Comparative Evaluation of the *Candida* Agglutinin Test, Precipitin Test, and Germ Tube Dispersion Test in the Diagnosis of Candidiasis

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Received for publication 6 August 1975

Normal sera and sera from burned patients were examined for *Candida* agglutinin titers, precipitin titers, and the ability to disperse germ tubes of *Candida albicans* in an attempt to determine whether germ tube dispersion is correlated with *Candida* infection as animal models have indicated. Other investigators have reported that immunoglobulin G antibody to *Candida* interferes with a serum clumping factor resulting in germ tube dispersion. Germ tube dispersion in sera from burned patients with varying degrees of *Candida* infection is significantly greater than that found in uninfected controls. In addition, the germ tube dispersion test indicated the presence of *Candida* infection in several patients who had clinical evidence of infection but no detectable agglutinins or precipitins.

Candida albicans is commonly found as part of the flora of the skin and gastrointestinal tract. However, in the burn patient with impaired ability to resist infection, this organism may become a serious problem (10). It is often difficult to determine whether the isolation of *C. albicans* from a patient represents colonization or systemic infection. There is no serological test available to clearly differentiate colonization from systemic infection. Recently, attention has focused on the development of a serological test to detect systemic *Candida* infection.

When yeast cells of *C. albicans* are incubated in normal serum, they produce short filaments known as germ tubes (14, 17). Germ tube-producing yeast cells (subsequently called germ tubes) of *C. albicans* are clumped in normal sera (2, 6, 8, 15) by a serum clumping factor, but this clumping is absent or decreased in sera from patients with systemic or chronic mucocutaneous candidiasis (2, 8) as well as in sera from animals experimentally infected with *Candida* (6, 15). The reduced ability of sera from infected patients or animals to clump *Candida* is reported to be due to immunoglobulin G antibody to *Candida* that interferes with the serum clumping factor (2, 15).

The term "dispersion" is used to indicate cells with germ tubes that are not clumped. A quantitative germ tube dispersion test has been developed to determine the increase in the number of freely dispersed germ tubes that occurs in

mouse sera after *Candida* infection (6). This procedure has been suggested as a method to detect systemic candidiasis. We have examined the ability of the germ tube dispersion test to detect *Candida* infection in a group of burn patients and compared it with *Candida* agglutinating and precipitating antibody tests.

MATERIALS AND METHODS

Sera. Serum samples were collected from three groups of subjects and stored at -70°C until tested. Group 1 consisted of healthy, nonburned, uninfected individuals. Group 2 consisted of burn patients without *Candida* infection. Several burn sites were repeatedly cultured on each patient and none yielded growth of *C. albicans* (Table 1). Group 3 consisted of burn patients with *Candida* infection. In these patients, *C. albicans* was repeatedly cultured from the burn wounds of numerous body sites (Table 1).

The two groups of burn patients were similar in all respects except for the presence or absence of *Candida* infection. Statistical analyses were performed with the Student's *t* test. The mean age of group 2 patients was 8.0 years, compared with 7.7 years in group 3. The serum samples were obtained from these children at random intervals during their hospitalization, but the day after the burn was not significantly different between the two groups. The severity of the burn was somewhat greater in group 3 than group 2, but it was only of borderline significance.

Agglutinin titers. Heat-killed cells of *C. albicans* for the determination of agglutinin titers were initially prepared according to the method of Chilgren

