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Elevated Aspartic Proteinase Secretion and Experimental Pathogenicity of *Candida albicans* Isolates from Oral Cavities of Subjects Infected with Human Immunodeficiency Virus

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Isolates of *Candida albicans* from the oral cavities of subjects at different stages of human immunodeficiency virus (HIV) infection or uninfected controls were examined for (i) production of aspartic proteinase(s), a putative virulence-associated factor(s); (ii) the presence in the fungal genome of two major genes (*SAP1* and *SAP2*) of the aspartic proteinase family; and (iii) experimental pathogenicity in a murine model of systemic infection. It was found that the fungal isolates from symptomatic patients secreted, on average, up to eightfold more proteinase than the isolates from uninfected or HIV-infected but asymptomatic subjects. This differential property was stably expressed by the strains even after years of maintenance in stock cultures. Moreover, representative high-proteinase isolates were significantly more pathogenic for mice than low-proteinase isolates of *C. albicans*. The characters high proteinase and increased virulence were not associated with a single molecular type or category identifiable through DNA fingerprinting or pulsed-field electrophoretic karyotype, and both *SAP1* and *SAP2* genes were present in both categories of isolates, on the same respective chromosomes. In conclusion, our data suggest that during HIV infection more-virulent strains or biotypes of *C. albicans* which are identifiable by direct analysis of virulence determinants are selected. It also appears that the biotype switch to increased aspartic proteinase and virulence properties occurs before the HIV-infected subject enters the symptomatic stage and overt AIDS.

Oropharyngeal candidiasis and esophageal candidiasis are frequent opportunistic infections during the course of AIDS (15, 19), but the mechanisms by which they occur are not exactly known. The transition of *Candida albicans*, the most frequent agent of candidiasis (9, 32), from asymptomatic carriage to pathogenic expression on the mucosal tissues matches the characteristic impairment of systemic T-lymphocyte functions occurring in subjects infected by human immunodeficiency virus (HIV), as assessed by defective responses to major, chemically defined *Candida* antigens (34, 41). Whether alteration in *Candida* virulence adds to the underlying immune dysfunction is, however, unknown. In particular, it is debated whether different biotypes of *C. albicans*, which could affect the incidence or severity of candidiasis, infect the oral cavities of HIV-positive (HIV^+) subjects (3–5, 20, 38, 40, 45).

We have previously assessed the prevalence of *Candida* spp. from the oral cavities of subjects at different stages of HIV infection, together with the secretion of aspartic proteinase (AP), a putative virulence factor of *C. albicans*, and the use of fluconazole to prevent recurrences of esophageal candidiasis (1, 9, 11, 37). In the present study, we have expanded these initial observations to another group of subjects and a higher number of *C. albicans* isolates, by studying AP production and the presence in the fungal genome of two genes encoding two AP isoenzymes, *SAP1* and *SAP2* (12, 21). Moreover, we have directly addressed the virulence of oral isolates of the fungus in a systemic murine infection model. Our findings support the notion that the strains of *C. albicans* infecting patients with AIDS produce particularly elevated AP levels and that at least some of them may be more virulent than the isolates colonizing the oral cavity of normal subjects.

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MATERIALS AND METHODS

Subjects. Three hundred forty-six consecutive HIV-infected patients admitted to the Department of Clinical Immunology of the University of Rome "La Sapienza" over the years 1992 to 1994 were examined clinically and microbiologically for the presence of oral candidiasis, which was diagnosed on the basis of the physical signs of oral thrush and fungus isolation (10, 11; see also below). The criteria for HIV infection and staging were those previously adopted (1, 11, 34). The controls were 29 HIV-negative (HIV^-) subjects belonging to risk categories (drug abusers, homosexuals, and sexual partners of HIV^+ subjects) as well as 21 uninfected, healthy subjects who were not in risk groups. All controls were matched for age and sex to HIV^+ subjects.

