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MiniReview

Therapeutic potential of yeast killer toxin-like antibodies and mimotopes

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Abstract

This review focuses on the potential of yeast killer toxin (KT)-like antibodies (KTAbs), that mimic a wide-spectrum KT through interaction with specific cell wall receptors (KTR) and their molecular derivatives (killer mimotopes), as putative new tools for trans-disease anti-infective therapy. KTAbs are produced during the course of experimental and natural infections caused by KTR-bearing micro-organisms. They have been produced by idiotypic vaccination with a KT-neutralizing mAb, also in their monoclonal and recombinant formats. KTAbs and KTAbs-derived mimotopes may exert a strong therapeutic activity against mucosal and systemic infections caused by eukaryotic and prokaryotic pathogenic agents, thus representing new potential wide-spectrum antibiotics.

Keywords: Yeast killer toxin; Killer antibodies; Killer mimotopes; Antimicrobial therapy; Anti-idiotypic antibiotics; Anti-idiotypic therapy

1. Introduction

Since the early 1970s, the optimistic believe of physicians that virtually all microbial infections were treatable was quenched by the emergence and the dramatic increase in the incidence and prevalence of resistance to antimicrobial agents among bacterial and fungal pathogens causing nosocomial as well as community-acquired infections [1–4].

The increasing frequency of antimicrobial-resistant strains and species has been attributed to a variety of factors that include the acquisition of resistant genes (via plasmids, transposons or integrons) and the heavy and often inappropriate use of antimicrobials. Their extensive use as growth enhancers in animal feed as well as the increase in regional and international travel allows the resistant strains to rapidly and easily cross geographic boundaries [5–7].

Resistant strains are often responsible for infections characterized by increased morbidity, mortality, and healthcare costs. Prevention and control of these infections require new vaccines, new antimicrobial agents and the prudent use of the existing ones [8]. Among the emerging pathogens, fungi have gained increasing medical importance during the past decades as causes of morbidity and mortality, mostly as opportunistic agents, due to the growing number of susceptible immunocompromised and otherwise modified hosts [9,10].

Overall, there is an urgent need to develop new therapeutic and preventative anti-infective strategies by looking for new microbial targets and novel antimicrobial agents.

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Among the plethora of efforts aimed to add new effective weapons to the armamentarium against microbial pathogens, a growing set of interesting observations has emerged in recent years on yeast killer toxin-like antibodies (KTAbs) and mimotopes as potential new antimicrobial compounds and mediators of protection in active vaccination.

In Fig. 1, a diagram depicts the interactions of a KT produced by the yeast *Pichia anomala* (*Pa*KT), characterized by a wide spectrum of antimicrobial activity mediated by its binding to specific microbial KT receptors (KTRs), and natural, anti-idiotypic polyclonal, monoclonal and recombinant antibodies, representing its internal image, and synthetic peptides (mimotopes). The dual identity occurring between *Pa*KT and its immunological derivatives (anti-KTR *Pa*KTAbs, anti-Id *Pa*KTAbs, *Pa*KTmAb, *Pa*KTscFv, P6 and KP) and *Candida albicans* KTR (as an example) and anti-*Pa*KT mAbKT4 in the idiotypic vaccination and anti-idiotypic therapy will be discussed in the following sections.

2. Anti-idiotypic and natural antireceptor yeast killer toxin-like antibodies

Yeast KT-like Abs, also defined as ''killer Abs'' and ''antibiobodies'' (antibiotic antibodies, KTAbs), are able to directly inhibit functions that are critical for survival of microorganisms, by mimicking the biological activity of KTs, thus exerting a microbicidal activity. Several fungi, especially yeasts, can produce and secrete proteinaceous KTs lethal to other taxonomically related or unrelated susceptible microorganisms. They represent a sophisticated biological mechanism of competition in natural ecosystems [11].

KTs differ in terms of genetic and molecular characteristics as well as mechanisms of action. Their lethal activity is mediated by an initial binding to specific KTRs on the surface of susceptible cells [12,13]. KTs produced by some *P. anomala* and *Williopsis saturnus* var. *mrakii* killer strains aroused obvious interest, because of their wide spectrum of activity including important pathogenic microorganisms, such as *C. albicans*, *Pneumocystis carinii* and *Mycobacterium tuberculosis* [14–17]. However, their instability at physiological pH and temperature, as well as antigenicity and toxicity, excluded their potential use as therapeutic agents, apart from topical applications [18,19].

