Indirect immunofluorescence assay for antibody to germ tube of *Candida albicans*—a new diagnostic test

Y. M. HO¹, M. H. NG¹, C. H. TEOH-CHAN¹, P. C. K. YUE², AND C. T. HUANG¹

From the Departments of Microbiology¹ and Surgery², University of Hong Kong, Hong Kong

SYNOPSIS Indirect immunofluorescent and agglutination assay were used to study the anti-*Candida albicans* reactivities in the serum of 13 normal subjects and 14 patients infected with *C. albicans*. A significant increase in anti-*C. albicans* seroreactivity was observed during infection with this organism but the increase in the anti-germ tube immunofluorescence titre was the more marked. It is evident that the anti-germ tube immunofluorescence assay is more discriminatory for *C. albicans* infection than the conventional agglutination assay.

Candida albicans has two growth phases. As commensal, it exists predominantly as blastospores; but, during active infection, it changes to the mycelial form (Gresham and Burns, 1960; Kozinn and Taschdjian, 1962). It has been shown that the mycelium and the blastospores are antigenically distinctive (Evans *et al*, 1973). Based on these two basic facts, the present study has been evolved with the development of an indirect immunofluorescence (IF) assay for the determination of antibodies to the mycelium and the application of this as a diagnostic method for *Candida* infection.

Material and methods

SERA

The sera from 27 subjects were studied. These consisted of 14 patients who had proven candidiasis with repeated culture, and 13 normal individuals serving as controls.

CULTURE METHODS

Blastospores were obtained by growing *C. albicans* (group A) on Sabouraud agar at 37°C for 48 hours. The organisms were harvested in and washed with saline. They were resuspended in saline, heated at 65°C for 6½ hours, and stored at 4°C until used (Hasenclever and Mitchell, 1960; 1961).

Culture medium consisting of swine serum diluted 1 in 2.5 v/v with a solution containing cysteine (50

Fig 1 Immunofluorescence staining of smear of acetone fixed germinating cells of *C. albicans* (group A) showing uniform staining at low serum dilution of both normal subjects and patients with candidiasis.

Fig 2a

Fig 2b

Fig 2 At high serum dilution: (a) partial immunofluorescence staining seen only in normal subjects, (b) staining of the germ tube only—as seen only in patients with candidiasis.

$\mu\text{g/ml}$) and sodium thioglycollate ($20 \mu\text{g/ml}$) was used to promote germination (Cassone *et al*, 1973). After inoculation the cultures were shaken continuously at 37°C for 2-3 hours. Germinating organism was then harvested, washed three times with saline, and stored at 4°C until used.

SEROLOGICAL METHODS

Agglutination

Blastospore suspension in saline was prepared containing 3×10^6 organisms per ml. The agglutinin titre of the serum was determined by mixing 0.5 ml of serially diluted serum with an equal volume of the prepared blastospore suspension. The mixtures were incubated at 37°C for 2 hours and allowed to stand overnight at 4°C . The agglutinin titre is the reciprocal of the maximum serum dilution giving a positive agglutination.

Indirect immunofluorescence

Anti-blastospore reactivity was determined by indirect immunofluorescence (IF-B). Smears of blastospores were prepared by adding a drop of yeast cell suspension onto glass slides, which were

allowed to air-dry and then fixed in acetone for 10 minutes. The smears were reacted successively for 1 hour each with aliquots of a serially diluted serum and a 1/16 diluted fluorescence conjugate goat anti-immunoglobulin (Hyland, USA) at 37°C in a moist chamber. The IF-B titre of a serum is expressed as the reciprocal of the maximum serum dilution which gave positive immunofluorescence.

Anti-germ tube reactivity (IF-T) was determined by the indirect immunofluorescence technique, as described above, except that acetone fixed smears of germinating yeast cells were used, and a positive reaction was scored by immunofluorescence staining of the germ tubes. The titre is expressed as the reciprocal of the maximum serum dilution that gave positive immunofluorescence staining of the germ tubes.

Results

In a preliminary study we have evaluated the anti-*Candida albicans* (group A) seroreactivity using acetone fixed smears of the germinating cells. At a low serum dilution, uniform staining of the organism

was observed (fig 1). But at a higher dilution of normal serum, immunofluorescence was found to be exclusively associated with the blastospores (fig 2a). Using the sera of patients with culturally confirmed candidiasis, however, the anti-blastospore reactivity was preferentially diluted out such that only residual germinating tube immunofluorescence was observed at high serum dilutions (fig 2b). This discordant seroreactivity therefore suggests that the antigenic contents of the blastospore and the germinating tube are distinctive.