TABLE 1. *Burn patient data*

Patient	Age	Percentage of burn total/third	Day after burn	Positive cultures of <i>C. albicans</i> :		
				Wounds	Urine	Blood
Group 2						
JH	8	22/4	15	0/7 ^a	0/3	0/2
TC	11	19/0	20	0/6	0/2	0/1
DD	6	6/2	116	0/10	0/1	0/1
OS	11	19/18	11	0/3	0/3	0/2
BK	4	10/10	17	0/2	0/1	0/1
RS	9	12/10	35	0/11		0/1
WB	5	56/41	41	0/12	0/6	0/9
Group 3						
DB	13	48/30	29	7/39	0/4	0/21
DC	13	36/29	23	5/7	1/3	0/1
MC	9	38/38	35	1/11	0/1	0/4
SC	4	31/23	30	5/13	0/3	0/8
BC	4	42/37	38	1/10	0/2	0/4
FC	13	66/48	69	43/58	10/11	0/28
SB	9	36/28	31	5/12	1/2	0/2
NB	5	38/18	17	6/8	0/8	0/6
KH	4	19/1	24	3/7	0/4	0/6
RC	12	69/52	17	1/26	0/5	0/14
DT	2	46/46	81	12/41	2/7	4/42
EB	5	64/28	51	11/32	1/7	1/15
JW	4	21/20	7	2/6	0/1	0/1
BB	11	93/93	359	75/212	25/88	5/166
LL	12	71/55	43	12/42	0/6	1/14

^a Number positive/number of cultures.

et al. (2). However, viability studies indicated the presence of viable cells. An additional 30 min of heating at 56 C was found to result in the complete absence of viable cells. Equal volumes of antigen and serum dilutions (1:3) were placed in a 37 C water bath for 3 h, and after an additional 18 h at 4 C the agglutinating antibody titer was read.

Precipitin titers. The presence of precipitating antibody was determined by double diffusion using 20 μ l of undiluted patient serum and 10 μ l of soluble antigen (Schering Corp., Bloomfield, N.J.). The immunodiffusion plates were incubated in a moist chamber at 25 C and examined after 72 h. The titer of any precipitating antibody observed in undiluted serum was determined using 20 μ l of twofold serum dilutions.

Germ tube dispersion test. An inoculum of 10^6 *C. albicans* was introduced into 0.3 ml of normal or patient serum and shaken at 77 rpm at 37 C (6). After 3 h of incubation, the tubes were agitated. Inasmuch as Katsura and Uesaka (6) did not specify the time or vigor of manual shaking, mechanical agitation was selected to ensure that all samples were equally agitated. Blending in a Vortex mixer less than 45 s does not significantly affect germ tube dispersion. The tubes were placed in a Vortex mixer for 15 s (Vortex Genie, Fisher Scientific), and the number of freely dispersed germ tubes was counted in a hemocytometer (Bright-line Neubauer, 0.1 mm deep). The inoculum corresponds to 250 cells per 10^{-4} ml in the hemocytometer (6).

RESULTS

Serological data from normal individuals and uninfected burn patients. Sera from normal individuals (group 1) and also from burn patients without *Candida* infection (group 2) clump *Candida* as indicated by the low number of freely dispersed germ tubes (Table 2). The difference in the mean number of dispersed germ tubes in these two groups is not significant. More of the uninfected burn patients demonstrated antibody titers than did the normal subjects. Agglutinins were present in five of seven uninfected burn patients, precipitins were detected in two compared with agglutinins which were found in only four of 12 normal individuals, and precipitins were present in none of the normal individuals.

Serological data from infected burn patients. Germ tubes of *C. albicans* were dispersed in sera from burn patients with varying degrees of *Candida* infection (Table 3). The mean number of dispersed germ tubes observed in sera from these patients is significantly greater than the number found in sera from uninfected burn patients ($P < 0.001$) or normal individuals ($P < 0.001$). Ten of 14 burn patients with *Candida* infection showed agglutinin titers, and five demonstrated precipitins. Every

TABLE 2. Serological data on normal subjects and burn patients without *Candida* infection