The first report, in 1988 [20], of KTAbs produced in rabbits through the idiotypic network opened new perspectives on the potential of the yeast killer phenomenon and KT-like Abs. This subset of protective antifungal Abs could be elicited in different experimental and natural conditions, as well as a variety of formats, as briefly described below.

A monoclonal Ab (mAbKT4) that neutralized the microbicidal activity in vitro of *Pa*KT against a susceptible *C. albicans* reference strain was produced by the

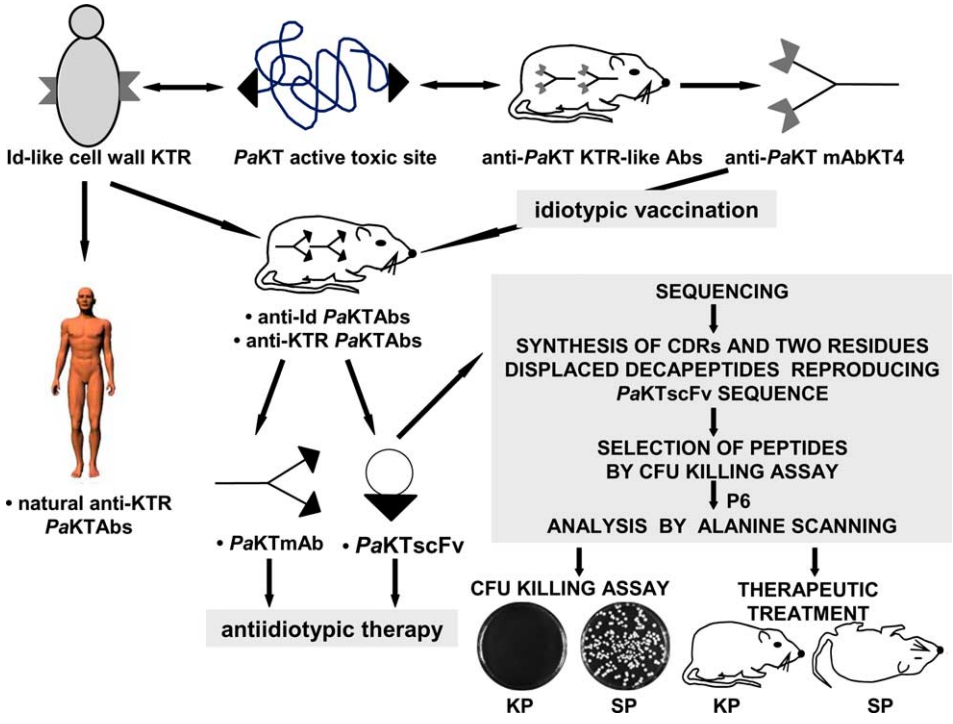

Fig. 1. Receptor (KTR)-mediated interactions of *Pichia anomala* killer toxin (*Pa*KT) and *Pa*KT-like natural (anti-KTR *Pa*KTAbs), anti-idiotypic polyclonal (anti-Id *Pa*KTAbs), monoclonal (*Pa*KTmAb), recombinant (*Pa*KTscFv) antibodies and mimotopes (P6, KP) in the idiotypic vaccination and anti-idiotypic therapy.

conventional hybridoma technology from mice immunized with *Pa*KT [21]. By using mAbKT4 as an idiotypic vaccine (''idiotypic vaccination''), protective systemic and mucosal polyclonal anti-idiotypic *Pa*KTAbs (anti-Id *Pa*KTAbs) in different animal models (rabbits, mice, rats) [20,22–25] as well as monoclonal (*Pa*KTmAb) [26] and recombinant (*Pa*KTscFv) [27] anti-Id *Pa*KTAbs have been produced.

Parenteral mAbKT4 vaccination in syngeneic mice resulted in the production of serum *Pa*KTAbs that were able to confer significant protection against lethal intravenous challenges with *Pa*KT-susceptible *C. albicans* cells [24]. Intravaginal mAbKT4 vaccination in oophorectomized and estradiol-treated rats elicited the production in the vaginal fluid of *Pa*KTAbs, mostly as secretory IgA, conferring mucosal immunoprotection against experimental vaginal candidiasis [25].