Isolation and identification of yeasts. A plain, cotton-tipped swab was used to take an oropharyngeal sample from each subject, kept in sterile physiological saline, and transferred to the laboratory, where the sample was seeded on plates of Sabouraud dextrose agar (BBL, Baltimore, Md.) with added chloramphenicol (50 μg/ml) and Mycosel agar.

The plates were incubated for 48 to 72 h at 30°C. All yeasts were identified through morphology on cornmeal agar with 1% Tween 80, the germ tube test in serum, and the API 20C system (BioMerieux). The identification was confirmed by assimilation, fermentation, and serological tests (Iatron, Tokyo, Japan) (28, 42).

AP detection. All *C. albicans* strains were tested for AP secretion in bovine serum albumin (BSA) agar (yeast carbon base [Difco], 1.17%; yeast extract [BBL], 0.01%; BSA [BDH, London, United Kingdom], 0.2%). The medium was adjusted to pH 5, sterilized by filtration, and added to a stock solution of autoclaved (2%) agar. Enzyme activity was scored as follows: − or ± when no visible or a very limited clarification of the agar around the colony was present, 1+ when appreciable proteolysis was observed (1 to 2 mm), and 2+ when agar clarification largely exceeded the margin of the colony (3 to 5 mm).

Proteinase antigen secreted in BSA broth was also detected by enzyme-linked immunosorbent assay (ELISA) (10, 11). Briefly, the microrganism was precultured in YEPD broth (1% yeast extract, 2% peptone, 2% glucose), the cultures

were centrifuged, and the cellular pellet was washed and incubated (10^6 cells in 25 ml of YEPD broth plus 0.2% BSA) at 30°C with slight agitation. After 40 h of incubation, the samples were centrifuged at 3,400 × *g* for 10 min, and the supernatants were treated with 0.5 ml of 50% trichloroacetic acid, incubated for 30 min in ice, and centrifuged at 3,400 × *g* for 10 min. The pellets were washed twice with 95% ethanol, dissolved in 1% sodium dodecyl sulfate (SDS), diluted in 0.2 M sodium carbonate buffer (pH 9.6), and applied to the MicroTest plates.

Purified rabbit antiproteinase serum (10) was added at a 1:100 dilution and incubated for 2 h at 37°C. The second antibody was phosphatase-conjugated goat anti-rabbit immunoglobulin G (1:1,000 dilution; Sigma, St. Louis, Mo.), which was added and incubated for 2 h at 37°C. The reaction was detected with the phosphatase substrate nitrophenol phosphate (Sigma). The amount of proteinase was calculated from a standard curve (1 to 110 ng) determined with the purified proteinase as a coating antigen. The plates were read with a Titer K Multiscan (Skatron, Oslo, Norway) at 405 μm blanked against air. The specificity of the ELISA for proteinase detection was confirmed by Western blotting (immunoblotting) (10).

AP was purified from *C. albicans* ATCC 10261 grown in YBD medium (yeast extract, 0.2%; albumin [BSA], 0.2%; and glucose, 2%) as previously described (10, 36). The purified enzyme (specific activity, 10 to 14.5 U/mg of protein) gave a single band with a molecular weight of 43,000 in SDS-polyacrylamide gel electrophoresis and an identical, single band on Western blotting with polyclonal antiserum and a monoclonal antiproteinase antibody (36; other data unpublished).

Electrophoretic karyotype and Southern blotting. The karyotype of *C. albicans* was determined by transverse, alternating-field gel electrophoresis (7). Briefly, the fungal cells were grown to the stationary phase in YEPD broth and then washed in 1.2 M sorbitol solution containing 20 mM EDTA, pH 8.0. The pellet was resuspended to a cell concentration of 10^9/ml in a solution of EDTA-sorbitol containing 14 mM β-mercaptoethanol and incubated for 15 min at 37°C. The samples were embedded in 1% low-melting-point agar (Bio-Rad, Hercules, Calif.) for spheroplast lysis and preparation of the chromosomal DNA (7, 44). Chromosome separation was achieved through periodic changes of polarity by four stages of 24 h each at 100 V.

