, Abs should be considered as containing many hidden peptides, known as "cryptides" (Ng and Ilag, 2006; Pimenta and Lebrun, 2007; Ueki et al., 2007; Samir and Link, 2011) in both their variable and constant regions. They can exert biological effects that cannot be predicted based on the activity of the precursor protein (Polonelli et al., 2012). These observations call into question the traditional distinction between acquired and innate immunity, suggesting a further close link between them. Ab-derived antifungal peptides, on the other hand, may be promising molecules for future therapeutic developments. Their easy production, engineering, and chemical optimization, through aminoacidic substitutions, peptidomimetics, etc., can greatly expand the possibilities of obtaining effective antifungal immunotherapeutic tools. These approaches may reasonably fall within the "fragment-based drug discovery," i.e., the design of good-quality lead compounds from fragment hits that can be developed into clinical candidates (Foloppe, 2011).

Future studies on Ab-derived peptides will be addressed to better clarify their molecular mechanisms of fungicidal action, presumably leading to the discovery of cellular targets for new therapeutic antifungal approaches in the never ending war against fungal diseases.

ACKNOWLEDGMENTS

Part of the work described in the manuscript has been supported by funding from the Italian Ministry of Health in the frame of the National AIDS Research Program (last grant number 40H39). The funder had no role in decision to publish or preparation of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 April 2012; paper pending published: 08 May 2012; accepted: 10 May 2012; published online: 01 June 2012.

Citation: Magliani W, Conti S, Giovati L, Zanello PP, Sperindè M, Ciociola T and Polonelli L (2012) Antibody peptide based antifungal immunotherapy. Front. Microbio. ***3****:190. doi: 10.3389/fmicb.2012.00190*

This article was submitted to Frontiers in Fungi and Their Interactions, a specialty of Frontiers in Microbiology.