Patient ^a	Agglutinin titer	Precipitin titer	No. of dispersed germ tubes
Group 1			
MM	Neg	Neg	80
SS	Neg	Neg	64
AK	1:10	Neg	28
JL	1:40	Neg	24
BS	Neg	Neg	32
SD	Neg	Neg	48
DO	1:10	Neg	24
FN	Neg	Neg	56
DW	1:40	Neg	70
FL	Neg	Neg	60
PY	Neg	Neg	68
MC	Neg	Neg	61
			51 ± 6 ^b
Group 2			
JH	1:80	Neg	42
TC	1:80	Neg	72
DD	1:160	Neg	32
OS	1:160	+ (Undiluted only)	10
BK	Neg	Neg	57
RS	1:160	1:4	8
WB	Neg	Neg	122
			49 ± 15 ^b

^aGroup 1, normal subjects; group 2, burn patients.^bMean ± standard error of the mean.

patient whose serum contained precipitating antibody was also shown to have agglutinating antibody of titer 1:80 or greater.

The four patients with *Candida* infection who yielded the highest number of dispersed germ tubes in their sera (DT, EB, BB, and LL in Table 3) also had *C. albicans* recovered in one or more of their blood cultures. Cultures of intravenous catheters were sterile in patients DT and EB, whereas *C. albicans* was recovered from the catheters of patients BB and LL. However, skin biopsy of a leg wound in patient LL revealed the presence of invasive *Candida* infection. The serum of this patient dispersed the greatest number of germ tubes.

The serum of patient JW (Table 3) was found to yield 104 dispersed germ tubes despite the absence of agglutinating or precipitating antibody. This serum sample was obtained on the day of hospital admission, at which time wound cultures of each arm yielded *C. albicans*. One week later, *C. albicans* was also recovered from the blood, and skin biopsy revealed the presence of invasive *Candida* infection.

DISCUSSION

The occurrence of *Candida* agglutinins (1, 5) and precipitins (12, 16) in normal sera, in unin-

fected hospital patients (11), and in burn patients without apparent *Candida* infection (Table 2) makes it difficult to evaluate patients with suspected *Candida* infection by these serological tests. Although agglutinin titers greater than 1:160 are considered significant, they fail to distinguish between superficial and visceral infections (18). In addition, precipitins have been reported to be absent in patients with terminal candidemia (7, 18) and systemic candidiasis (19). These communications illustrate the problems that may be encountered in attempting to diagnose systemic candidiasis with currently available serological methods.

The data presented here indicate that the quantitative germ tube dispersion test may be useful in detecting *Candida* infection. It has been demonstrated (Table 2) that the low number of dispersed germ tubes observed in sera from uninfected burn patients, despite the presence of agglutinin and precipitin titers, is comparable to the number observed in normal sera, suggesting that the presence or absence of *Candida* infection may be better indicated by the germ tube dispersion test than by agglutinin or precipitin tests. Four burn patients with *Candida* infection (DB, SC, KH, and JW in Table 3) had no detectable antibodies by the agglutinin and precipitin tests, yet the number of dispersed germ tubes in their sera is twice that found in sera from uninfected burn patients. This suggests that the dispersion test correlates better with clinical evidence of infection than

TABLE 3. Serological data on burn patients with *Candida* infection

Group 3 patient	Agglutinin titer	Precipitin titer	No. of dispersed germ tubes
DB	Neg	Neg	108
DC	1:80	Neg	151
MC	1:80	+ (Undiluted only)	137
SC	Neg	Neg	132
BC	1:80	Neg	122
FC	1:80	1:4	101
SB	1:80	+ (Undiluted only)	85
NB	1:80	Neg	142
KH	Neg	Neg	83
RC	1:80	1:2	34
DT	ND ^a	ND	174
EB	1:160	Neg	175
JW	Neg	Neg	104
BB	1:320	1:4	152
LL	1:160	Neg	186
			126 ± 11 ^b

^a ND, Not determined.^b Mean ± standard error of the mean.

agglutinin or precipitin tests.