After the electrophoresis, the gel was stained with ethidium bromide (0.5 μg/ml; 30 min), destained, and photographed with Polaroid T57 film. For chromosome hybridizations, the gels were treated with 0.25 M HCl for 5 min, transferred onto nylon membranes (Bio-Rad), and hybridized with a ^{32}P-labelled random primed *SAP1* or *SAP2* probes (12, 21). Hybridization and initial washing steps were carried out as described elsewhere (21). The final washing was performed in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 70°C for 30 min. Filters were exposed on 3M (St. Paul, Minn.) XDA plus films with 3M Trimax screens at −80°C.

Experimental pathogenicity. For experimental infection, the yeast inoculum was prepared from cells grown in Winge broth (0.2% glucose, 0.3% yeast extract) for 48 h at 28°C on a reciprocal shaker before being washed, counted in a hemocytometer, and diluted to an appropriate cell number in physiological saline. Then graded inocula of each *C. albicans* isolate were used to produce systemic (by the intravenous route) infections in 18- to 21-g male CD2 F_1 mice (Charles River, Calco, Italy). Dead animals were examined at necroscopy, and target organs (kidneys and heart) were cultured for the presence of invading *C. albicans.*

RESULTS

Prevalence of *C. albicans* in HIV$^-$ and HIV$^+$ subjects. *C. albicans* was isolated from the oral cavities of 140 of 346 HIV$^+$ patients and from 19 of the 50 HIV$^-$ subjects, either not belonging or belonging to risk categories for HIV infection. Among HIV$^+$ subjects, 17 isolates were from 44 Centers for Disease Control and Prevention (CDC) stage II subjects, 82 were from 208 CDC stage III subjects, and 41 were from the 94 CDC stage IV (AIDS) patients. The mean ± standard deviation CD4$^+$ cell numbers were 514 ± 89.7, 491 ± 38.4, and 281 ± 40.5 for subjects at stages II, III, and IV, respectively. The isolates were from oral disease in 0, 4.8, and 85.2% of the subjects at the above CDC stages, respectively.

Proteinase (AP) secretion. AP secretion was tested in 135 of 159 isolates of *C. albicans* from the different subjects. The enzyme was assessed both by expression of proteolytic activity on BSA agar and by ELISA detection in BSA broth (confirmed by Western blotting [10]). At the stationary phase of growth, all cultures reached comparable amounts of dry mass per milliliter of culture (data not shown). All isolates showed the presence of proteinase enzyme activity on BSA medium (score, 1+ or 2+), and all secreted a measurable quantity of proteinase antigen in ELISA.

TABLE 1. Proteinase secretion by *C. albicans* isolates from subjects at different stages of HIV infection

Subject group (CDC stage)	No. of strains	AP production by:	
		ELISA (ng/ml [mean ± SE])	BSA hydrolysis (no. scoring 2+/total no. [%])
HIV$^-$	19	34 ± 6	3/19 (16)
HIV$^+$ (II)	13	74 ± 39	2/13 (15)
HIV$^+$ (III)	70	262 ± 45[a]	31/70 (44)[b]
HIV$^+$ (IV)	33	228 ± 59[a]	14/33 (42)[b]

[a] $P < 0.01$ by Student's *t* test, comparing with HIV$^+$ CDC stage II or HIV$^-$ subjects.

[b] $P < 0.01$ by the χ^2 method, comparing with HIV$^+$ CDC stage II or HIV$^-$ subjects.

However, as shown in Table 1, the isolates from HIV-infected patients at CDC stages III and IV secreted much more enzyme than the isolates from HIV$^-$ and HIV$^+$ but asymptomatic subjects, as coherently indicated by both methods used to assess AP production. On average, the strains from AIDS subjects (CDC stage IV) produced about eightfold more AP (evaluated as antigen in ELISA) than the isolates from the HIV$^-$ group.

