

Immunogenic and protective *Candida albicans* constituents

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Candida commensalism, immunogenicity and protection: a problematic issue

Candida albicans is an opportunistic fungus which has become an increasingly common cause of disease in the immunocompromised or otherwise modified host. Mucocutaneous involvement is especially common in patients with defective cell-mediated immunity (CMI), such as those with AIDS, whereas deep-seated candidiasis is more prevalent in neutropenic patients (Klein *et al.*, 1984; Bodey *et al.*, 1986). Although the fungus possesses an array of low-penetrance "virulence" factors (Cutler, 1991), a defective immune response is usually a prerequisite for serious candidal infections to occur. On the other hand, *C. albicans* is an ordinary commensal of the human gastrointestinal (GI) tract, and its presence is sensed by the immune system of the healthy subject which responds in terms of intense *Candida*-specific and non-specific immunomodulatory effects (Cassone, 1995). Thus, *Candida* is a microorganism which interacts safely and, perhaps, even beneficially (as non-specific immunomodulator) in a normal host, but pathologically in a predisposed subject.

In order to understand host-fungus interactions in both categories of subjects, it is essential to study: i) the fungal components which elicit, modulate or suppress host immune responses; ii) how these components are expressed during *Candida* growth and morphogenesis *in vitro* and *in vivo*; iii) the immunoregulatory mechanisms underlying protective and non-protective antifungal responses. These studies are fundamental to the design and realization of candidal diagnostics and vaccines. Although the commensal nature of *C. albicans* is clearly an obstacle to the idea itself of a classical prophylactic vaccine (i.e. one which protects an uninfected individual), there

are no conceptual lags to the use of a therapeutic vaccine, meaning the capacity of particular candidal components to stimulate specific immunity against the passage from a commensal or latent infectious state to overt disease, and/or fostering a protective immune response during the disease itself.

In the above context, the particular, opportunistic nature of the host-fungus relationship should be recognized. It is unlikely that we will ever discover a highly penetrant virulence factor of the type produced by some virulent bacterial species, or an elimination-oriented protective immunogen. Opportunistic agents, in particular those living commensally with their host, are rather checked by a network of natural and adaptive immune responses whose main function is to keep the agent under control (low numbers, in non-aggressive form, at permitted body sites) rather than eliminating it. The presence of a strongly protective antigen in these microorganisms would be surprising, since a number of contributory immunogens are more likely to induce immunosurveillance against microbial spread and tissue invasion (Cassone *et al.*, 1997a).

Another point to consider in the host-parasite relationship in candidiasis is the wide spectrum of diseases caused and the adaptation of a very versatile organism such as *C. albicans* to different host niches. There are essentially no host tissues which are precluded from colonization or invasion by this fungus, but the underlying host conditions largely dictate the nature of the disease. In particular cases, probably only minimal deviations from the normal physiologic conditions provoke the *Candida* attack. This may happen, for instance, in acute vaginitis (Sobel, 1992), whereas candidal esophagitis (but not systemic invasion) is typical of heavily immunodeficient AIDS patients (Klein *et al.*, 1984), and finally, hepatosplenic

Received April 9, 1998.

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This review is wholeheartedly dedicated to Francesco Pocchiari, Director General of the Istituto Superiore di Sanità (National Health Institute) for many years until his premature death in 1988. Francesco called me to the Istituto in 1980 trusting in the progress of medical mycology and immunology and its increasing impact on public health. I will remain grateful to him. (A. C.)

candidiasis is prevalently seen during recovery from neutropenia in bone-marrow-transplanted subjects (Bodey *et al.*, 1986; Odds, 1988). The fungus is not expected to express a similar level of its low-penetrance virulence factors and major immunogens in such different pathologic conditions. There is clear though preliminary evidence that the factors leading to systemic infection and the expressed antigenic repertoire are different from those involved in cutaneous-mucosal diseases (De Bernardis *et al.*, 1998a).