Importantly, mAbKT4-affinity chromatography purified *Pa*KTAbs, as well as *Pa*KTmAb (produced by conventional hybridoma technology from rats immunized with mAbKT4) and *Pa*KTscFv (selected from a phage-display Ab library constructed from splenocytes of mice immunized with mAbKT4), were able to passively immunoprotect naive rats against experimental mucosal candidiasis (''anti-idiotypic therapy'') [26,27].

Irrespective of their format (polyclonal, monoclonal or recombinant), isotype (be it IgA, IgG or IgM), and method of production, purified anti-idiotypic *Pa*KTAbs were able to directly kill in vitro microbial cells susceptible to *Pa*KT, such as *C. albicans*, thus acting as true functional internal images of the active toxic site of *Pa*KT. This candidacidal activity was totally abolished by previous absorption with mAbKT4. Immunofluorescence studies suggested that the killer activity of *Pa*KT and its immunological derivatives was mediated by the interaction with a cell wall KTR, which in *C. albicans* is mainly expressed on growing cells and particularly on budding cells and germ tubes. Significantly, *Pa*KTAbs competed with *Pa*KT for binding to yeast cells [22,24–27].

The identification of β-glucans as KTR for the KT secreted by *Williopsis saturnus* var. *mrakii* [28], which is antigenically related to *Pa*KT in that it is neutralized by mAbKT4 [29], and our previous observations discussed below strongly suggest that β-glucans are involved in the structure, entirely or in part, of *Pa*KTR.

The availability of *Pa*KTscFv allowed its genetic manipulation to engineer *Streptococcus gordonii*, a safe, human-commensal bacterium, in order to produce microbicidal molecules directly at mucosal sites. When used to treat experimental rat vaginitis caused by *C. albicans*, both recombinant strains obtained, one secreting and the other one displaying *Pa*KTscFv on the surface, were able to stably colonize rat vaginas and showed a relevant therapeutic anti-candidal activity, comparable to the one observed with a full therapeutic course of fluconazole [30,31].

From a theoretical point of view, the ascertained functional similarities of anti-idiotypic *Pa*KTAbs with *Pa*KT suggested a similar homology between the idiotype of mAbKT4 and KTR. The immune system can recognize the KTR of infecting microorganisms as the idiotype of mAbKT4, by producing, among a plethora of other antimicrobial Abs, a sub-set of antireceptor *Pa*KTAbs. This was first demonstrated in rats previously intravaginally immunized with mAbKT4. Subsequent intravaginal or intragastric administration of KTR-bearing *C. albicans* cells to mAbKT4-primed animals induced a dramatic booster effect, as protective *Pa*KTAbs were recalled at high titres in the vaginal fluids. Even more significantly, anti-KTR *Pa*KTAbs were detected in the vaginal fluids of rats, never immunized with mAbKT4 but experimentally and repeatedly infected with *C. albicans* cells, and of women particularly experiencing recurrent vaginal candidiasis [32], as well as in the serum and secretions of HIV-infected individuals with mucosal candidal infections (unpublished data). MAbKT4-affinity chromatography purified rat and human *Pa*KTAbs exerted the same in vitro candidacidal activity and ability to transfer passive immunoprotection to naive animals as anti-Id *Pa*KTAbs [27,32].

The precise clinical relevance of these microbicidal Abs in human disease remains to be elucidated, since their indubitable presence apparently did not confer immunoprotection, at least in some natural infections. A possible explanation for this apparent discrepancy was recently suggested by the demonstration that the outcome of experimental disseminated candidiasis relies on the interplay between protective, certainly inclusive of anti-KTR *Pa*KTAbs, and inhibitory Abs, elicited by the wide antigenic array of the infecting microorganism [33]. On the contrary, such interfering Abs cannot be produced following idiotypic vaccination.

3. Yeast killer toxin-like mimotopes

On the basis of the previous observations and the availability of the entire *Pa*KTscFv's nucleotide sequence, peptides reproducing the complementarity determining regions (CDRs) of both light and heavy chains of *Pa*KTscFv, as well as decapeptides containing parts of CDRs, were synthesized and tested for candidacidal activity in vitro. A number of them displayed candidacidal activity in vitro similar to the one exerted by the whole molecule [34]. The amino acid sequence of *Pa*KTscFv is shown in Fig. 2, where the positions of the CDRs, which had a peptide concentration corresponding to the 50% inhibitory concentration (IC_{50}) always higher than 10^{-4} $moll^{-1}$, and of two other candidacidal decapeptides (P6, characterized by the

