The relationship between fluorescent, agglutinating, and precipitating antibodies to *Candida albicans* and their immunoglobulin classes

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SYNOPSIS A parallel study of fluorescent, agglutinating, and precipitating antibodies to *Candida albicans* revealed that precipitating antibodies belong to the IgG class, whereas agglutinating antibodies reside in the IgG, IgM, and IgA classes. The three types as well as the three classes of antibodies were found in *Candida* endocarditis and mucocutaneous candidiasis. Immuno-absorption studies suggest that the three serological tests estimate antibodies to mannan determinants of *Candida albicans*.

A variety of tests for antibodies to *Candida* have been used in different laboratories and the most common of these are agglutinating tests (Winner, 1955; Murray, Buckley, and Turner, 1969), precipitating tests (Murray *et al.*, 1969; Stallybrass, 1964; Taschdjian, Kozinn, and Caroline, 1964; Chew and Theus, 1967; Pepys, Faux, Longbottom, McCarthy, and Hargreave, 1968), and immuno-fluorescent tests (Lehner, 1966, 1970; Esterly, 1968). The relationship between these antibodies and their immunoglobulin classes has not been defined, although this is of considerable interest in view of the diverse diagnostic claims made for some of these antibodies. Winner (1955) found that up to 64% of the apparently uninfected population had agglutinating antibodies to *Candida* and this led him to question their diagnostic value. Precipitating antibodies, however, have been found predominantly in systemic candidiasis (Stallybrass, 1964; Taschdjian *et al.*, 1964), though others (Chew and Theus, 1967; Pepys *et al.*, 1968) have found them in healthy subjects or in the absence of systemic candidiasis (Murray *et al.*, 1969).

A quantitative relationship between the level of serum antibodies in patients with candidiasis, as compared with carefully selected controls, was established for agglutinins (Comaish, Gibson, and Green, 1963) and for fluorescent antibodies (Lehner, 1966 and 1970). These results suggested that the diagnosis of candidiasis was dependent on the sensitivity and possibly immunoglobulin class of antibodies and that significant titres could be found

to differentiate infection from controls. Indeed, the aims of this investigation were to compare three methods of antibody estimation with special reference to the immunoglobulin class of antibody.

Patients and Methods

SERA

The series of 31 samples of blood were divided into three groups.

Group I

Sera from 10 patients with chronic mucocutaneous candidiasis.

Group II

Seven sequential sera from three patients who had cardiac surgery and developed *Candida* endocarditis.

Group III

Fourteen sequential sera from four patients who had cardiac surgery without developing endocarditis.

FLUORESCENT ANTIBODY TEST

The indirect technique was used to determine the titre of the specific immunoglobulin class of *Candida* antibodies by using monospecific IgG, IgA, and IgM conjugates as described previously (Lehner, 1966, 1970). Sheep antirabbit serum conjugate (Wellcome reagents) was used to estimate the fluorescent antibody to *Candida* in the antisera raised in rabbits. The smears were made by using yeast cells of *Candida albicans* group A, suspended

in 0.4% formaldehyde saline. Doubling dilutions of sera from 1 in 2 were made in buffered saline to determine the antibody titre.

AGGLUTINATION TEST

This was carried out with whole yeast cells of another strain of *Candida albicans* group A as described previously (Murray *et al.*, 1969). The agglutination titre was determined by using serially diluted sera.

PRECIPITATION TEST

The organisms were disrupted by a hydraulic press, centrifuged at 40000 *g*, and the supernatant was used in a gel diffusion precipitation test (Murray *et al.*, 1969). The results were expressed as positive or negative precipitation reactions. In order to be certain that the strain of *Candida albicans* group A used in the fluorescent antibody test did not differ from that applied to the agglutination and precipitation reactions, a number of sera were tested in parallel by the fluorescent antibody test with smears prepared from both strains of *Candida*. Precipitating antibodies to mannan and protein components of the yeast cells were tested by double diffusion tests as described previously (Buckley, Lapa, and Hipp, 1970).

DEAE CELLULOSE CHROMATOGRAPHY

A pooled serum from four patients with oral candidiasis was fractionated by the method described previously (Levy and Sober, 1960; Lehner, 1969). The resulting three fractions were examined for IgG, IgA, and IgM by immunoelectrophoresis and each fraction was tested for *Candida* antibodies.

