Correlation between germ tube production, phospholipase activity and serotype distribution in *Candida albicans*

Valerio Vidotto[1], Cristiane Yumi Koga-Ito[1], Rosangela Milano[2], Barbara Fianchino[2] and José Pontón[3]

[1]Laboratorio Micologia Medica, Dipartimento Discipline Medico-Chirurgiche, Sez. Malattie Infettive, Università di Torino, Italia; [2]Laboratorio Microbiologia, Ospedale Amedeo di Savoia, ASL 3; [3]Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Odontología, Universidad del País Vasco, Bilbao, España

Summary

One-hundred and thirteen *Candida albicans* strains isolated from patients infected by the human immunodeficiency virus (HIV) and twenty five from HIV-negative individuals were studied. The *C. albicans* strains isolated from different sites of the body were tested for germ-tube (GT), phospholipase production and serotype. The results obtained indicate that the serotype A was predominant in all the groups except for the vaginal strains. No correlation was observed between phospholipase activity and serotype distribution. Germ tube (GT) production was higher among the serotype B strains. A positive correlation between GT induction and phospholipase activity was observed only for the isolates from the oral cavity. It is possible that the correlation between phospholipase activity and high GT production in *C. albicans* strains can facilitate the penetration through the mucosa.

Key words *Candida albicans*, Germ tube, Phospholipase, Serology, Virulence

Correlación entre producción de tubo germinal, actividad fosfolipasa y distribución de serotipos en *Candida albicans*

Se ha estudiado la producción de tubo germinal (TG), la producción de fosfolipasa y la distribución de serotipos en cepas de *Candida albicans* aisladas de 88 pacientes con sida y 25 controles sin infección por el VIH. El serotipo A fue predominante en todos los grupos de aislamiento de *C. albicans* considerados, excepto en los aislamientos vaginales. No se encontró ninguna correlación entre la produción de fosfolipasa de las cepas y su distribución serotípica. En las cepas aisladas en la cavidad oral se observó una correlación positiva entre la produción de fosfolipasa y la produción de TG. Según los resultados obtenidos es posible que una elevada actividad fosfolipasa de cepas de *C. albicans* y una buena produción de TG puedan facilitar su penetración en la mucosa.

Candida albicans, Tubo germinal, Fosfolipasa, Serología, Virulencia

Candida albicans is the most frequently isolated yeast in immunocompromised patients, particularly in those with AIDS [1]. Two serotypes, A and B, have been identified in *C. albicans* and the identification of these serotypes have been used for epidemiological purposes [2,3].

The serotype A is the most frequently isolated in patients with candidiasis, although the latest epidemiological studies have reported an increase in isolation of serotype B in HIV positive patients. Among the different virulence factors described in *C. albicans*, such as germ tube (GT) production, growth at 37°C, protease and phospholipase production, the latter seems to be more related to *C. albicans* pathogenicity and virulence [1,4-13].

For epidemiological purposes it would be interesting to find a relationship between GT production, phospholipase activity and different *C. albicans* serotypes distribution. Knowledge of this distribution among different clinical isolates could also provide more interesting information on *C. albicans* pathogenicity and epidemiology.

Dirección para correspondencia:
Dr. Valerio Vidotto
Dipartimento Discipline Medico-Chirurgiche,
Sez. Malattie Infettive, Università di Torino,
Laboratorio Micologia Medica, Corso Svizzera 164,
10149, Torino, Italia
Tel: +39 11 439 3866; Fax: +39 11 740 829
E-mail: vvidott@tin.it

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MATERIALS AND METHODS

Eighty-eight *C. albicans* strains isolated from patients infected by the human immunodeficiency virus (HIV) and twenty five from HIV-negative individuals were studied. The strains were identified by the API 20 C AUX kit (Biomérieux, Italia). The *C. albicans* strains from HIV-positive patients were isolated from oral cavity, digestive, respiratory, urinary tracts, vagina, nails and skin. The HIV negative-specimens were from the oral cavity. The HIV-negative individuals were patients with evident clinically lesions. All the strains were transferred onto fresh malt agar slants and incubated at 37°C for 3 days.

Phospholipase production was performed according to Polak [7]. The test medium consisted of malt agar containing 1M sodium chloride, 0.005M calcium chloride and 2% egg yolk. Each strain was inoculated in triplicate. Phospholipase activity (Pz) was measured in terms of the ratio of the diameter of colony plus zone of precipitation, according to the method described by Price *et al.* [14].

To study the GT induction, bovine adult serum (Sigma, USA) and minimal synthetic medium were used in order to obtain GT production from the strains after their incubation at 37°C for 4 h. The composition of the MSM [15] was the following in g/l: glucose 0.5; KH_2PO_4 2; $CaCl_2$ 0.05M, $MgSO_4$ 0.05; $(NH_4)_2SO_4$ 0.5. The final pH was adjusted to 6.75 by the addition of 2M KOH.

Serotyping was performed by indirect immunofluorescence according to Barturen *et al.* [16]. Each strain was inoculated at the concentration of 1 x 10^6 cell/ml into 1 ml of physiologic saline solution (0.9%). Then, 10 ml of this cellular suspension were inoculated into immunofluorescence slide wells. After fixation of the slides, 10 ml of the monoclonal antibody B9E (AMB9E) [3] were added to the wells and the slides were incubated for 30 min at 37°C. After this period of incubation, the slides were washed with physiologic saline solution (0.9%) and the 10 ml of anti-mouse IgM (µ-chain specific) conjugated to fluorescein isothiocyanate (Sigma) diluted at 1:100 was added to the wells. After an incubation of 30 min at 37°C the slides were observed by fluorescent equipped microscope. *C. albicans* strains NCPF 3153 (serotype A) and NCPF 3156 (serotype B) were used as controls, by fluorescence microscopy equipped with filters for fluoresceine.