To examine whether the elevated AP production by the isolates of *C. albicans* from symptomatic HIV$^+$ subjects was a stable characteristic, five strains with high proteinase production (hereafter designated HAP strains or biotypes) from patients with AIDS were subcultured several times in non-proteinase-inducing medium. Three consecutive determinations of proteinase secretion were performed with each isolate (with the exception of isolate 90, which was subjected to two determinations) over a period of 3 years. The results of these assays (Table 2) show that elevated AP production was rather stably maintained by the isolates from patients with AIDS during the repeated subcultures, as compared to the enzyme production by similarly subcultured isolates of *C. albicans* with low proteinase production (hereafter designated LAP strains or biotypes) from HIV$^-$ subjects.

AP genes in the HAP and LAP isolates of *C. albicans* from HIV$^-$ and HIV$^+$ subjects. To examine whether there were any gross differences in the presence or chromosomal localization of two genes of the AP family (*SAP1* and *SAP2*) between HAP and LAP isolates of *C. albicans*, genomic DNA was digested with various restriction endonucleases (*Pst*I, *Bgl*II, *Bam*HI, and *Eco*RI) and hybridized with the *SAP1* and *SAP2* clones (12, 21) under both high- and low-stringency conditions. These experiments were performed with 10 HAP isolates and an equal number of LAP isolates. As shown for representative strains in Fig. 1, identical hybridization patterns were obtained with *Eco*RI DNA digests, hybridized at high stringency, in all isolates. Namely, *SAP1* hybridized to a couple of bands at 2.3 and 1.1 kb while *SAP2* hybridized to a band of 9.5 kb.

In Southern blotting to chromosome-sized bands after pulsed-field electrophoresis, the *SAP2* gene mapped to the largest chromosomal band (chromosome 1 or R) (22), whereas the *SAP1* gene mapped to a band of about 1.0 Mb, corresponding to the position of chromosome 6 in most karyotypes (Fig. 2). When hybridization was performed under low-stringency conditions with the *SAP2* gene, three bands were detected, two corresponding to the chromosomes hybridized under high-stringency conditions with *SAP1* and *SAP2* and the third at the apparent position of chromosome 4 (around 1.75 Mb) (data

TABLE 2. Consecutive determinations of AP secretion by isolates of *C. albicans* from HIV$^-$ subjects belonging to risk groups or patients with AIDS

Source group	Isolate no.	BSA score[a]			AP production by ELISA (ng/ml)[b]		
		1st	2nd	3rd	1st	2nd	3rd
HIV$^-$ subjects in risk group	167	1+	1+	1+	14	18	13
	170	1+	1+	1+	15	13	14
	171	1+	1+	1+	15	13	12
	177	1+	1+	1+	14	25	14
	180	1+	1+	1+	14	13	12
	Mean ± SE				14.3 ± 0.3	16 ± 2.4	12.9 ± 0.4
AIDS patients	11	2+	2+	2+	1,135	1,200	937
	13	2+	2+	2+	987	350	512
	16	1+	1+	1+	510	132	480
	85	1+	2+	2+	1,125	1,075	487
	90	1+	1+	1+	540	360	ND[c]
	Mean ± SE				859 ± 139	623 ± 214	604 ± 111

[a] On the first determination.
[b] $P \ll 0.01$, comparing, in each determination, the mean ± standard error AP production by the isolates from HIV$^-$ and HIV$^+$ subjects.
[c] ND, not determined.

not shown). This pattern was consistently observed in all strains examined, despite the differences shown by these strains in the DNA digestion or electrophoretic karyotype patterns, thus demonstrating that distribution and chromosomal localization of *SAP1* and *SAP2* genes in these clinical isolates of *C. albicans* were identical to those seen in laboratory strains of the fungus (10, 21).

Experimental pathogenicity. Having established that high or low proteinase production is a rather stable characteristic of the isolates of *C. albicans* from HIV$^+$ and HIV$^-$ subjects, four HAP isolates and an equal number of LAP isolates were selected to experimentally infect, by the intravenous route, different groups of mice with graded inocula of fungal cells. The criteria for selection were the demonstrated stable proteinase production (see above) and the identical electrophoretic karyotype of all isolates, in an attempt to ensure the best possible genetic relatedness among the isolates tested.