RABBIT ANTIBODY TO CANDIDA ALBICANS

Serum IA was raised by two subcutaneous injections of 260 γ of soluble antigen in incomplete Freund's adjuvant at monthly intervals, and the rabbits were bled after three weeks. Serum RAS was raised by intravenous injections of 2% formalized cells, three times a week for four weeks, and the rabbits were bled one week after the last injection.

IMMUNOABSORPTION

Antisera IA, RAS, and a serum from a patient with systemic candidiasis, containing antibodies to *Candida albicans*, were absorbed. One ml of serum was mixed with 0.4 ml of a *Candida* suspension,

Fig. 1 Comparison of fluorescent and precipitating antibody tests.

Fig. 2 The relationship between agglutination and fluorescent antibody titres.

with a PCV of 70%; this was kept at 37°C for three hours and then at 4°C overnight. The absorbed serum was centrifuged and then reabsorbed. The absorbed and unabsorbed sera were tested by the three methods for *Candida* antibodies.

Results

RELATIONSHIP BETWEEN THE IMMUNOGLOBULIN CLASSES OF CANDIDA ANTIBODIES

The immunoglobulin class of fluorescent antibodies is compared with precipitating and agglutinating antibodies in Figures 1 and 2. The highest titres were found with the agglutination test up to 1 in 512, followed by fluorescent antibodies, up to 1 in 256 for IgG, and 1 in 128 for IgA and IgM. A very significant relationship was established by means of the Chi square test (with Yate's correction) between precipitating and IgG fluorescent antibodies ($P < 0.001$), but not with IgM or IgA. However, agglutinating titres were significantly correlated with all three immunoglobulin classes of fluorescent antibody titres: IgM ($r = 0.615$, $P < 0.001$), IgG ($r = 0.534$, $P < 0.01$), IgA ($r = 0.461$, $P < 0.01$). The fluorescent antibody titre against the two strains of *Candida albicans* was almost identical.

The immunoglobulin class of antibodies was determined directly by using the chromatography fractions (Fig. 3). IgG was found in the first fraction, IgM in the third fraction, and IgA (with some IgG) in the second fraction. The corresponding class of fluorescent antibodies was found in each of these fractions but precipitins were detected only in the IgG fraction. Agglutinins were present mostly in the IgM fraction (titre of 1:64), to a less extent in IgG (1:16), and a low titre of (1:4) was also detected in the IgA fraction.

ANTIBODIES IN SYSTEMIC AND MUCOCUTANEOUS CANDIDIASIS AND IN RABBIT ANTISERA A rise in antibody titre and the appearance of precipitins was found in patients with endocarditis (Table I). This was also present in two of the four patients who did not develop endocarditis, although the fluorescent antibody titre rose only to low levels. The group of patients with chronic mucocutaneous candidiasis revealed significant agglutinating and fluorescent antibodies, and precipitating antibodies were found in six out of 10 patients (Table II). Both rabbit antisera showed agglutinating and fluorescent antibodies (Table III) but serum IA revealed precipitating antibodies to mannan and protein

Fig. 3 The distribution of IgG, IgA, and IgM classes of *Candida* antibodies, as determined by the fluorescent, agglutination, and precipitation antibody tests with DEAE cellulose chromatography fractions.

Patients	Pre- cipitating Antibody	Agglutinating Antibody	Fluorescent Antibody		
			IgG	IgA	IgM
<i>With Candida Endocarditis</i>					
1 (before surgery)	—	0	4	2	0
1a (after surgery)	+	128	64	128	8
2	+	256	128	32	32
3	+	256	256	32	64
<i>Without Endocarditis</i>					
1 (before)	—	0	2	4	4
1a (after)	+	128	16	4	16
2 (before)	—	0	2	4	4
2a (after)	+	512	32	16	16
3	—	128	2	2	16
4	—	0	0	0	0

Table I Comparison of fluorescent, precipitating, and agglutinating antibodies to *Candida albicans* in patients having cardiac surgery

Serum	Precipitating Antibody	Agglutinating Antibody	Fluorescent Antibody		
			IgG	IgA	IgM
1	+	128	32	16	128
2	+	64	16	2	8
3	+	64	128	32	32
4	+	64	128	16	16
5	+	32	64	16	1
6	+	32	256	64	0
7	—	32	8	2	0
8	—	32	0	64	16
9	—	4	64	0	0
10	—	4	8	2	4

Table II Comparison of fluorescent, precipitating, and agglutinating antibodies in patients with chronic mucocutaneous candidiasis

components, whereas serum RAS had only antibodies to mannan.