Thus, the immunological mechanisms underlying anti-*Candida* protection cannot be simply individuated and categorized, as those, for instance, involved in the protection against frankly aggressive, encapsulated or toxigenic bacterial strains. The relationship between a commensal and its host rather resembles that occurring in a latent infection. Humans are

colonized by *C. albicans* during or soon after birth and live commensally with the fungus for the whole lifespan. Under these conditions, the fungus expresses a variety of immunogenic and immunomodulatory components and, in response to them, the host mounts a wide variety of immune responses. As an example, others and we have found that heat-inactivated *Candida* cells are capable of inducing T-cell proliferation, activation of NK cells against tumour target, production of a varied array of cytokines-chemokines, and activation of antimicrobial effects by macrophages and neutrophils (Damer, 1989; Arancia *et al.*, 1995; Cassone, 1995; Cassone *et al.*, 1997b) (fig. 1). There is initial evidence that all these different biologic properties are expressed by different *Candida* molecules (Gomez *et al.*, 1993; Torosantucci *et al.*, 1993; Cassone *et al.*,



Fig. 1. A SEM view of the close interaction between hyphae of *C. albicans* and a human natural killer cell.

For further details, see Arancia *et al.*, 1995. Magnification: $\times 16,000$. (Courtesy of Dr. G. Arancia, Istituto Superiore di Sanità, Rome).

1997b). In conclusion, it remains highly improbable that unique *Candida* immunogens and a limited set of immune responses are responsible for the whole set of protective mechanisms which must be site- or organ-specific and be directed against a possible plethora of distinct immunogens.

Complications, in common with other eukaryotic pathogens, arise from morphologic transition, switching and antigenic variation (reviewed by Cutler, 1991). In *C. albicans*, this is strictly associated with the dimorphic transition, i.e. the ability to convert from a yeast habit of growth to a mycelial one. There are a number of immunodominant polysaccharide epitopes that are abundantly expressed on the cell surface in yeast, but not in mycelial cells (Ponton *et al.*, 1993; Cassone *et al.*, 1988; De Bernardis *et al.*, 1994) (fig. 2). Since yeasts are the commensal forms, most predefined potentially protective immunity recognizes yeast form antigens as targets. During tissue

invasion, mycelial forms outgrow and yeast-targeted immune responses may lose their advocacy for protection because of epitope changes. This is also a way of diverting both humoral and cellular host immune response to dominant but protection-irrelevant targets, as these dominant epitopes are secreted and are no longer present on the fungal cell. Mycelial cells also express their unique antigens (Ponton *et al.*, 1993) and in view of their more aggressive potential, vaccination-useful (vaccine candidates) molecules should be sought for in these rather than in yeast-form cells of *C. albicans*.

Finally, immunogenicity (and protective value) is not an intrinsic or absolute property of a molecule like its molecular weight or chemical composition. As recently emphasized by Deepe (1997), no canonical sequences exist dictating the degree of immunogenicity or its protective nature. Thus, not only may it occur that an antigen is protective in one model of



Fig. 2. Ultrastructural localization of the mAb AF1 epitope (Cassone *et al.*, 1998) and its modulated expression on yeast and mycelial cell of *C. albicans*.

For further details, see De Bernardis *et al.* (1994). Magnification: $\times 36,000$. (Courtesy of Dr. G. Arancia, Istituto Superiore di Sanità, Rome).

disease and not in another (e.g. mucosal versus deep-seated candidiasis), but that the modalities of its administration and the cofactors (adjuvants and others) which, deliberately or not, are associated with the antigenic component exert a critical role.

Candida cells as immunogens

Once the framework in which *Candida* immunogens and potentially protective antigens should be placed is established, it may be easier to say that the most potent *Candida* immunogen is the whole *Candida* cell itself.