Table 3 shows that the four HAP strains of *C. albicans* from patients with AIDS were, as a whole, more pathogenic than the LAP isolates from HIV$^-$ subjects. In particular, the former caused an appreciable lethality even at a moderate inoculum size (10^4 cells) that was nonlethal with the isolates from the latter subjects. At 10^5 *C. albicans* cells, the isolates from HIV$^-$ subjects killed 25% of the animals, with a median survival time (MST) of >30 days, whereas the isolates from patients with AIDS killed 80% of the mice, with an average MST of less than

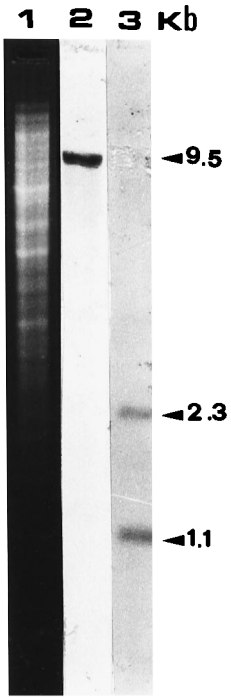

FIG. 1. Southern blots of genomic DNA with *SAP1* and *SAP2* genes. Genomic DNA of an isolate (isolate 11) of *C. albicans* from a patient with AIDS was digested with *Eco*RI (lane 1) and, after electrophoresis on an agarose gel, blotted to a nylon membrane and hybridized at high stringency with *SAP2* (lane 2) or *SAP1* (lane 3) probes. Kb, kilobases. The arrowheads point to the hybridization bands.

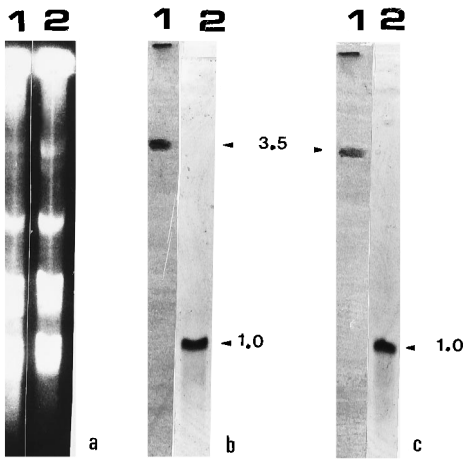

FIG. 2. Southern blotting of chromosomal DNA of *C. albicans* with *SAP1* and *SAP2* genes. (a) DNAs of two isolates of *C. albicans*, one (LAP) from an HIV$^-$ subject (lane 1) and one (HAP) from a patient with AIDS (lane 2) were subjected to pulsed-field gel electrophoresis to separate chromosome-sized bands. (b and c) After transfer to nylon membranes, the blots of the chromosomal DNA from LAP (b) or HAP (c) isolates were hybridized under high-stringency conditions with *SAP2* or *SAP1* probes (lanes 1 and 2, respectively). Arrowheads indicate molecular sizes (in megabases) of hybridized chromosomal bands.

TABLE 3. Mouse lethality after intravenous infection (of five mice per strain) with strains of *C. albicans* from patients with AIDS or HIV$^-$ subjects

Subject group	Isolate no.	Result with inoculum of:					
		10^3 cells		10^4 cells		10^5 cells	
		MST (days)	No. of mice dead	MST (days)	No. of mice dead[a]	MST (days)	No. of mice dead[b]
AIDS patients	11	—[c]	—	>30	1	4	4
	13	>30	1	18	3	2	4
	85	>30	1	>30	1	21	3
	16	>30	0	>30	2	12	5
HIV$^-$	180	>30	0	>30	0	>30	1
	177	>30	0	>30	0	>30	1
	167	>30	0	>30	0	>30	2
	170	>30	0	>30	0	>30	1

[a] $P < 0.05$ (χ^2 method, both tails) by cumulating the mortality of all mice inoculated with isolates from patients with AIDS and comparing with the cumulated mortality following the challenge with isolates from HIV$^-$ subjects.
[b] $P < 0.01$ (one tail, as in footnote *a*).
[c] —, not determined.