IMMUNOABSORPTION

After absorption of the three antisera the precipitating and fluorescent antibodies were undetectable except for a titre of 1 in 8 (no. 6; Table III) and the agglutinating antibodies were depressed to ≤ 1 in 16.

Serum	Precipitating Antibody to:		Agglutinating Antibody	Fluorescent Antibody		
	Protein	Mannan		IgG	IgM	IgA
1 Human 562	+	+	128	32	16	16
2 Human 562 absorbed	+	—	< 16	0	0	0
3 Rabbit 1A	+	+	> 100		γ globulin	
4 Rabbit 1A absorbed	+	—	< 16		32	
5 Rabbit RAS	—	+	> 1000		0	
6 Rabbit RAS absorbed	—	—	16		256	
					8	

Table III Comparison of four types of antibody in two rabbit and one human antisera to *Candida albicans* before and after absorption

These findings were particularly significant as antibodies to mannan components were absent, though antibodies were present to protein components in the absorbed sera (nos. 2 and 4; Table III). Antiserum RAS (no. 5; Table III) showed antibodies only to mannan components and the highest agglutinating and fluorescent antibody titres. It appears from these results that mannan and not protein antibodies are responsible for the agglutination and fluorescent reactions.

Discussion

Direct testing of the immunoglobulin fractions of human serum has revealed that precipitins to *Candida* belong to the IgG class and that they are very significantly related to the IgG class of fluorescent antibodies ($P < 0.001$). This is consistent with the view that IgG antibodies are very effective precipitins (Pike, 1967). Agglutinins, however, were found in highest titre in the IgM fraction (1:64) of fluorescent antibodies ($r = 0.615$, $P < 0.001$). Agglutinating antibodies were also detected in the IgG (1:16) and IgA (1:4) fractions, and were significantly related to the IgG ($r = 0.534$, $P < 0.01$) and IgA ($r = 0.461$, $P < 0.01$) fluorescent antibodies. Although the IgA chromatography fraction contained a small amount of IgG, there was no detectable IgG in the IgA fluorescent conjugate, so that agglutinating antibodies to *Candida* may belong to the IgA in addition to the IgM and IgG classes. Thus, the agglutination test for *Candida* estimates the three major classes of antibodies and the high agglutination titre may represent a summation of the IgM, IgG, and IgA titres. Indeed, the agglutination titre showed the best correlation with the three summated fluorescent antibody titres ($r = 0.695$, $P < 0.001$). These results are consistent with the findings of others that for agglutination smaller amounts of IgM than IgG are required and that agglutinating activity is shown by the IgA class of antibodies (Pike, 1967).

A differential diagnosis could not be made between systemic and mucocutaneous candidiasis by any one of the three immunological tests. Precipitins were

found in six out of 10 patients with chronic mucocutaneous candidiasis and this differed from other results (Stallybrass, 1964; Taschdjian *et al*, 1964). It is now very likely that the specificity ascribed to precipitins can be accounted by their belonging to the IgG class, unlike agglutinins that are found in the IgM, IgG, and IgA classes.

In the three patients with *Candida* endocarditis precipitins and raised agglutinating and fluorescent antibody titres were found. This was particularly evident in the patient in whom serum antibodies could be estimated before and after endocarditis developed (no. 1; Table I). An unusual feature in this patient was that the IgM class of fluorescent antibodies rose only from 0 to 8, though IgA antibodies rose from 2 to 128. The appearance of precipitins and a rise of agglutinating titre from 0 to 128 and to 512 in two out of four patients who have undergone cardiac surgery without developing endocarditis could not be interpreted satisfactorily. It is of some interest that the fluorescent antibody test has not shown a corresponding rise in titre in these patients and reached only IgG levels of 1:16 and 1:32, and IgM of 1:16. This suggests that the immunofluorescent test is more reliable than the precipitating and agglutinating tests and that a rise in IgG fluorescent antibody titres to $\geq 1:64$ should be considered as evidence in favour of the diagnosis of systemic candidiasis.