Heat-inactivated cells, and, even more so, a living attenuated *Candida* vaccine, induce, in animals not colonized by *C. albicans*, a highly protective response against a virulent fungal challenge both in mucosal infection models and in systemic ones. By the use of a low-virulence, agerminative strain of *C. albicans* (CA-2 or PCA-2), Bistoni *et al.* (1986) first showed that a non-lethal, slowly eliminated primary infection induces a high degree of resistance to a lethal challenge with a highly virulent *Candida* strain. Starting from that seminal paper, Romani and collaborators therefore demonstrated that the protection was linked to the induction of a type 1, T helper cytokine pattern (Romani *et al.*, 1992a,b, 1994; Puccetti *et al.*, 1995; Romani and Howard, 1995). The role of early involvement of natural immunity, in particular IL4, IL12 and IL6-dependent, on the activation of the protective type 1 CD4⁺ T-cell response has also been recently emphasized (Mencacci *et al.*, 1998; Romani *et al.*, 1996b). Induction of protective immunity after resolution of a primary self-limited infection has also been clearly demonstrated by Cassone *et al.* (1995) and Fidel *et al.* (1995) in rodent models of vaginal candidiasis. Interestingly enough, while T cells are clearly required for protection in this latter model (De Bernardis *et al.*, 1997), the ultimate effector mechanisms seem, rather, attributable to antibodies, mostly IgA and IgG, which were produced in the vaginal cavity during the primary self-healing infection and were able to passively transfer the protection from immunized to naive rats (De Bernardis *et al.*, 1997) (see also below).

Before attempting any inference about the different nature of protective responses, the large differences in the experimental models and in the disease itself should be taken into account. In systemic *Candida* infection by parenteral challenge, the fungus disseminates through blood and lymphatics and induces a strong inflammatory response, mostly in the target organs, of which the kidney seems the principal one. The abscessual reaction is typical of this infection and hyphae of *C. albicans* are seen surrounded by polymorphonucleate cells and macrophages at the site of

infection. Inflammation is the mandatory source and mediators of those cytokines such as IL6 and probably also TNF α , whose critical role for induction of the protective axis natural immunity — CMI, is clearly inferred by experiments with gene-knocked-out mice (Romani *et al.*, 1994, 1996b). In contrast, *Candida* vaginitis, either in mouse or rat, is only induced by oestrogen administration, which causes pseudo-oestrus and the associated profound alteration of the vaginal epithelia, with massive keratinization. The infecting *C. albicans* adheres to the keratinocytes, produces hyphae which only rarely invade the subepithelial tissue and no or a moderate degree of inflammation is seen (fig. 3).

Aside from the localization, the expressed *Candida* antigens are not expected to be the same, nor is the immune response in such different situations the same either. Interestingly, vaginal infection is able to induce a clear systemic Th-type 1 response but this is largely uncoupled to protection at the vaginal level, for which site-specific mechanisms, including the activity of T cells with $\gamma\delta$ receptors, have been suggested (Fidel *et al.*, 1996). Whatever the mechanisms of immunoregulation and effector activity, in both models of mucosal and systemic infections, major protective antigens have been actively sought for with only limited success, especially for the latter model.

Some key *Candida* antigens

Table I lists the principal and better defined antigens of *C. albicans* used as immunogens or test antigens to reveal or expand immune responses. The putative yeast-killer toxin receptor (YKTR) is not included here despite its potential for induction of highly protective candidacidal antibodies (Cassone *et al.*, 1997a), because it has not yet been identified (see the contribution by Polonelli and collaborators). The table indicates that very few definite *Candida* antigens have been characterized and only partially assessed for their immunogenicity and protective ability. Mannoproteins (MPs) and mannan polysaccharides have probably been the most extensively studied. MPs have been selected for our studies since they are excellent candidates to play a major role in host-*Candida* relationship. MPs are cell-surface-expressed, abundant, natural glycoconjugates which, by virtue of their rich B- and T-cell antigenic repertoire, may be particularly relevant for the host's adaptive immunity. In addition, their mannan moieties and their degradation products have convincingly been involved in modulatory effects on both adaptive and natural immune responses either by direct interaction with lymphocytes or monocytes or even by binding essential mediators of the immune responses (Trinel *et al.*, 1993; Cassone, 1995; Cassone *et al.*, 1997b).