10 days. All isolates were comparably lethal when the inoculum was $\geq 10^6$ *Candida* cells. Both the cumulated overall mortality and average MST significantly differed between the two groups of isolates ($P < 0.01$ by the χ^2 and Mann-Whitney U tests, respectively). In all cases, the lethality was attributable to deep-seated *C. albicans* infection, as shown by necroscopic examination that was routinely performed in all dead animals and showed abundant proliferation of fungal cells in two target organs (such as kidney and heart).

DISCUSSION

Various forms of mucosal candidiasis are common in HIV-infected subjects (15, 17, 19). Some authors have suggested a sort of hierarchical sequence of vaginal, oropharyngeal, and esophageal disease, depending on CD4$^+$ cell number and the associated immunodepression level (1, 17). As growth and morphogenesis of *C. albicans* on the mucosa appear to be under the control of the cell-mediated immune response (6, 35), the first functional and then numerical deletion of CD4$^+$ cells that is so typical of HIV infection (13) may explain frequency and localization of such infections in patients with AIDS. Related to this, HIV-infected patients have a major defect in the in vitro lymphocyte response to an immunodominant, protective mannoprotein antigen of *C. albicans* (26, 34, 41).

However, *C. albicans* has a degree of virulence (9), and mucosal candidiasis (oral and vaginal) is also a frequent disease even in nonimmunodepressed subjects. Moreover, *C. albicans*, i.e., the most pathogenic species of *Candida*, is almost invariably isolated from patients with AIDS, whereas many other less virulent *Candida* species may colonize the mucosa of healthy people (29, 32). Finally, oro-esophageal candidiasis of patients with AIDS is difficult to eradicate, and recurrences of the disease are frequent even with appropriate antifungal treatments (2, 5). This may suggest the selection of strains with increased potential for adaptation to the mucosal environment that could coexist with, or add to, progressively impaired host local defenses in determining extensive mucosal colonization and disease.

A number of authors have indirectly addressed the above problems by looking at the different biotypes of *C. albicans* (in HIV- or non-HIV-infected subjects), with contrasting results (3, 4, 20, 38, 40, 45). On the basis of preliminary data (11), we have more directly addressed the issue of virulence differences among the isolates of *C. albicans* from AIDS patients and those from HIV$^-$ or asymptomatic HIV$^+$ subjects. We focused particularly on AP(s), which among the virulence-associated traits of *C. albicans* (9) is the best characterized and the one with more convincing, though not yet definitive, evidence for involvement in the pathogenesis of candidiasis (9, 25, 36, 37). Many experimental and clinical studies do indeed suggest that elevated production of this enzyme is a very relevant aspect of *C. albicans* adaptation to host tissues and virulence (37).

The evaluation of AP secretion by our *C. albicans* isolates was coupled to the determination of mouse lethality by selected fungal isolates sharing the same electrophoretic karyotype but largely differing in AP production in vitro. The selection of only those isolates belonging to the same electrophoretic karyotype was taken to ensure a degree of genetic relatedness among the strains, so as to diminish the bias due to other unknown strain differences. Karyotype determination is one of the most selective and resolving biotyping characters of *C. albicans*, although karyotype identity does not guarantee clonality (23, 27, 43). Importantly, the selected strains were identical in all classical characters of *C. albicans* species, and they did not show any feature, either biochemical or in the electrophoretic karyotype, that could classify them as atypical strains (3, 24).