The results of the absorption studies suggest that the present serological tests estimate antibodies to mannan determinants. Thus, high titres of agglutinating and fluorescent antibodies were found in the presence of precipitating antibodies to mannan determinants and absence of protein determinants (Table III). Absorption of antibodies to mannan determinants from antisera in which antibodies to protein determinant were left unabsorbed was associated with a loss of or a significant fall in agglutinating and fluorescent antibodies. Mannan antibodies were also recorded in precipitation tests by others (Chew and Theus, 1967; Pepys *et al*, 1968). As mannans are probably cell wall components of *Candida* (Kessler and Nickerson, 1959; Kemp and

Solotorovsky, 1964), and may reside on the surface of yeast cells (Hasenclever and Mitchell, 1964), they would be directly accessible to whole cell agglutination and to antibody binding in the immunofluorescent test.

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References

- Buckley, H. R., Lapa, E. W., and Hipp, S. S. (1970). Immunological significance of some antigens of *Candida albicans*. In *Proceedings of the 5th International Congress of Infectious Diseases, Vienna*.
- Chew, W. H., and Theus, T. L. (1967). *Candida* precipitins. *J. Immunol.* **98**, 220-224.
- Comaish, J. S., Gibson, B., and Green, C. A. (1963). Candidiasis: serology and diagnosis. *J. invest. Derm.*, **40**, 139-142.
- Esterly, N. B. (1968). Serum antibody titers to *Candida albicans* utilizing an immunofluorescent technic. *Amer. J. clin. Path.*, **50**, 291-296.
- Hasenclever, H. F., and Mitchell, W. O. (1964). A study of yeast surface antigens by agglutination inhibition. *Sabouraudia*, **3**, 288-300.
- Kemp, G., and Solotorovsky, M. (1964). Localisation of antigens in mechanically disrupted cells of certain species of the genera *Candida* and *Torulopsis*. *J. Immunol.*, **93**, 305-314.
- Kessler, G., and Nickerson, W. J. (1959). Glucomannan—protein complexes from cell walls of yeasts. *J. biol. Chem.*, **234**, 2281-2285.
- Lehner, T. (1966). Immunofluorescence study of *Candida albicans* in candidiasis carriers and controls. *J. Path. Bact.*, **91**, 97-104.
- Lehner, T. (1969). Characterization of mucosal antibodies in recurrent aphthous ulceration and Behcet's syndrome. *Arch. oral Biol.*, **14**, 843-853.
- Lehner, T. (1970). Serum fluorescent antibody and immunoglobulin estimations in candidosis. *J. med. Microbiol.*, **3**, 475-481.
- Levy, H. B., and Sober, H. A. (1960). A simple chromatographic method for preparation of gamma globulin. *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 250-252.
- Murray, I. G., Buckley, H. R., and Turner, G. C. (1969). Serological evidence of *Candida* infection after open-heart surgery. *J. med. Microbiol.*, **2**, 463-469.
- Pepys, J., Faux, J. A., Longbottom, J. L., McCarthy, D. S., and Hargreave, F. E. (1968). *Candida albicans* precipitins in respiratory disease in man. *J. Allergy*, **41**, 305-318.
- Pike, R. M. (1967). Antibody heterogeneity and serological reactions. *Bact. Rev.*, **31**, 157-174.
- Stallybrass, F. C. (1964). *Candida* precipitins. *J. Path. Bact.*, **87**, 89-97.
- Taschdjian, C. L., Kozinn, P. J., and Caroline, L. (1964). Immune studies in candidiasis. III. Precipitating antibodies in systemic candidiasis. *Sabouraudia*, **3**, 312-320.
- Winner, H. I. (1955). Study of *Candida albicans* agglutinins in human sera. *J. Hyg. (Lond.)*, **53**, 509-512.