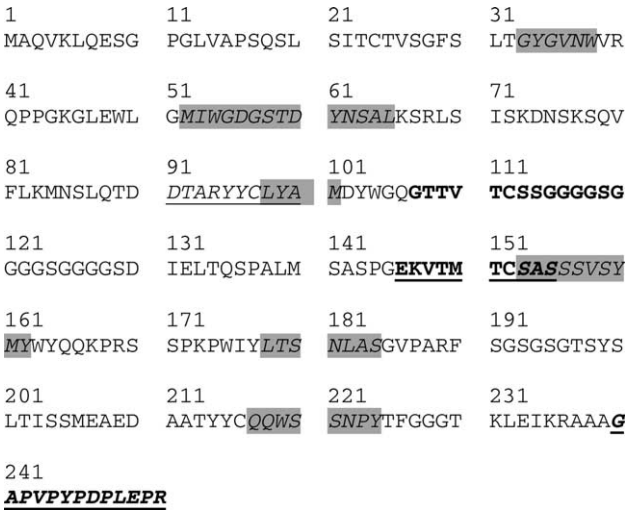

Fig. 2. The *Pa*KTscFv amino acid sequence. Indicated are the positions of the CDRs [italics, light grey: CDR_1 V_H(33–38); CDR_2 V_H (52–65); CDR_3 V_H (98–101); CDR_1 V_L (153–162); CDR_2 V_L (178–184); CDR_3 V_L (217–224)], P8 [underlined (91–100)] and P6 [bold, underlined (146–155)] decapeptides, linker [bold (107–120)], and E-tag [bold, underlined (240–252)] sequences.

sequence EKVTMTCSAS, inclusive of the first three residues of the CDR-L1, and P8, characterized by the sequence DTARYYCLYA, inclusive of the first three residues of the CDR-H3) are indicated.

The decapeptide P6 demonstrated the highest candidacidal activity in vitro ($IC_{50} = 1.06 \times 10^{-5}$ moll^{-1}) and it was selected for analysis by alanine scanning, in order to identify the functional contribution of each residue. In Table 1 the in vitro candidacidal activities of P6 and peptides obtained by its alanine scanning are shown in comparison with the one of a scramble decapeptide, properly synthesized as altered sequence of P6 (SP0, MSTAVSKCET), which showed no in vitro candidacidal activity at all, used as a control. All the substituted decapeptides retained some activity, but the one with alanine replacing E, named KP (No 2, AKVTMTCSAS), showed a surprisingly increased dose-dependent activity, with 100% of killing at a concentration of 6.25 μgml^{-1} ($IC_{50} = 5.6 \times 10^{-8}$ moll^{-1}), as determined by CFU assays after incubation for 6 h at 37 °C.

The reduction of KP by COOH-terminal deletion up to three residues to establish the ability of the shortened derivatives to retain their candidacidal activity caused a drop of the IC_{50} values of about three orders of magnitude, in particular with the deletion of the COOH-terminus serine.

Due to the presence in the KP molecule of a cysteine residue, potentially responsible for oxidation and polymerization processes, stability of KP was evaluated. KP proved to be very stable in its lyophilized form. In non-reducing conditions, solubilized KP can easily dimerize by formation of disulfide bridges. This, however, does not affect the candidacidal activity of the peptide, which is maintained unaltered over a long period of time under different storage conditions (4 °C, room temperature, 37 °C). Furthermore, a stable dimeric peptide (2KP) synthesized as such can exert even a stronger candidacidal activity n vitro in comparison with its own scramble dimeric peptide (2SP). Finally, P6 and KP peptides synthesized by using the same amino acid residues in the D rather than L conformation retained their in vitro candidacidal activity (unpublished data).

On the basis of the previous observations, KP was further investigated in comparison with its scramble peptide, named SP (MSTAVSKCAT). As shown in Fig. 3, the time-killing curves, determined by incubation of *C. albicans* with KP at four different concentrations, demonstrated a clear, rapid candidacidal effect of the decapeptide. In particular, KP achieved more than 90% killing within 30 min at the highest concentration (100 μgml^{-1}, 16× minimal fungicidal

Table 1
In vitro activity against *Candida albicans* of the products obtained by alanine scanning from the killer synthetic decapeptide P6