GT percentage production and phospholipase activity (Pz) among the different *C. albicans* isolates strains was performed in triplicate and the results are reported in Table1.

The statistical Student t test was performed to check a significant difference between the Pz phospholipase average values.

RESULTS

The strains isolated from the oral cavity presented the greatest phospholipase activity expressed as Pz values (average Pz = 0.642 ± 0.145) while the lowest was observed in the strains isolated from the urinary tract (average Pz = 0.854 ± 0.164) (Table 1). The strains isolated from digestive and respiratory tracts showed similar Pz average values with no statistically significant differences observed among them (p=0.548).

The vaginal strains showed the greatest GT percentage production after 2 h in serum as well as in MSM (Table 1). The strains isolated from the oral cavity and respiratory tract showed the greatest percentage production of GT in serum after 4 h (93% and 85%, respectively). Strains from the digestive tract presented lower GT percentage production in serum in relation to the other groups (65%) (Table 1). On the other hand, in MSM media, the vaginal strains and those isolated from the respiratory tract showed the greatest percentage production of GT after 4 h (48% and 45%, respectively).The strains isolated from nails and skin presented a lower GT production in MSM (11%). All the strains duplicated or tripled their GT percentage production between the 2nd and 4th hour both in serum and in MSM (Table 1).

Table 1. Phospholipase activity (Pz) and percentage of germ tube production in serum and in MSM of the *C. albicans* strains.

Origin	Number	Phospholipase (Pz) (average ± SD)	% Germ Tube			
			Serum		MSM	
			2h	4h	2h	4h
Oral cavity	32	0,642 ± 0,145	37	93	12	27
Digestive tract	21	0,780 ± 0,190	23	65	8	33
Respiratory tract	23	0,788 ± 0,145	34	85	14	45
Urinary tract	7	0,854 ± 0,164	30	85	8	19
Vagina	24	0,726 ± 0,128	42	83	27	48
Nails and skin	6	0,758 ± 0,194	28	67	3	11

The serotype A was prevalent in all the isolated strains examined, except for the vaginal strains. The serotype A isolates among these groups ranged between 71% and 100% (Table 2). On the contrary, the serotype B was not infrequently observed among the isolates. The percentages of *C. albicans* serotype B ranged between 0% and 50%. The lowest percentage of this serotype was observed for the strains isolated from nails and skin and respiratory tract (0% and 8.7%, respectively) (Table 2).

Table 2. Serotypes A and B distribution in the *C. albicans* strains studied.

Origin	HIV		Serotypes			
			A		B	
	Pos	Neg	n	(%)	n	(%)
Oral cavity	25	-	20	(80)	5	(20)
Oral cavity	-	7	5	(71.4)	2	(28.6)
Digestive tract	21	-	19	(90.48)	2	(9.52)
Respiratory tract	23	-	21	(91.3)	2	(8.7)
Urinary tract	7	-	5	(71,43)	2	(28.57)
Vagina	24	-	12	(50)	12	(50)
Nails and skin	6	-	6	(100)	0	(0)

No correlation between GT production and phospholipase activity was observed among the strains isolated from the digestive and respiratory tract, vagina, nails and skin (Table 1). An inverse correlation was observed among the urinary tract strains, that presented a high GT production after 4 h in serum (85%) and a low phospholipase activity (high Pz average = 0.854 ± 0.164) (Table 1). The strains isolated from the oral cavity presented the higher phospholipase activity (0.642 ± 0.145) and a high GT production in serum (93%) (Table 1).

The percentage production of GT was slightly higher among serotype B strains (83.1%) in relation to the serotype A strains (77.69%). No correlation was observed between phospholipase activity and serotype distribution. Phospholipase activity was similar for the serotype A strains (average Pz = 0.747 ± 0.165) and serotype B strains (average Pz = 0.738 ± 0.139).

DISCUSSION

Most published studies have reported a predominance of serotype A in healthy individuals and patients with candidiasis [3,17-19]. However, there is some recent evidence showing an increase in the isolation of serotype B in immunocompromised patients, particularly in HIV positive patients, where serotype B was prevalently isolated from vagina and oropharynx [3, 20]. In the present study, all the patients examined, both HIV-negative and HIV-positive subjects, showed a clear predominance of the serotype A in all the groups of *C. albicans* studied, except for the vaginal group that presented the same number of isolates of the serotype A and B. According to the literature [20], the high incidence of serotype B in HIV patients has also been confirmed.

The virulence in *C. albicans* is believed to be due not to a single virulence factor but to the combination of several factors [21]. Evidence of association between potential virulence factors has been recently described. Gale *et al.* [22] have linked adhesion to hyphal growth and pathogenicity to the gene *INT1*. In the present study, we found a correlation between high phospholipase activity and GT production in the oral isolates examined which suggests that these capabilities are needed for the colonization and infection of the oral cavity by *C. albicans*. It is possible that the high phospholipase activity and GT production can facilitate the penetration through the mucosa, since the phospholipase activity is particularly concentrated at the tips of the hyphae [23]. On the other hand, if *C. albicans* isolates from sites other than the oral cavity, no positive correlation was observed between GT induction and phospholipase activity. Since hyphae are commonly observed in infected tissues, it is possible that phospholipases are less important in tissues other than the oral cavity. Since hyphae are often observed during invasion of tissue, it may be speculated that phospholipases are less important in tissue other than oral mucosa. In fact it has been shown that phospholipase production in *C. albicans* is limited to acidic growth conditions which may be attained in the oral milieu [24].

Although these experiments were carried out *in vitro*, it would be worthwhile to carry them out in living organisms, because the results could be different.

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