Fig. 3. Histopathological aspects of experimental candidal vaginitis in rats.
Magnification: $\times 500$.

Table I. Major *C. albicans* constituents investigated as defined antigens.

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- Heat shock proteins (HSP90 and HSP70)
 - Enolase
 - Mannoproteins and mannan
 - MP65
 - mannan adhesin
 - Aspartyl proteinase (Sap2)
 - Soluble mixtures of more or less chemically defined antigenic extracts (e.g. MPF2, etc, etc)
-

For references, see text.

MP immunogenicity

There is a general consensus that the MP constituents of the cell wall of *C. albicans* similar to those of *Saccharomyces cerevisiae*, have a globular protein moiety, to which a main chain of α -1-6 mannan is linked through the characteristic tunicamycin-sensitive, asparaginyl-di-N-acetylchitobiose bridge, and bears a number of secondary mannoside chains through the alkali-labile, beta-

eliminable, O-glycosidic linkage with hydroxy amino acids.

The main chain itself has both acid stable regions and side chains which are attached to the main chain via a phosphodiester linkage, and thus are acid-labile. Since the seminal works by Shibata Suzuki, and their collaborators (see, for instance, Shibata *et al.*, 1995), these polysaccharide regions of MP are relatively well-known and are contributing essential information on antigenicity and serological specificity (in part reviewed by Cutler herein).

What has long remained a mystery is the protein constituents of MP, despite the clear fact that easily detectable CMI responses in humans and experimental animals are mostly directed against the protein moiety of MP (Torosantucci *et al.*, 1993; Gomez *et al.*, 1996). In our laboratory, we addressed this issue, and specific MP components have been extracted, purified and tested for their immunogenicity and protective efficacy both in the mouse systemic infection model and in rat vaginitis. A previously characterized MP-F2 fraction (Torosantucci *et al.*, 1993) has recently been dissected into its major antigenic constituents. In particular, a 65-kDa component was purified by immunoaffinity chromatography with a monoclonal antibody recognizing a

protein moiety of MP-F2 and obtained by a complex, novel approach of MP preparation and immunization (Gomez *et al.*, 1996). This glycosylated protein (MP65) has been shown to be the main, if not the sole, antigenic target of the MP-F2 mixture in experiments of human lymphocyte proliferation *in vitro* (Gomez *et al.*, 1996). Incubation with the MP65-specific mAb4C8 inhibited lymphoproliferation, showing that recognition of the peptide epitope of MP65 was critical for human T-cell activation. MP65-induced T-cell proliferation required MHC class-II-restricted antigen presentation and did not occur in cord blood cell cultures, showing that the molecule acted as a true antigen rather than as a mitogen or superantigen. Amino acid composition showed MP65 to be particularly rich in serine/threonine, which together account for >20% of the total composition, thereby justifying the numerous O-glycosidic linkages present in the molecule. Its sequence shows a large homology with the deduced amino acid sequence of an ORF of a *S. cerevisiae* with unknown function. Based on this homology, the MP65 gene of *C. albicans* (provisionally designated CA-MP65) has been cloned and studied for its expression. Both the recombinant protein of *S. cerevisiae* and that of *C. albicans* have recently been obtained, together with three peptide sequences of the natural MP65 molecule identified as major antigenic epitopes for human T-cell stimulation (Gomez *et al.*, in preparation). The synthesis of these peptides as well as the availability

of the recombinant protein will now allow for an extensive appropriate test of the immunogenicity and protective ability of this molecule in appropriate experimental models.