Our results demonstrate that *C. albicans* strains with particularly high proteinase production (HAP strains) are significantly more likely to be isolated from HIV$^+$ symptomatic patients and patients with AIDS than from HIV$^-$ or even HIV$^+$ but asymptomatic subjects. We also show here that representative HAP isolates are significantly more virulent for the normal mouse than are representative LAP strains. The difference in AP production between some isolates from patients with AIDS and some of those from HIV$^-$ subjects could reach 1 or even 2 orders of magnitude. This difference was apparently maintained on repeated passages on artificial media which could not induce proteinase expression. Thus, when selected in vivo, HAP production seems to be a stable genetic trait that could be related to acquisition of differential gene expression. Although direct gene expression through mRNA analysis has not been studied here, differences in actual enzyme production were clear-cut, suggesting either a different level of mRNA expression or other regulatory mechanisms leading to the AP synthesis and secretion. In this context, phenotypic switching could be involved, in view of the demonstration that *SAP1* gene

transcription is under the regulation of the switching (reviewed in reference 39). Whether and to what extent this is true for all natural isolates of *C. albicans* which may vary in many other phenotypic characteristics are not known. Moreover, there are conflicting results concerning *SAP1* expression in vivo (12, 16).

Finally, there are other *SAP* genes (*SAP3* to *SAP7*) present in *C. albicans* that were not probed here (21, 30, 31). It is possible that the strong selection-induction conditions of in vivo growth of *C. albicans* in subjects with AIDS favor the expression of one or several *SAP* genes which are not usually expressed in vitro or under commensal but noninfecting conditions. This possibility could not be discriminated here because the antiproteinase serum used for enzyme immunoassay cross-reacts with several AP isoenzymes which have substantial sequence homology (30, 31). Overall, further investigations are required to clarify all the complex issues mentioned above.

Whatever the mechanisms and AP isoenzymes involved are, our data suggest that the possession or expression of increased AP activity by *C. albicans* isolates from patients with AIDS confers more virulence upon *C. albicans* under natural conditions. Although the mouse infection experiments could not be performed with more than a few isolates of each category, there was a much more pronounced and accelerated lethality in mice which were experimentally infected by the HAP strains than in those infected by the LAP strains. Our data are consistent with past observations that mutagenized, AP-deficient strains of *C. albicans* are less pathogenic than parent strains for systemically infected mice (25, 36, 37). They also demonstrate that, at least for some strains of the fungus, selection for increased AP production under natural conditions could lead to increased virulence. Experiments with a rat model of *Candida* vaginitis are in progress to further substantiate this issue, also in consideration of the well-established association between mucosal candidiasis and proteinase expression (8, 10, 12).

The isolation of HAP strains from symptomatic HIV^+ subjects who do not yet have AIDS suggests that selection of the HAP level is associated with a phase of oral colonization that precedes oral disease (11, 15). In fact, less than 5% of the isolates from patients at CDC stage III of HIV infection were associated with oral disease, while the large majority of them were only colonizing the oral cavity. In this context, the data of Schmid et al. (38) concerning *C. albicans* biotype replacement in the oral cavity early in the manifestation of AIDS are consistent with our findings, as also are the recent data of McCullough et al. (24) about the isolation from HIV-infected patients of a distinct subgroup of *C. albicans* endowed with increased adhesion to buccal epithelial cells and proteinase production as well. However, there was no evidence that our highly proteolytic isolates of *C. albicans* formed a distinct subgroup. As a matter of fact, elevated AP production was largely shared among most of the isolates from symptomatic HIV^+ subjects, independently of their electrophoretic karyotype class. It should be stressed here that only longitudinal studies in the same subjects during progression of HIV infection could lead to unequivocal conclusions about the presence and replacement of typical or atypical strains of *C. albicans* in the oral cavity of HIV-infected subjects during the course of HIV infection.

Whatever the mechanisms are, our results favor the idea that more-virulent strains of *C. albicans* may be isolated from AIDS patients and that this characteristic may be bound to an elevated production of a putative virulence enzyme and could be stably maintained. Together with increased resistance to well-tolerated azole drugs or possibly other virulence-associated properties like adherence (14, 24, 29, 33), this means that more aggressive strains of this opportunistic human commensal may be present in the setting of patients with AIDS. Moreover, since APs of *C. albicans* are produced in vivo (9, 12) and are able to degrade a number of important, defensive host proteins such as immunoglobulins and complement (18, 37), the presence of HAP strains of this fungus could theoretically aggravate the condition of the immunodepressed host.

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