Decapeptide	100 μgml^{-1}	25 μgml^{-1}	6.25 μgml^{-1}
1. EKVTMTCSAS	5.7±0.2*	29.8±10.6	67.1±13.8
2. AKVTMTCSAS	0*	0*	0*
3. EAVTMTCSAS	9.9±3.3	42.7±2.0	53.4±7.0
4. EKATMTCSAS	9.3±2.4*	19.7±2.9	60.1±5.2
5. EKVAMTCSAS	9.2±4.1*	26.6±4.2	63.2±4.8
6. EKVTATCSAS	0.1±0.1*	10.1±3.0*	40.4±16.0*
7. EKVTMACSAS	52.9±3.9	55.5±3.7	58.1±8.2
8. EKVTMTASAS	55.7±10.2	59.7±4.8	64.3±6.7
9. EKVTMTCAAS	2.6±0.5*	23.1±3.6*	72.9±7.4
10. EKVTMTCSAA	11.9±0.6*	32.9±0.8*	70.3±9.1
11. MSTAVSKCET	100	100	100

Decapeptide 1 is P6; decapeptides 2–10 are derived from P6 by alanine scanning; decapeptide 2 is KP; 11 is the scramble decapeptide (SP0) derived from P6 and used as a control. The activity of each decapeptide is expressed as percentual growth in comparison with the control in a colony forming unit (CFU) assay, essentially carried out as previously described, after incubation for 6 h at 37 °C with the respective reagents [34].
Statistically significant difference ($P < 0.005$) in CFU counts in comparison with the control (each test performed in triplicate) is indicated by *. The statistical significance was assessed by the two-tailed Student's *t* test.

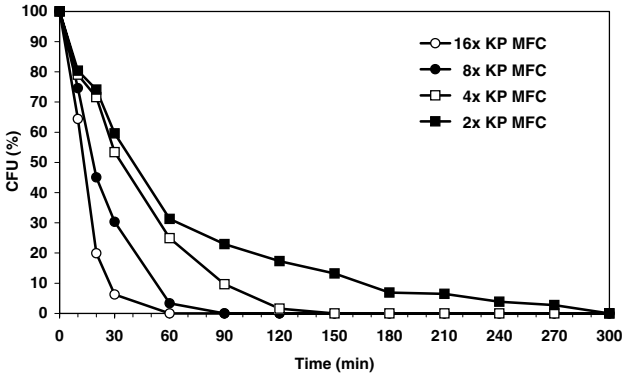

Fig. 3. Time kinetics of KP-mediated killing of *C. albicans*. Viable *Pa*KT-susceptible germinating *C. albicans* cells (approximately 3×10^2) were incubated with KP or SP at different concentrations (100, 50, 25, 12.5 $\mu g\,ml^{-1}$, corresponding to 16×, 8×, 4× and 2× minimal fungicidal concentration, MFC), at 37 °C up to 6 h [34]. At different times (0, 10, 20 min, then 30-min intervals), the treated yeast suspensions were dispensed and streaked on the surface of Sabouraud dextrose agar plates which were then incubated at 30 °C, and colony forming units (CFUs) were enumerated after 48 h. The killing was expressed as percentage of CFU, calculated as: (average number of CFU in the KP-treated test group/average number of CFU in the SP-treated control group)×100. Each experiment was performed in triplicate.

concentration, MFC) and a complete killing within 5 h even at the lowest concentration (12.5 $\mu g\,ml^{-1}$, 2× MFC).

Besides its recognized candidacidal activity, KP was able to compete with *Pa*KTAbs for binding to KTR of germinating cells of *C. albicans*. Its candidacidal activity was inhibited, in a dose-dependent fashion, by the soluble β-1-3 glucan laminarin, but not by the soluble β-1-6 glucan pustulan, suggesting that KP would be a functional mimotope of *Pa*KT.

Most importantly, KP demonstrated remarkable therapeutic activity against experimental rat vaginal and systemic mouse candidiasis, similar to that of a therapeutic course of fluconazole, even when the challenge strain was a fluconazole-resistant strain of *C. albicans*. Noteworthy, KP was similarly highly effective in normal, immunocompetent and SCID mice, suggesting that its activity did not require any crucial participation of the host's adaptive immunity [34].

4. Transdisease therapeutic potential of yeast killer toxin-like antibodies and mimotopes

An exciting corollary of these observations is represented by the potentially wide spectrum of activity of *Pa*KT, *Pa*KTAbs and *Pa*KT-mimotopes. Unlike *Pa*KT, its immunological derivatives, such as natural, monoclonal, recombinant *Pa*KTAbs and mimotopes, are much more chemically stable, reproducible and available to quite an unlimited extent, to be easily tested in vitro at physiological conditions and administered as parenteral or mucosal therapeutic agents in different animal models of infection.