Meanwhile, some immunological properties have been studied by the use of the MP65-containing MP-F2 fraction and the deproteinated polysaccharide moiety, e.g. mannan. In mice immunized with whole cells of a low-virulence strain of *C. albicans*, thus protected against a challenge with a highly virulent strain of this fungus (see above), MP-F2 was able to elicit a strong DTH response that was accompanied by splenocyte proliferation *in vitro* in the presence of *Candida* antigen. The supernatants of MP-F2-stimulated splenocyte cultures contained IFN γ , but no IL4. IFN γ was produced by CD4⁺ cells *in vitro*, and its level could be increased by adding anti-IL4 or, mostly, anti-IL10 antibodies to the CD4⁺ cell cultures (fig. 4). MP-F2 was also effective for primary mouse immunization, eliciting a vigorous DTH response that could be efficiently reproduced in naive recipients by transfer of CD4⁺ cells from the spleens of MP-F2 immunized mice. The above immunization conferred upon mice a moderate but consistent degree of protection against a virulent *Candida* challenge, both in terms of median survival time and in the number of *Candida* cells in the kidney (Mencacci *et al.*, 1994). However, while DTH induction by MP-F2 was as strong as that induced by immuniza-

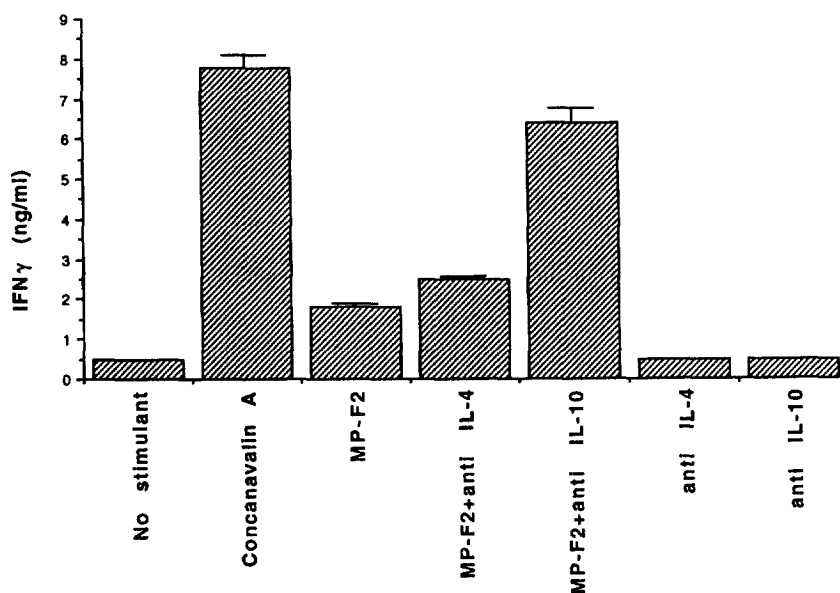


Fig. 4. IFN γ production by CD4⁺ cells, from *Candida*-immunized mice, cocultured with accessory spleen cells, and stimulated *in vitro*, as indicated.

For technical details, see Mencacci *et al.* (1994).

tion with whole *Candida* cells, MP-F2-induced protection was significantly weaker. We therefore concluded that DTH elicitation was only partially related to the protection and that the MP-F2 fraction contained one or more, major immunogens of *C. albicans*, capable of interfering with the balance of CD4⁺ Th1/Th2 responses relevant in the mechanisms of *Candida*-specific protection (Mencacci *et al.*, 1994).