The majority of the burn patients evaluated in this study received intravenous fluid therapy (plasma expanders, blood, blood products) during their hospitalization, presenting the possibility that such fluids may affect the dispersion of germ tubes. Katsura and Uesaka have presented evidence (6) that with increasing dilution of serum the number of dispersed germ tubes decreases. However, the most marked reduction occurred with large serum dilutions (1:64 and 1:256). No burn patient received a volume of fluids that would effectively dilute his total blood volume 64 times. It is therefore unlikely that the fluid therapy concomitant with the management of burn patients would affect the dispersion of germ tubes.

In an experimental animal model, germ tube dispersion increased in mouse sera with increasing severity of macroscopic lesions in the organs (6). In the burn patients described in this report, it is difficult to determine the severity of the *Candida* infections inasmuch as there were no deaths and subsequent postmortem examination of the organs was not possible. Without tissue biopsy, the presence or absence of systemic *Candida* infection cannot be documented in the patients in group 3. Blood cultures for *Candida* were negative in 11 of these 15 burn patients, suggesting that the majority most likely have local infections. Four patients had episodes of candidemia that cleared upon amphotericin B therapy. Although tissue candidiasis may occur in less than 50% of instances of candidemia (4), at least one of the patients (LL) had histological evidence of invasive *Candida* infection. Although evidence for tissue infection is not available in the other three patients, sera from these four patients dispersed the highest number of germ tubes. These results may indicate that the introduction of *Candida* into the systemic circulation is of sufficient antigenic stimulus to produce an increase in the number of dispersed germ tubes that could signal the potential for the development of systemic *Candida* infection.

The ability of yeasts to form germ tubes in sera after 2 to 3 h of incubation is limited to *C. albicans*, *C. stellatoidea*, *C. utilis*, *C. rugosa*, and *Schizosaccharomyces fragilis* (3, 9, 17). Of these, only *C. albicans* and *C. stellatoidea* are encountered in human infections. Katsura and Uesaka (6) have demonstrated that, whereas animals infected with *C. albicans* disperse germ tubes of both *C. albicans* and *C. stellatoidea*, those animals infected with *C. stellatoidea* dispersed germ tubes of the homologous organism to a greater extent than those of the

heterologous organism. There were no burn patients with *C. stellatoidea* infection available for this study, but patients with such infections should be tested to determine the amount of germ tube dispersion in their sera.

A decrease in clumping activity was demonstrated when agglutinating and precipitating antibody titers were maximal in the sera of rabbits immunized with heat-killed *C. albicans* (15). However, the antibody titers of the patients described in this study show no clear relationship to germ tube dispersion. Although it has been reported that immunoglobulin G antibody to *Candida* interferes with the serum clumping factor (2, 15), an alternative explanation may be that *Candida* antigen in the serum binds the clumping factor, resulting in dispersion. Indeed, Weiner and Yount have reported that transient mannan antigenemia occurred in three of four patients with systemic candidiasis up to 10 days before the development of precipitins to *Candida* cytoplasmic or cell wall antigens (Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, F4, p. 86).

The data presented here indicate that the germ tube dispersion test correlates better with clinical evidence of *Candida* infection than the agglutinin or precipitin tests. Sera from normal subjects dispersed a low number of germ tubes. Similarly, sera from burn patients with no clinical evidence of *Candida* infection also dispersed a low number of germ tubes despite the presence of *Candida* agglutinins and precipitins. Furthermore, sera from burn patients with clinical evidence of *Candida* infection dispersed a significantly greater number of germ tubes than did the sera from uninfected patients. Although the majority of the infected burn patients had agglutinins, the germ tube dispersion test indicated the presence of *Candida* infection in four additional patients who demonstrated no detectable antibodies in agglutinin and precipitin tests. In addition, it has been demonstrated that germ tube dispersion was greatest in patients with candidemia, suggesting its value in detecting potentially serious infections. The results of this study indicate that germ tube dispersion is significantly greater in sera from burn patients with *Candida* infection and that the testing of additional patients with other disorders is warranted.

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