Thus, it was demonstrated that a surprisingly wide spectrum of epidemiologically important eukaryotic and prokaryotic microbial pathogens exhibits a remarkable susceptibility to these molecules (Table 2). Besides *C. albicans*, other fungi, such as *Aspergillus fumigatus* [35], *P. carinii* [36,37], a large number of clinical strains of *Candida* spp., regardless of their species and pattern of resistance to conventional antifungal agents (manuscript in preparation), *Cryptococcus neoformans* [38], and *Paracoccidioides brasiliensis* (manuscript in preparation), were susceptible in vitro to *Pa*KT-derivatives. Furthermore, besides the above reported in vivo activity against candidiasis, the *Pa*KT-derivatives exerted significant therapeutic activity in different animal models of systemic and mucosal fungal infections. Treatment with *Pa*KTmAb protected T-cell-depleted allogeneic bone marrow-transplanted mice from experimental invasive pulmonary aspergillosis [35]. Aerosol administration of *Pa*KTmAb exerted a strong therapeutic effect in *P. carinii*-infected nude rats [37]. KP selectively impaired or retarded the synthesis/release of fungal virulence factors and exerted a relevant therapeutic effect in experimental murine systemic cryptococcosis. It significantly reduced fungal load in target organs and prolonged survival [38]. Finally, treatment with KP exerted a dramatic protective effect in mice experimentally infected with a virulent strain of *Pa. brasiliensis*. After 8 days from challenge and three parenteral KP administrations, tissues and organs from KP-treated animals had no detectable fungal cells and were almost preserved.

Analogous killer effects of *Pa*KT-derivatives have been demonstrated in vitro against bacterial pathogens of major epidemiological interest, such as multidrug-resistant strains of *M. tuberculosis* [39], vancomycin-resistant strains of *Enterococcus* spp., penicillin-resistant strains of *Streptococcus* spp., and methicillin-resistant strains of *Staphylococcus* spp. [40]. Inhibition and reduction of dental colonization by *Streptococcus mutans* and other oral streptococci have been observed by treatment with *Pa*KTmAb, in an ex vivo model of human teeth [41]. Finally, a significant and dose-dependent microbicidal activity of *Pa*KT-derivatives has been observed in vitro against relevant species of protozoan pathogens, such as *Leishmania major* and *L. infantum* [42] and *Acanthamoeba castellanii* (manuscript in preparation). No resistant strain has been found among the microorganisms tested so far.

Regardless of microbial system and nature, all of the *Pa*KT-derivatives competed with *Pa*KT and/or each other for putative microbial cell wall KTR. Their microbicidal activity was abolished by previous adsorption with mAbKT4 and laminarin. These observations

Table 2
Recognized antimicrobial activity of yeast killer toxin-like antibodies and synthetic mimotopes

Fungi[a]		Bacteria[a]		Protozoa
In vitro	In vivo	In vitro	Ex vivo	In vitro
Candida spp.	*Candida albicans* [23,25,27,30,34]	*Mycobacterium tuberculosis* [39]	*S. mutans* [41]	*Leishmania major* [42]
C. albicans [20,22–27,30–32,34]	*Aspergillus fumigatus* [35]	*Staphylococcus aureus* [40]	*Oral streptococci* [41]	*L. infantum* [42]
C. dubliniensis[b]	*Pneumocystis carinii* [37]	*S. haemolyticus* [40]	(*S. intermedius*, *S. mitis*, *S. oralis*, *S. Salivarius*)	*Acanthamoeba castellanii*[b]
C. glabrata[b]	*Cryptococcus neoformans* [38]	*Enterococcus faecalis* [40]		
C. guillermondi[b]	*Paracoccidiodes brasiliensis*[b]	*E. faecium* [40]		
C. krusei[b]		*Streptococcus pneumoniae* [40]		
C. lusitaniae[b]		*S. mutans* [41]		
C. parapsilosis[b]		*Oral streptococci* [41]		
C. tropicalis[b]		(*S. intermedius*, *S. mitis*, *S. oralis*, *S. salivarius*)		
Aspergillus fumigatus [35]		*Escherichia coli*[c]		
Cryptococcus neoformans [38]		*Salmonella enterica*[c] (serovar Derby)		
Pneumocystis carinii [36,37]		*Pseudomonas syringae pv tomato*[b]		
Paracoccidiodes brasiliensis[b]		*Pseudomonas corrugata*[b]		

[a] Including drug-resistant strains.
[b] Manuscript in preparation.
[c] Unpublished data.

strongly suggest the occurrence of a transphyletic KTR in different taxonomically unrelated and epidemiologically important eukaryotic and prokaryotic pathogenic microorganisms, possibly consisting of glucans or glucan-like molecules, whose nature and function need further definition.