Whether the immunogenicity and the (limited) protection afforded by the immunization with MP-F2 is mediated by MP65 or other immunodominant molecules is not known, but is now being intensely investigated. MPF2 is indeed a complex antigenic fraction with a possible combination of both stimulatory and inhibitory constituents (Domer, 1989). In parallel studies with human polymorphonuclear cells, MP-F2 proved to be a very active stimulator of IL6 production, particularly in HIV⁺ subjects (Torosantucci *et al.*, 1997), that has been considered critical for protection as inferred by experiments with IL6 gene knockout mice (Romani *et al.*, 1996b). However, other possibly contrasting cytokines (e.g. IL10) are also stimulated by the MP-F2 fraction in macrophage cultures, still highlighting the complexity of the anticandidal response and cytokine elicitation by *Candida*-stimulated cells (Cassone *et al.*, 1997). The polysaccharide material contained in the MPF2 fraction, in particular, needs to be investigated more in depth. Aside from its immunomodulatory properties (Domer, 1989) mannan proved to be a protective immunogen in rat vaginitis (De Bernardis *et al.*, 1997; see also below), a finding of particular interest if seen together with the report by Han and Cutler (1995) of a protective mannan adhesin. Both mannan fractions are indeed components of the phosphomannan complex.

Secretory materials and virulence factors

Besides structural constituents, secreted molecules play an important role in the host-parasite relationship. As for other pathogens these molecules may constitute the true antigens naturally eliciting a protective mechanism, or can be exploited to induce that response. Besides mannan and mannoprotein, we have devoted a great deal of research to secretory aspartyl proteinase (Sap) enzymes, encoded by a family of at least nine genes (Hube, 1996), because of their previously supposed, now formally demonstrated (De Bernardis *et al.*, 1998b) role in virulence of *C. albicans* (Cassone *et al.*, 1987; De Bernardis *et al.*, 1995). SAP production is elevated during active mucosal disease, mostly in HIV-infected subjects (De Bernardis *et al.*, 1996), and the enzyme(s) induce the production of antibodies which are protective, possibly by inhibiting the enzymatic degradation of defence-relevant host component. Active immunization with a chemically inactivated, polysaccharide-free Sap preparation has been attempted in the rat vaginitis model. Following intravaginal administration in adjuvant, the Sap vaccine was able to induce anti-Sap antibodies and a degree of protection similar to that conferred upon rats by immunization with mannan (table II; De Bernardis *et al.*, 1997). It is unlikely that the Sap vaccine is similarly useful for protection against systemic disease, as Sap is generally not or is only scantily expressed at physiological pH, and antibody induction has in this case proved to be low or moderate. It has been shown by others that antibodies against immunodominant peptide epitopes of Sap2 were not protective in a model of disseminated candidiasis (Ghadjari *et al.*, 1997).

Among the constituents which are present both in the cytoplasm and in the cell wall and can also be found in the secretory materials, there are two other

Table II. Protection conferred by active immunization with Sap2 and mannan against rat vaginitis by *C. albicans*.

Immunization ^(a)	<i>C. albicans</i> burden in rat vagina CFU/ml × 10 ³ on days:			Ab ^(b)	
	1	7	14	Anti-mannan	Anti-Sap2
None (saline)	>100	>100	81 ± 9	Absent	Absent
Sap2	48 ± 5*	32 ± 4*	11 ± 3*	Absent	Present
Mannan	55 ± 10*	41 ± 7*	31 ± 7*	Present	Absent
Adjuvant	>100	86 ± 12	78 ± 11	Absent	Absent

^(a) None: saline; Sap2, 100 µg of protein in adjuvant weekly for three weeks before challenge. Mannan, 100 µg of polysaccharide, in adjuvant weekly, for three weeks before challenge; adjuvant, IFC standard. Ten rats per group. Administration: intravaginal.

^(b) Ab, antibodies measured in ELISA one month after vaccination and before challenge.

For other technical details, see De Bernardis *et al.*, 1997.