The above results also reflect a qualitatively different antigenic stimulation caused by the organism as a parasite or as a commensal. To test this possibility further we have studied the anti-*C. albicans* (group A) seroreactivity of normal subjects and of patients with current *C. albicans* infection. Table I shows that the agglutinin (A) and the anti-blastospores (IF-B) and anti-germinating tube (IF-T) immunofluorescence titres were significantly increased in the infected subjects. The increase in anti-T (IF-T) was, however, the most marked.

	Mean Titre		
	Patients	Controls	P
IF-T	2582 (± 474)	113 (± 22)	<0.001
IF-B	931 (± 368)	160 (± 22)	<0.05
A	325 (± 109)	86 (± 22)	<0.05

Table I Anti-*C. albicans* seroreactivities in normal subjects and patients infected with *C. albicans*

The anti-*C. albicans* seroreactivities, as determined by the two different serological methods, were compared by analysing the linear regression of the immunofluorescence titres on the agglutinin titres (A). The IF-B titres of both normal and patients' sera were thus found to correlate with the corresponding agglutinin titres (coefficient of correlation = 0.700) (fig 3). The IF-T titres of the patients and the normal subjects were found to regress differently on the corresponding agglutinin titres (A) although the slopes of the two linear regression lines were similar (fig 4).

Discussion

Infections with commensal organisms have become increasingly prevalent in recent years. Because of their ubiquitous presence, it is often difficult to ascertain the exact significance of laboratory findings such as positive isolations of these organisms from clinical specimens or an increase in serum antibodies against these organisms. Although an increase in agglutinin or IF-B titres has been observed during *C. albicans* infection, the persistent level of the same

Fig 3 Linear regression of anti-blastospore immunofluorescence titres (IF-B) on agglutinin titres of sera of normal subjects (○) and patients (●).

Fig 4 Linear regression of anti-germ tube immunofluorescence titre (IF-T) on agglutinin titre of sera of normal subjects (○) and patients (●).

seroreactivity observed among the normal subjects renders it difficult to discriminate infected individuals. The anti-germ tube immunofluorescent assay, in contrast, appeared to provide a more acute reflection of the infected state since the increase in IF-T titre in *C. albicans* infection was more pronounced than the corresponding increases in the IF-B or agglutinin titres. This is consistent with the histological findings that the mycelial growth phase invariably occurs during infection with *C. albicans* (Winner, 1969).

On the basis of the present data it is apparent that the immunofluorescent and agglutinin assays are

related in their specificities, the immunofluorescent assays being the more sensitive. The results of this analysis also indicate that the IF-T titres are the more acute reflection of *C. albicans* infection, and an IF-T titre of 640 may be regarded as presumptive evidence of infection. Allowing an assay error of $\pm \log 2$, it may be anticipated that the detection rate of *C. albicans* infection will occur maximally at $\frac{1}{14}$ and minimally at $\frac{1}{13}$. The maximum and minimum rate of occurrence of 'false positives', on the other hand, may be anticipated to be $\frac{1}{13}$ and $\frac{0}{13}$ respectively. By setting presumptive IF-B and agglutinin titres at 160 and 80 respectively, it is apparent that these titres are less discriminatory for *C. albicans* infection (table II).

Serological methods	Presumptive titre	Rate of detection		Rate of false positive	
		Min	Max	Min	Max
Agglutination	80 (40-160)	$\frac{11}{14}$	$\frac{14}{14}$	$\frac{2}{13}$	$\frac{11}{13}$
IF-B	160 (80-320)	$\frac{12}{14}$	$\frac{14}{14}$	$\frac{2}{13}$	$\frac{13}{13}$
IF-T	640 (320-1280)	$\frac{13}{14}$	$\frac{14}{14}$	$\frac{0}{13}$	$\frac{1}{13}$

Table II Anticipated rates of detection of *C. albicans* infection by different serological methods

The different linear regression patterns of IF-T titres on agglutinin titres observed in the normal and

patients' sera may be used further to discriminate *C. albicans* infection. Thus, a line drawn parallel to but at +3 standard deviations from the linear regression line obtained with the normal subjects has delineated all but one of the patients. Both criteria, based on absolute IF-T level and discordant seroreactivities, are now being applied in the routine serological detection of *C. albicans* infection.

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