5. Conclusions and perspectives

These observations on the direct microbicidal activity of *Pa*KT-derivatives open new exciting perspectives in the production of novel antimicrobial therapeutic agents and vaccines. Engineered low molecular weight peptides, such as KP, could represent standardized compounds easily produced and less expensive than other immunological *Pa*KT-derivatives. Such killer mimotopes can display a broad receptor-mediated antimicrobial spectrum with a cellular target conserved through natural evolution, hopefully not rejectable by the microorganisms and, as such, not involved in mutations resulting in drug resistance.

As expected for peptides derived from physiological molecules, such as Abs, KP demonstrated the lack of any detectable toxicity to in vitro cultured cell lines and white blood cells (unpublished data).

After more than a decade since this approach has been under study, and one year since the production of killer peptides, KP has been patented and is now entering clinical trials, initially in patients with mucosal candidiasis, to determine its metabolism, side effects and effectiveness. The assessment of efficacy and safety, based on clinical and mycological responses, will enable to further progress to the treatment of deep-seated candidiasis as well as other fungal, bacterial or protozoal infections, even resistant to conventional drugs, according to the in vitro and in vivo wide spectrum of activity of KP.

Molecules able to selectively interact with microbial cell wall components, which are not present in mammalian cells, such as KTAbs and KP, should be rationally considered as putative antimicrobial agents. From this point of view, few other promising candidacidal Abs have been described. Matthews et al. [43,44] demonstrated that different Abs against fungal heat shock protein 90 (HSP90) were therapeutically active in murine models of invasive candidiasis. In particular, the preclinical assessment of the efficacy of a human recombinant Ab against an epitope of HSP90 (Mycograb®) demonstrated its activity against a wide range of yeast species. Mycograb® showed intrinsic in vitro antifungal activity (MICs ranging from 128 to 256 $\mu g ml^{-1}$) with a mechanism of action involving inhibition of HSP90. Synergy with amphotericin B was demonstrated in the therapy of *Candida* infections [45].

More recently, Moragues et al. [46] described a mAb (mAbC7) directed against a protein epitope of a cell wall stress mannoprotein expressed in different fungal agents, which, besides its ability to inhibit adherence and germination of *C. albicans* cells, exerted a direct in vitro candidacidal activity (25 $\mu g\,ml^{-1}$ caused an 80% reduction in the number of CFU).

Unlike Mycograb® and mAbC7, KP is a small peptide, devoid of antigenicity, which is characterized by a potent candidacidal activity (killing concentration of 6.25 $\mu g\,ml^{-1}$).

The generation of different natural, monoclonal as well as recombinant killer Abs supports the concept of a family of microbicidal Abs, which could be used as adjuvant therapy in addition to the commonly used chemotherapy, opening new promising perspectives in the control of microbial infections [47].

Finally, the possibility of deriving antimicrobial peptides, like KP, from microbicidal Abs could provide a unique approach for the production of a new class of wide-spectrum peptide antibiotics, eventually deliverable directly at mucosal sites by safe transgenic commensals permanently expressing them. They can be also active against pathogenic microorganisms that are currently resistant to conventional drugs.

As discussed before, a spectacular approach could be pursued to produce candidate vaccines to elicit KTAbs in vivo. Thus, innovative vaccine and therapeutically potential anti-infective strategies can be envisaged against infections caused by pathogenic microorganisms that are difficult to prevent and treat by mimicking a natural process, such as the yeast killer phenomenon [48–52]. This could help dealing with the growing problem of the emergence of antimicrobial resistance among normally susceptible pathogenic agents as well as the spread of less susceptible species, including epidemiologically important pathogenic microorganisms.

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Luciano Polonelli, Walter Magliani and Stefania Conti want to dedicate this work to the memory of their mentor and friend Dr. Libero Ajello.

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