The asterisk denotes a statistically significant difference ($P < 0.01$, Student's two-tailed *t* test) between the asterisked value and those of animals receiving saline or adjuvant.

antigens which have been associated with the immune mechanism of protection, i.e. heat shock proteins (Hsp) and enolase. As mostly expected of Hsp, both constituents were highly immunogenic. However, only for the 47-kDa antigen, a degradation product of Hsp90, there is some convincing evidence that protective antibodies can be elicited during immunization. Mathews and Burnie (1992) discuss this evidence elsewhere. They argued that the presence of this antibody may be critical for the otherwise unexplained rarity of disseminated infection in AIDS patients who are severely affected by mucocutaneous forms of the disease (Mathews *et al.*, 1988). It is now clear that AIDS patients, though affected by a wide range of dysregulated anti-*Candida* responses, nonetheless maintain a high degree of anti-*Candida* activity of neutrophils (Cassone *et al.*, 1997b), which are also important to avoid *Candida* dissemination, but not mucosal invasion (Bodey *et al.*, 1986; Romani and Howard, 1995). A synergy between special antibodies, their opsonizing power and *Candida* killing by neutrophils and other natural immunoeffectors, is possibly involved in antibody-mediated protection but has not been sufficiently demonstrated.

Another clear role for protective antigen is offered by the work of Cutler's group on a special *Candida* mannan adhesin. When encapsulated into a liposome and as such used to vaccinate normal or SCID mice, a protective response was clearly elicited and was attributable to antibodies (Han and Cutler, 1995). As for the protective anti-Sap response (see above), anti-adhesin antibodies were able to passively transfer the protection and some monoclonal antibodies against the polysaccharide moiety of the adhesin conferred passive protection. The adhesin protective epitopes have been identified and discussed elsewhere in this issue.

Highly immunogenic, non-protective antigens

Microbial Hsp70 are also highly immunogenic proteins and because of their transdisease vaccination potential, they are being intensely studied. We have recently cloned and expressed in *E. coli* one member of the Hsp70 gene family of *C. albicans* (CaHsp70) (La Valle *et al.*, 1995). As expected, the recombinant protein was indeed capable of eliciting particularly strong humoral and CMI responses. The pattern of cytokines produced by splenic CD4⁺ cells, cocultured with accessory cells, from CaHsp70-immunized mice was predominantly of type 1. Moreover, *in vivo* administration of CaHsp70 promoted rapid production of IL6, a protective cytokine, by natural immunoeffectors. Nonetheless, the animals were not only non-protected against a lethal *C. albicans* challenge, but were indeed more susceptible to it (Bromuro *et al.*, 1998). These results somewhat parallel those reported by Allendoerfer *et al.* (1996) with animals immunized with *Histoplasma capsulatum* Hsp70, and

challenged with this fungus. Altogether, they provide clear evidence that high immunogenicity and induction of apparently protective cytokine patterns are not necessarily linked to protection, and once again demonstrate that immunological correlates of anticandidal protection cannot be unequivocally determined. Although clear data are lacking, the same conclusion may hold true for enolase (Sundstrom *et al.*, 1994), another immunodominant *C. albicans* antigen which proved to be highly immunogenic but non-protective in models of disseminated *C. albicans* infections (unpublished data).

Antibody versus cell-mediated immunity in anti-candidal protection: is that a useful distinction?

Although an exhaustive discussion of the role of natural and the two arms of adaptive immunity (humoral and cell-mediated) is beyond the scope of this review (see other contributions to this book), some aspects must necessarily be dealt with here.

From the data and considerations summarized above, it should be clear that whenever well defined experimental models and specific responses to definite fungal targets have been used, evidence has been provided that both humoral (antibody) and CMI responses play a critical role in anti-*Candida* protection. From the clinical point of view, there has always been a strong belief that CMI was the predominantly protective arm of the adaptive immunity, and certainly all recent evidence with AIDS strongly favours this assumption. However, it has already been noted how candidiasis in AIDS is limited to some highly disturbing but organ-limited mucocutaneous diseases. Even in these cases, disturbances in antibody isotype regulation and production occur and should be considered in assessing the relative importance of the two arms and possibly of their interaction.

A key point here is acknowledging the redundancy of anticandidal immune responses with respect to the protection. As critically discussed elsewhere (Cassone *et al.*, 1997a; Casadevall *et al.*, 1995, 1998), many of the previous uncertainties about the distinct roles of CMI and Ab responses in candidiasis are probably explainable in terms of inability to precisely define specific immune targets and effector mechanisms. The same, widely accepted paradigm whereby a type 1 T helper response equals protection should be taken to mean a framework of immunoregulatory mechanisms within which precise targets and effectors are still largely unknown. From this point of view, a common definition such as "anti-*Candida* immune response" is exceedingly vague, and speaking of anti-*Candida* serum or CMI may be rather meaningless. From a comparison of the data in the table III,

Table III. A schematic, thought-provoking comparison between antibody- and cell-mediated immunity in anti-*Candida* protection.

Antibody-mediated	Cell-mediated
• Reported to occur both in mucosal and systemic experimental <i>Candida</i> infections • Limited evidence in humans • Transfer of protection (passive) to naive animals demonstrated to be effective in various models • Antigen targets well defined in most cases • Presence of evidence that monoclonal antibodies are protective	• Reported to occur both in mucosal and systemic experimental <i>Candida</i> infections • Strong but indirect evidence in humans • Transfer of protection (adoptive) to naive animals demonstrated to be only rarely effective • Antigen targets not well defined or totally unknown in most cases • Absence of evidence that T-cell clones are protective

one can clearly perceive that the role of CMI is as important as that of humoral immunity, but also that the role of the latter is somewhat strengthened by more precise evidence of fungal targets.

It should also be clearly realized that the above exercise (which is the more important of the two) may be misleading. It is now well established that CMI plays an important role in protective antibody formation. Rats unable to respond to T-dependent antigens did not raise protective anti-mannan or anti-Sap responses in the vagina (De Bernardis *et al.*, 1997) and there is initial evidence that early antibody production and reaction with definite fungal antigens may affect T helper pattern polarization (Vecchiarelli, personal communication). On the other hand, two critical type 1 cytokines (IFN γ and IL2) promote the switching to an IgG2a isotype and comodulate with IL5 IgA synthesis (Coffman *et al.*, 1993). IL6, a well-know promoter of antibody response, is critical for CMI-driven protection in both mucosal and systemic experimental candidiasis, as inferred from work in IL6 gene-deficient mice (Romani *et al.*, 1996a). IL4, the activity of which must be neutralized to achieve protection (Romani *et al.*, 1992), opposes IFN γ in isotype switching and polarizes the response towards harmful or ineffective IgE systems (Murray *et al.*, 1985). Thus, CMI and antibody responses are not mutually exclusive events, as most literature in microbial immunology would appear to suggest. They indeed collaborate, between themselves and with natural immunity, in the defence against *Candida*. We continue to believe that a major issue now in candidiasis (as well as in other fungal diseases) is not the relative importance of antibody and CMI responses, but rather the identification of the components which induce or modulate these responses, the mechanisms by which the two arms of the immune system work and cooperate

(or interfere) and how this knowledge can lead to new tools (e.g. vaccines, immunotherapeutics) for combating fungal disease.

Acknowledgements

The authors are grateful to the secretarial staff of the Laboratory of Bacteriology and Medical Mycology for their continuous support in manuscript and research program preparations. The authors' data reviewed in this paper were from research granted through the National AIDS Program (Contract N° 10/A/U).

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The *Candida albicans* phosphomannan complex in *Candida*-host interactions

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Introduction

The *Candida albicans* cell wall surface makes first contact with the host during establishment of colonization, and this fungal surface is intimately involved in pathogenesis of candidiasis. A chemical understanding of the *Candida* surface is required for vaccine development against disseminated and

mucocutaneous forms of disease. In earlier studies, others and we had shown that cell wall epitopes are variably expressed depending on the growth phase and environmental conditions (Brawner and Cutler, 1986; Brawner and Cutler, 1984; Brawner and Cutler, 1987; Deslauriers *et al.*, 1996; Okawa *et al.*, 1996; Barturen *et al.*, 1995; Cutler and Kanbe, 1994; Poulain *et al.*, 1985; Poulain *et al.*, 1983).